horticulturae

Article

Obtaining Vegetable Production Enriched with Minor Micronutrients Using Fullerene Derivatives

Gayane G. Panova [1,*], Konstantin N. Semenov [2], Anna S. Zhuravleva [1], Yuriy V. Khomyakov [1], Elena N. Volkova [1,3], Galina V. Mirskaya [1], Anna M. Artemyeva [4], Nailia R. Iamalova [1,2], Victoriya I. Dubovitskaya [1] and Olga R. Udalova [1]

1 Agrophysical Research Institute (AFI), 195220 Saint-Petersburg, Russia; zhuravlan@gmail.com (A.S.Z.); himlabafi@yandex.ru (Y.V.K.); ele-ven@yandex.ru (E.N.V.); galinanm@gmail.com (G.V.M.); yamalova.nailya@gmail.com (N.R.I.); vikot85@mail.ru (V.I.D.); udal59@inbox.ru (O.R.U.)
2 Department of General and Bioorganic Chemistry, Pavlov First Saint Petersburg State Medical University, 197022 Saint-Petersburg, Russia; semenov1986@yandex.ru
3 Department of Environment and Rational Nature Management, Saint Petersburgs State University of Industrial Technologies and Design, 198095 Saint-Petersburg, Russia
4 Vavilov All-Russian Research Institute of Plant genetic Resources (VIR), 190000 Saint-Petersburg, Russia; akme11@yandex.ru
* Correspondence: gaiane@inbox.ru; Tel.: +7-(812)535-79-09

Abstract: Elaborating on the methods and means of enriching nutrition, including that of plants, with a number of microelements that are vital for humans is now very important due to the unresolved acute problems of micronutrient deficiency and imbalance, which affect the majority of the population of various countries in the world. Promising solutions for the implementation of biofortification in terms of safety, efficiency, size, biocompatibility, and transportability are the water-soluble derivatives of C_{60} or C_{70} fullerene. By now, the use of water-soluble fullerenes ($C_{60}(OH)_{22–24}$ or $C_{70}(OH)_{12–14}$ fullerenols, C_{60} fullerene with glycine or with arginine: C_{60}-*L*-Gly or C_{60}-*L*-Arg) with various functional groups for plant enrichment is pioneering. Experimental research work was carried out at the agrobiopolygon of the Agrophysical Research Institute under controlled microclimate conditions. This work constituted an assessment of the influence of $C_{60}(OH)_{22–24}$ fullerenol introduction into the soil on the content of macro- and microelements in the soil and in plants, for example, cucumber, as well as on the plants' physiological state (photosynthetic pigments, the intensity of lipid peroxidation, the activity of peroxidase and catalase enzymes), growth, and element content. Its aim was to study the possibility of enriching the plants' production (Chinese cabbage, tomato, and cucumber) with compositions of the fullerene derivatives (C_{60}-*L*-Gly or C_{60}-*L*-Arg, $C_{60}(OH)_{22–24}$ or $C_{70}(OH)_{12–14}$ fullerenols) and selenium or zinc compounds by introducing them into a nutrient solution or by foliar treatment of plants. It was revealed that the introduction of solutions of C_{60} fullerenol in various concentrations (1 mg/kg, 10 mg/kg, and 100 mg/kg) into soddy-podzolic sandy loamy soil contributed to the activation of the processes of nitrogen transformation in the soil, in particular, the enhancement of the process of nitrification, and to the increase in the content of mobile forms of some macro- and microelements in the soil as well as of the latter in plant organs, for example, in cucumber plants, especially in their leaves. Along with this, the plants showed an increase in the content of photosynthetic pigments, a predominant decrease in the activity of the oxidative enzyme peroxidase and in the intensity of lipid peroxidation, and an increase in the content of the reducing enzyme catalase. The improvement in the physiological state of plants had a positive effect on the growth rates of cucumber plants. The compositions of solutions of amino acid fullerenes (C_{60}-*L*-Gly or C_{60}-*L*-Arg) and sodium selenate as well as C_{60} or C_{70} fullerenols and zinc sulfate, selected on the basis of different charges of molecules or functional groups of fullerene derivatives, showed higher efficiency at low concentrations in enriching the plant products of Chinese cabbage, tomato, and cucumber with selenium and zinc, respectively, compared with mineral salts of the indicated elements and control (edible part of Chinese cabbage: by 31.0−89.0% relative to that in the control and by 26.0–81.0% relative to the treatment of plants with a sodium selenate; tomato fruits: by 33.7–42.2% relative to that in the control and by 10.2–17.2% relative to the treatment of plants with a sodium

check for updates

Citation: Panova, G.G.; Semenov, K.N.; Zhuravleva, A.S.; Khomyakov, Y.V.; Volkova, E.N.; Mirskaya, G.V.; Artemyeva, A.M.; Iamalova, N.R.; Dubovitskaya, V.I.; Udalova, O.R. Obtaining Vegetable Production Enriched with Minor Micronutrients Using Fullerene Derivatives. *Horticulturae* **2023**, *9*, 828. https://doi.org/10.3390/horticulturae9070828

Academic Editor: Miguel Guzmán

Received: 1 June 2023
Revised: 9 July 2023
Accepted: 14 July 2023
Published: 20 July 2023

selenate; cucumber fruits: by 42.0–59.0% relative to that in the control and by 10.0–23.0% relative to the treatment of plants with a zinc sulfate). At the same time, the quantitative characteristics of growth, productivity, and/or quality of the obtained products increase and improve accordingly. The prospects for further research include an in-depth study into the mechanisms of the compositions of fullerene derivatives and various compounds of trace elements' influence on the plants, as well as the synthesis and study of the various exo- and endo derivatives of fullerenes' properties, including C_{60} complex compounds with transition metals and fullerenes, which, inside their carbon networks, contain atoms of various chemical elements, such as lanthanum and others.

Keywords: biofortification; trace elements; selenium; zinc; Chinese cabbage; tomato; cucumber; polyhydroxylated fullerenes C_{60}; C_{70}; amino acid derivatives C_{60}; soil–plant system; soil substitute–plant system; plant growth; quality; productivity

1. Introduction

The problems of micronutrient deficiency and imbalance in the majority of the population of various countries in the world, leading to various dangerous diseases and immune status disorders, are acute and require the search for an environmentally safe solution. According to the WHO, various human diseases, including cancer, are associated with certain nutritional disorders [1,2]. Specialists in the field of nutrition reported that the elimination of vitamin D deficiencies, a decrease in the content of simple carbohydrates in the diet, and an increase in natural antioxidants, vitamins A, C, E, B_6, B_{12}, folic acid, microelements (iron, iodine, copper, selenium, manganese, zinc, chromium, etc.), and polyunsaturated fatty acids of the ω-3 family can ensure the genetic stability of cells and slow down the processes of biological aging and the development of age-associated diseases [3,4]. Among these micronutrients, the trace elements selenium and zinc play the most important roles in the human organism. The most exhaustive reviews on the anticarcinogenic properties of the listed substances are given in the following works: selenium [5,6] and zinc [7,8]. In experiments with induced cancer, all of these substances showed an ability to inhibit the development of tumors in the so-called period of promotion of carcinogenesis and are usually used for a long time. The mechanism of their anticarcinogenic action is diverse.

The above-mentioned diet nutrition includes the regular consumption of fruit and vegetables containing the necessary amounts of biologically active compounds with a complex of useful properties, including selenium and zinc. However, in order to obtain the recommended daily doses (for example, selenium: ~0.030–0.055 mg/day; zinc: ~9–19 mg/day) [6,9–11] of useful micronutrients within these plant products (for example, vegetable crops contain, in mg per kg of the plants' edible part, selenium:~0.0001–0.022 and zinc: ~0.8–9.1) [9,12], it is necessary to consume several kg or more per day, which is not possible for the human organism.

One of the ways to solve this problem is to increase the production of plant raw materials and high-quality products containing minor micronutrients in quantities that are safe for the human organism. The latter can be achieved through the development and implementation in the agroindustrial complex of new environmentally friendly and highly effective biofortification methods, techniques, means, technologies, and others.

In this regard, the task of enriching agricultural crops with biologically active substances was declared a priority by the world scientific community in the 21st century. A fairly large number of research papers is devoted to the consideration of the problem of the environmentally safe targeted enrichment of plant production with useful ingredients and the search for ways to solve it [13]. In particular, they note the relevance of research aimed at improving the safety and controllability of the process of plant enrichment and the development of new methods and means to achieve this goal.

The enrichment of plants with microelements is carried out by introducing their compounds into the root environment (soil or nutrient solution) or through a pre-sowing treatment of the seed material and foliar treatment of vegetative plants during the growing season [14,15]. Many preparations of microfertilizers and preparations containing zinc and other trace elements chelated with organic compounds, in particular, citric acid, humic acids, and synthetic polymers, have been created [13,16–19]. With regard to selenium, the market is significantly less saturated with preparations containing it. There are known mineral fertilizers containing sodium selenate [20–23], selenium-containing preparations (sodium selenite, sodium biselenite, sodium selenate, etc.) for introduction into the solution during hydroponic plant cultivation [23,24], and seed- and foliar plant treatments [23,25–28]. A number of researchers have shown the dependence of the efficiency of plant enrichment with selenium on the type of soil or substrate, natural or climatic environmental conditions, applied agricultural technologies, species, varieties, or hybrids of plants, as well as on the form of the applied selenium-containing compound [21,23,29]. Thus, some researchers believe that selenate is 33 times more effective than selenite when fertilizing plants [30]. The best accumulation of selenium was observed when using Se in the form of selenate (VI) for potatoes [29], carrots [31], common buckwheat [32], winter wheat [33], lettuce [34], and turnips [35], while selenite (IV) was more effective in rice biofortification [36].

However, there are serious problems in implementing technology for enriching plants with selenium and zinc microelements, namely: the toxicity of microelements in high concentrations to plants and other biological objects; varietal differences in response to the gradient content of the microelement in the environment; environmental pollution [19,23,37]. In this regard, it is very important to search for and to develop an environmentally safe, biologically active, and biodegradable preparation process that ensures the effective delivery of the necessary macro- and microelements and other physiologically active compounds to the plants. The currently developed carbon nanomaterials, in particular, fullerenols and other water-soluble adducts of fullerenes (for example, amino acids), can serve as promising sources for obtaining such preparations. Fullerenols are a mixture of polyoxyhydroxylated water-soluble fullerene derivatives [38,39]. The high lipophilicity of the carbon core of fullerenols ensures their penetration through biomembranes, their nanosize ensures steric correspondence to biomolecules, and the "cloud" of π- electrons on the surface ensures their participation in free radical reactions [38,40–42]. Due to these properties, fullerenols have a great potential for application in medicine and pharmacology, as well as in other fields in the science and economic sectors [38].

However, the use of water-soluble fullerene derivatives has not yet become widespread in crop production due to a lack of sufficient knowledge about the mechanisms of their influence on agro- and ecosystems and their living components, including plants. Research work in this direction has emerged recently [40–56]. At the same time, the information in the literature is not systematic and is often contradictory, which is due to the experiments carried out by different research groups under different conditions using different forms and concentrations of the studied substances. Thus, in the works of various authors, the effects of polyhydroxylated fullerene were shown in different directions: from damaging onion cells [42] to increasing the density of the green algae culture *Pseudokirchneriella subcapitata*, as well as accelerating the growth of the hypocotyl in Arabidopsis [41], increasing the biomass, yield, and content of useful substances of bitter melon plants after seed treatment [43]. The effect of fullerene derivatives, as well as of other nanomaterials, is determined by their size, composition of their functional groups, concentration, and species differences in plant response and environmental conditions [44–57]. The positive effect of these compounds on plants is presumably associated with antioxidant activity, namely, the ability to bind reactive oxygen species, as was clearly shown in barley and rape plants [47,49,55–57].

Also, the issues of the absorption of fullerene derivatives and their distribution, transformation, or decomposition in the plant organism have not been practically studied. Thus, the ability of plants, for example, rice, radish, onion, bitter melon, and wheat, to absorb and accumulate C_{60} or C_{70} fullerene derivatives is reported in the literature [44–46,58]. Water-soluble C_{60} fullerene derivatives penetrate animal and plant membranes either as lipophilic ions or in a neutral form after protonation [40,58]. In the example of wheat

(*Triticum aestivum* L.) and radish (*Raphanus sativus* L.) seedlings, it was shown that the absorption of C_{60} and C_{70} fullerene derivatives by plants depends on their concentration in the root environment and that these compounds accumulate mainly in roots [50,58]. Almost nothing is known about the mechanisms of the possible indirect effect of water-soluble fullerene derivatives on plants after they enter the soil.

Since 2011, our research group, represented by specialists in the field of fullerenoid structure chemistry from the Pavlov First Saint Petersburg State Medical University and agrobiologists from the Agrophysical Research Institute and from Vavilov All-Russian Research Institute of Plant genetic Resources, has developed new compositions of substances with unique properties based on C_{60} or C_{70} fullerene derivatives. Our work showed the high efficiency of the created substances in low concentrations in intensifying the production process and increasing the stress resistance of plants. At the same time, the working solutions of the created substances are safe for living organisms. We have found that an increase in the net productivity of plants and their resistance to oxidative stress after the introduction of polyhydroxylated, carboxylated, and amino acid derivatives of C_{60} into the root environment (nutrient solution for hydroponic plant cultivation) or after a foliar treatment of the plant is associated with established changes in the structure and efficiency of the photosynthetic apparatus, as well as with the effect on the processes of plant metabolism, exchange, and their antioxidant defense systems. Indicators of the antioxidant defense system's state were the intensity of lipid peroxidation, the activity of superoxide dismutase, and the generation of reactive oxygen species [47–53].

The revealed potential ability of fullerene derivatives to increase the content of macro- and microelements in plants [51,53] testified to the expediency of using them as potential mediators to contribute to the enrichment of plants with physiologically active substances useful for humans. Similar studies with fullerene derivatives have not yet been carried out; there is no information about their targeted use for enriching plants with specific micronutrients in the available literature. To fulfill this need, a comprehensive research study was carried out by us for the first time, the results of which are presented in this article.

The research hypothesis is as follows: the compositions of water-soluble fullerenes and microelement compounds, having different charges, are very effective in increasing the content of the applied microelement in the edible part of the used vegetable crops and in significantly improving the physiological state of plants when their solutions are introduced into the soil–plant (or soil substitute–plant) system. The noted effects are expected to be observed at a very low concentration of the compound in solutions due to the above-mentioned unique properties of water-soluble fullerenes.

This paper shows the results of pioneering studies on the effect of polyhydroxylated fullerene C_{60} and C_{70} and their composition or the composition of C_{60} amino acid derivatives with selenium or zinc microelement compounds on the content of a number of microelements in plants, their physiological state, and the quality and safety of the edible part of plant products when the test substances are introduced into the soil, into liquid root media, and via foliar treatment of the plants. The obtained results made it possible to evaluate, for the first time, the ability of the tested fullerene derivatives as mono-solutions and as part of a composition with solutions of mineral salts of microelements (sodium selenate and zinc sulfate) to enhance the enrichment of the edible part of plants, both with themselves and with other micronutrients useful for humans and animals.

The purpose of this work is to study the direct and indirect influence of water-soluble derivatives of C_{60} and C_{70} fullerenes on a number of vegetable crops and the content of trace elements in their products, including selenium or zinc, when these test substances, in the form of mono-solutions or compositions with mineral compounds of selenium or zinc, are introduced into the soil, into liquid root media, or via foliar treatment of plants.

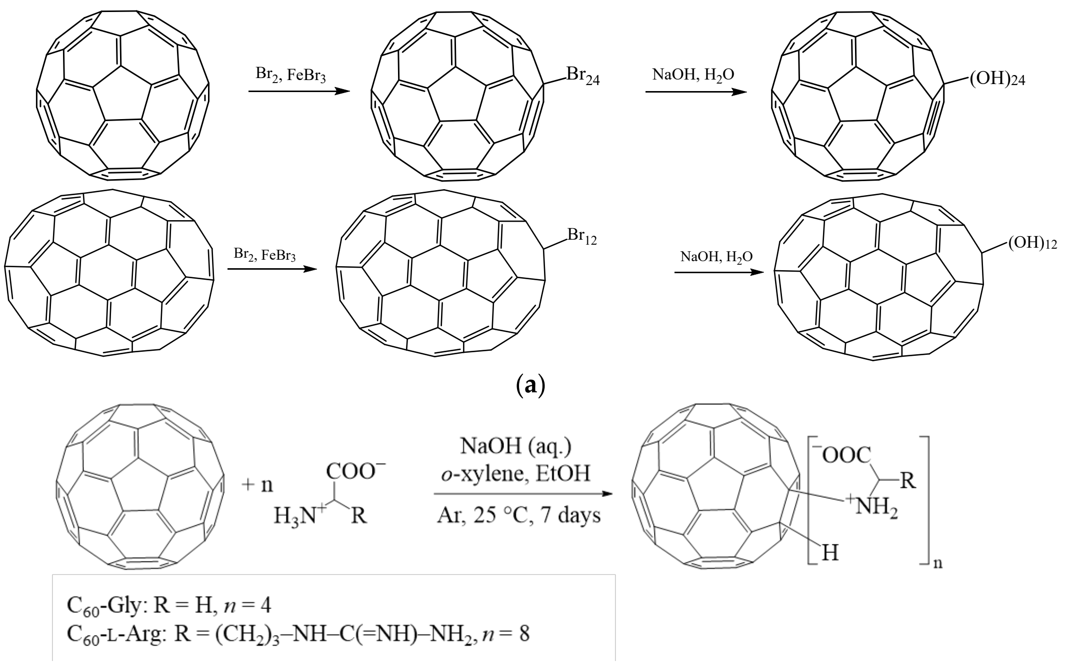
2. Materials and Methods

The subject of research was water-soluble derivatives of fullerenes: polyhydroxylated (fullerenol) $C_{60}(OH)_{24}$ and $C_{70}(OH)_{12}$, amino acid C_{60} with arginine (C_{60}-L-Arg), and C_{60}

with glycine (C_{60}-*L*-Gly), synthesized by a previously developed one-step method from individual fullerenes, a fullerene mixture, or fullerene soot using an aqueous solution of alkali and a phase transfer catalyst (tetrabutylammonium hydroxide—TBAH) [39,59–61]. The synthesis of C_{60} fullerene adducts with amino acids was carried out in accordance with the developed procedure. To carry out the synthesis, sodium hydroxide (14.75 g) was dissolved in water (54 mL), and after cooling the solution, amino acids (26.7 mmol) and ethanol (270 mL) were added. Then, a saturated solution of fullerene C_{60} (1 g) in *o*-xylene (131.5 mL) was added to the resulting solution, and the reaction mixture was stirred at room temperature for seven days under argon. Next, solvents (water, ethanol, *o*-xylene) were removed from the reaction mixture using an RV3V rotary evaporator (IKA, Königswinter, Germany), during which the temperature was not raised above 65 °C. To remove unreacted fullerene, the precipitate was dissolved in water, and the resulting heterogeneous system was filtered (blue ribbon filter). The solution was then neutralized with hydrochloric acid to pH 7 and purified by dialysis using dialysis membranes (Viscase, Darien, CT, USA, 1 kDa cut-off) against deionized water. Next, water was removed from the resulting solution by a rotary evaporator. The resulting precipitate was dried at 65 °C for 4 h. The yield of synthesis was 91% for C_{60}-*L*-Gly derivative and 85% for C_{60}-*L*-Arg derivative.

Synthesis of fullerenols was carried out in two stages: first, the bromo derivatives $C_{60}Br_{24}$ and $C_{70}Br_{12}$ were obtained by the reaction between the corresponding fullerene (C_{60} or C_{70}) and bromine in the presence of a $FeBr_3$ catalyst, after which the resulting products were hydrolyzed with an aqueous solution of NaOH. Excess alkali was removed by dialysis using dialysis membranes (Viscase, USA, cut-off mass 1 kDa) against deionized water. The yield of the final products was 95%.

The reaction schemes for obtaining fullerenols and fullerene derivatives with amino acids are shown in Figure 1.



(**b**)

Figure 1. Scheme for obtaining $C_{60}(OH)_{24}$ and $C_{70}(OH)_{12}$ fullerenols (**a**) and C_{60}-*L*-Gly and C_{60}-L-Arg derivatives (**b**).

The concentration of C_{60} fullerene derivatives was in the range of 0.1–1.0 mg/L of water.

The objects of research were test vegetable crops with different varieties or hybrids—Chinese cabbage *Brassica rapa* subsp. *Pekinensis* (Lour.) Hanelt cv. Daqingkou, tomato

Solanum lycopersicum L. cv. Natasha, and cucumber *Cucumis sativus* L. (hybrid F_1 Neva). Plant seeds were obtained from the collection of the Federal Research Center All-Russian Institute of Plant Genetic Resources, named after N.I. Vavilov (VIR), Federal Scientific Center for Vegetable Growing, seed company: joint stock company "Sortsemovoshch". The choice of cultivars of Chinese cabbage, tomato, and cucumber's test crops was due to their possessing the greatest adaptability to the regulated conditions of intensive light culture.

The studies were carried out at the AFI agrobiopolygon under controlled microclimate conditions (Saint-Petersburg, Russia). Plants were grown in a series of vegetation experiments under favorable light, root, and air conditions in the original experimental samples of plant growing light equipment (PGLE) [62].

2.1. Experimental Design

The following series of experiments were carried out:

1. Evaluation of the influence of introducing C_{60} fullerenol into the soil on the cucumber plant and the content of the main macro- and microelements in the soil and plants.
2. Study of the influence of introducing C_{60}-*L*-Gly or C_{60}-*L*-Arg and Na_2SeO_4 compositions into the nutrient solution on Chinese cabbage and the selenium content in the plants.
3. Study of the influence of foliar treatment with C_{60}-*L*-Gly or C_{60}-*L*-Arg and Na_2SeO4 compositions on tomato and the selenium content in its production.
4. Study of the influence of foliar treatment with compositions of the fullerenol $C_{60}(OH)_{22–24}$ or $C_{70}(OH)_{12–14}$ and $ZnSO_4$ on cucumber and the zinc content in its production.

Experimental conditions at the AFI agrobiopolygon are reflected in Table 1.

1. *Evaluation of the influence of introducing C_{60} fullerenol into the soil on the cucumber plant and the content of the main macro- and microelements in the soil and plants*

The cucumber plants (hybrid F_1 Neva) were grown in our PGLE experimental samples [62] in 1 L containers with soddy-podzolic sandy loamy soil (an arable layer). Chalk was evenly introduced into the soil at a rate of 2.5 g per 1 kg of soil, after which there was a period of rest for 7 days. The mineral background was formed at a level of $N_{90}P_{90}K_{90}$, and soil moisture was maintained at the level of 60–70% throughout the experiment.

The solutions of C_{60} fullerenol were introduced into the soil at various concentrations of 1 mg/kg, 10 mg/kg, and 100 mg/kg of soil. In the control variant, distilled water was introduced into the soil at the same volume as the volume of C_{60} fullerenol solutions introduced into the soil. Four days later, cucumber plants were sown in the soil with dry seeds, with four plants per container. The variants of the experiment had 20 plant replicates. The experiment was repeated twice.

Phenological observations of plant development were carried out during the vegetation experiment. The growing period was 38 days. At the end of it, the raw and dry mass as well as the dry matter of leaves, stems, and roots of cucumber seedlings were determined. The area of the leaf assimilating surface, morphometric indicators, including the height of plants, the number of leaves, and the cross-sectional area of the stem, the content of photosynthetic pigments, indicators of the activity of antioxidant systems in the plant organs, and the biochemical composition of the resulting plant production were determined.

At the end of the experiment, pH_{KCl} of the soil (an arable layer) was determined. In the soil, the content of ammonium nitrogen, nitrate nitrogen, mobile phosphorus and potassium, exchangeable calcium and magnesium, and trace elements (mobile iron, mobile copper, mobile zinc, and mobile manganese) were determined.

Table 1. Experiments conditions at the AFI agrobiopolygon.

Experiment Series	Growing Equipment [62]	Method of Plant Cultivation	Method of Treatment with Test Substance	Condition					
				Air Temperature: Day/Night	Air Humidity	Light Source/Duration of the Light Period	Light Intensity	pH of Root Inhabited Zone, Relative Units	EC, mS cm^{-1}
1	Plant growing light equipment	Geoponic	Introduction into soil	+22–+24 °C/+18–+20 °C	75–80% (soil humidity: 60–70%)	High-pressure sodium lamps (DnaZ-400, "Reflax" LLC, Moscow, Russia)/14 h per day	70–75 W/m^2 in the PAR	Soil: 5.8	Soil: no data
2	Rhizotron	Panoponic [63]	Introduction into nutrient solution	+22–+24 °C/ +18–+20 °C	60–70%		75–80 W/m^2 in the PAR region	Nutrient solution 6.0	Nutrient solution 1.0
3	Plant growing light equipment		Foliar treatment	+ 23–+25 °C / +21–+23 °C	60–70%.	High-pressure sodium lamps (DnaZ-400, "Reflax" LLC, Moscow, Russia)/16 h per day	95–105 W/m^2 in the PAR region	Nutrient solution 5.6–5.8	Nutrient solution 1.5
4	Plant growing light equipment		Foliar treatment	+22–+24 °C/ +18–+20 °C	75–80%.	High-pressure sodium lamps (DNaZ-400, "Reflax" LLC, Moscow, Russia)/14 h per day	70–75 W/m^2 in the PAR area	Nutrient solution 6.0–6.2	Nutrient solution 1.6

2.2. Plants' Biofortification with Se or Zn

2. *Study of the influence of introducing C_{60}-L-Gly or C_{60}-L-Arg and Na_2SeO_4 compositions into the nutrient solution on Chinese cabbage and the selenium content in the plants*

In order to evaluate the effect of amino acid derivatives of C_{60}-*L*-Gly *or* C_{60}-*L*-Arg (0.0001%) and Na_2SeO_4 (0.0001%) on the Chinese cabbage plants' state and selenium content in their roots and aerial parts, a vegetative experiment was carried out where these substances enter the root systems as part of Knop's nutrient solution while growing plants in a rhizotron [62,63] under controlled favorable conditions for 30 days.

The rhizotron is a piece of plant-growing light equipment for the year-round cultivation of plants that has the ability to visualize the growth and development of root systems along with the above-ground parts of plants. The plants were grown by the panoponics method, developed at AFI, on a thin-layer analog of the soil. This analog is a reusable hydrophilic material made of polyethylene terephthalate, which is placed on the vertical plates of the rhizotron. The seeds are placed and fixed on the surface of the hydrophilic material at the top of the plate, and the roots of the plants grow down, like in the soil layer. The hydrophilic material provides plant root systems with a circulating nutrient solution through the flat, slotted capillaries.

Other conditions of the experiments are shown in Table 1. Replications per variant: 10 plants. The experiment was repeated twice.

At the end of the growing season, the mass of the edible parts of Chinese cabbage plants was measured, and the selenium content in them was estimated.

3. *Study of the influence of foliar treatment with C_{60}-L-Gly or C_{60}-L-Arg and Na_2SeO_4 compositions on tomato and the selenium content in its production*

The effect of foliar treatment of cv. Natasha tomato plants with mono- and mixed solutions of C_{60}-*L*-Gly or C_{60}-*L*-Arg (0.0001%) and sodium selenate (0.0001% Na_2SeO_4) was evaluated in a vegetation experiment under the conditions shown in Table 1. Seeds of the plants were put into the layer (1 mm thick) of suspension based on the Cambrian clay, on the surface of the abovementioned hydrophilic material placed in the plant growing equipment tray. The material provides plant root systems with Knop's nutrient solution circulating over the tray bottom through the flat, slotted capillaries [62,63].

Foliar treatment with solutions of the tested substances was carried out three times during the period of budding–flowering. Plants treated with distilled water served as controls. Replications per variant: 10 plants. The experiment was repeated twice. The duration of each experiment was 110 days.

At the end of each vegetation experiment, the yield of tomato fruits per unit area and their quality and safety characteristics (the content of dry matter, vitamin C, and the amount of sugars, monosaccharides, disaccharides, carotene, and nitrates) were evaluated according to generally accepted and standard methods [64–68], as well as selenium content [69].

4. *Study of the influence of foliar treatment with compositions of the fullerenol $C_{60}(OH)_{22–24}$ or $C_{70}(OH)_{12–14}$ and $ZnSO_4$ on cucumber and the zinc content in its production*

A series of vegetation experiments under controlled conditions in the AFI agrobiopolygon was carried out using plant-growing light equipment for long-stemmed crops [62,63]. The conditions of the experiment are presented in Table 1. The method of growing plants (panoponics) is similar to that described above.

Composition of modified Knop's nutrient solution for cucumber is shown in Table 2.

Table 2. Composition of the modified nutrient solution for cucumber.

Substance Name	Amount of Substance, mmol/L
Calcium nitrate	4.2
Potassium nitrate	3.6
Potassium phosphate monosubstituted	1.8
Magnesium sulfate heptahydrate	1.0
Ammonium nitrate	1.8
Urea	0.3
Iron citrate ammonium	0.0178
Boric acid	0.0467
Manganese sulfate pentahydrate	0.00789
Zinc sulfate heptahydrate	0.00070
Copper sulfate pentahydrate	0.00068

There were six variants of foliar treatment: (1) control of distilled water, (2) $ZnSO_4$ solution at the concentration of 160 mg/L distilled water, (3) fullerenol C_{60} solution at the concentration of 1 mg/L distilled water, (4) fullerenol C_{60} (1 mg/L) + $ZnSO_4$ (160 mg/L), (5) fullerenol C_{70} at the concentration of 0.1 mg/L distilled water, (6) fullerenol C_{70} (0.1 mg/L) + $ZnSO_4$ (160 mg/L).

Foliar treatment with these solutions was carried out three times during the period of the plants' vegetative growth: from the phase of the 3rd true leaf to the 10th true leaf. The abovementioned concentrations of solutions of fullerenols $C_{60}(OH)_{22–24}$ or $C_{70}(OH)_{12–14}$ are favorable for cucumber plants (previously established by us in preliminary experiments). The concentration of an aqueous solution of zinc sulfate at 160 mg/L was chosen after a preliminary experiment as a compromise, providing the accumulation of zinc with the least pronounced inhibition of plant growth.

Each cucumber plant was formed into one stem, and, upon reaching a specified vertical length (2 m), the top of the stem with leaves was pinched.

During the growing season, daily monitoring of the state of plants and phenological observations were carried out.

Replications per variant: 10 plants. The experiment was repeated twice. The duration of each experiment was 65 days.

After finishing the vegetation experiments, the plant growth biometrics were measured: leaf area, stem cross-sectional area, and mass of the plant organs (culms and leaves). The main indicators of productivity were also evaluated: the number of cucumber fruits, the mass of one fruit, the mass of fruits on the plant, and ultimately, the yield of fruits per unit area. The values of quality and safety indicators of plant production were evaluated using standard and generally accepted methods [64–68].

2.3. Soil Analyses

To determine the soil pH by the potentiometric method according to [70], a salt extract was prepared at a ratio of soil-to-potassium chloride solution (with a concentration of 1 mol/L) of 1:2.5, followed by measurement with an ion-selective electrode.

Ammonium nitrogen (as according to [71]) was measured in the salt extract after measuring the pH by staining with indophenol reagent. Calibration was carried out using standard solutions of ammonium chloride. After staining, the optical density of the calibration solutions and soil samples was measured on a spectrophotometer at a wavelength of 655 nm.

Nitrates were extracted according to [72] with a 1% potassium aluminum sulfate solution at a soil-to-solution ratio of 1:2.5, followed by measurement with a nitrate-selective electrode. Calibration was carried out using standard solutions of potassium nitrate.

Mobile compounds of phosphorus and potassium (according to [73]) were extracted from the soil with a solution of hydrochloric acid with a concentration of 0.2 mol/liter at a soil-to-solution ratio of 1:5: phosphate solution. In the same extract, the content of exchangeable potassium was determined on a flame photometer calibrated with standard potassium ion solutions.

Exchangeable calcium and magnesium (according to [74]) were determined in the salt extract after measuring the pH by the titrimetric method using a solution of the sodium salt of ethylenediaminetetraacetic acid and the indicator eriochrome black T.

Trace elements (mobile iron, mobile copper, mobile zinc, mobile manganese) were determined by the method of atomic absorption spectrophotometry in the flame atomization mode after extraction with an ammonium acetate buffer with pH 4.8.

2.4. Plant Analyses

2.4.1. Morphology Measurements

The plants' raw and dry mass (after drying at 105 °C in a thermostat) by weight and area of the leaf assimilating surface (leaf area) was determined taking into account the mass of one cut of a certain area per wet mass of leaves from a plant according to the formula [75]:

$$S_{leav.} = (S_{cut}/m_{cut}) * m_{leav.},$$

where

$S_{leav.}$—leaves' area;
S_{cut}—cut area;
m_{cut}—cut mass (average value);
$m_{leav.}$—raw mass of leaves from a plant.

The stem cross-sectional area was then defined as the product of the square of the stem radius and the number π. The stem radius was calculated by dividing the diameter values in half. Stem diameter was measured with a caliper.

2.4.2. Photosynthetic Pigment Analysis

Spectrophotometric quantitative determination of chlorophyll a and b and carotenoids was carried out by extraction in acetone and measuring the optical density of the obtained extracts at wavelengths of 662, 644, and 440.5 nm, respectively [76]. The above-described spectrophotometric studies were carried out using a spectrophotometer PE-3000UV ("Promekolab" LLC, St. Petersburg, Russia).

2.4.3. Activity of Antioxidant Systems

The assessment of the activity of antioxidant systems in the roots and aerial parts of plants was carried out on the basis of determining the intensity of lipid peroxidation (LPO) as well as the activity of peroxidase and catalase enzymes. LPO was determined by the accumulation of malondialdehyde (MDA) in plants [77]. The content of MDA was assessed by the degree of accumulation of the product from its reaction with thiobarbituric acid (TBA) [77]. Peroxidase activity was determined photometrically with the formation of benzidine blue, according to A.N. Boyarkin [78]. The method is based on determining the reaction rate of benzidine until the formation of a blue oxidation product at a certain concentration that is pre-set on the spectrophotometer. Catalase activity was determined by iodometric method [79]. The studied tissue was triturated with phosphate buffer (pH 7) and diluted with water to 50 mL. An aliquot of the suspension was taken, then it was brought to a temperature of 20 °C, and hydrogen peroxide was added. After 5 min, the remaining undecomposed hydrogen peroxide was determined by the iodometric method.

2.4.4. Quality and Safety Indicators of Plant Production

The values of quality and safety indicators of plant production were evaluated using standard and generally accepted methods [64–68]. The dry matter content was determined by the thermostat–weight method by weighing the averaged sample before and after drying in a thermostat at a temperature of 105 °C for 6 h. Analysis for ascorbic acid (vitamin C) was carried out by direct extraction from plants with 2% metaphosphoric acid, followed by high performance liquid chromatography [64]. Water-soluble carbohydrates (mono- and disaccharides and sum of sugars) were determined by the Bertrand method. A plant sample weighing 1 g was placed in a conical flask with a capacity of 250–300 cm^3, 60 cm^3 of distilled water preheated to 50–60 °C was added, and it was shaken for 15–20 min at a shaking frequency of 200 vibrations per minute. After precipitating the protein with a solution of lead acetate and filtering the sample through a paper filter, an extract of soluble carbohydrates was obtained. In the extract, the content of carbohydrates was determined with Felling's reagent and subsequent permanganometric titration.

Nitrate content was determined by ionometric method [65]. A crude sample portion was extracted with a 1% solution of potassium aluminum sulfate at a plant-to-solution ratio of 1:5.0, followed by measurement with a nitrate-selective electrode. Calibration was carried out using standard solutions of potassium nitrate.

For raw ash and macro- and microelement analysis, plant samples were dried in an oven to constant weight at a temperature of 105 °C, ground in a mill, and sieved through a sieve with a mesh diameter of 1 mm until complete passage. To analyze samples for raw ash, a weight of 1 g was taken on an analytical balance, transferred to pre-calcined porcelain crucibles, and dry ashing was carried out in a muffle furnace at a temperature of 520 °C for 5 h until complete ashing. Trace elements were determined by atomic absorption spectrometry (AAS) after microwave treatment [66–68]. For analysis, a weighed portion of 1 g was taken on an analytical balance, placed in Teflon autoclaves, and subjected to microwave decomposition in the presence of 10 mL of concentrated nitric acid. After decomposition and cooling of the samples, the obtained extracts were transferred with deionized water into a 100 mL volumetric flask. The resulting solution was filtered through an ash-free blue ribbon filter. The measurements were carried out on a Varian AA240FS atomic absorption spectrometer with flame atomization. The device was calibrated using standard solutions of elements with a given concentration. Trace elements and magnesium were measured at the most sensitive wavelengths of Fe (248.7 nm), Mg (324.6 nm), Zn (213.7 nm), and Mn (279.5 nm) using hollow cathode lamps.

Determination of the selenium content in plant samples was carried out according to the standard method [69] on a NovAA atomic absorption spectrophotometer from Analytik Jena. The essence of the method lies in the fact that selenium ions react with sodium borohydride in an acidic medium to form selenium hydride, which is carried by a gas flow into a heated measuring cell, where it is atomized. Quantitative analysis of selenium is carried out by atomic absorption at a wavelength of 196.0 nm.

Determination of macronutrients was carried out after wet ashing. A 0.2 g plant sample was taken on an analytical balance, placed in test tubes, and concentrated acid was added in the presence of hydrogen peroxide [68]. Total nitrogen was determined by the photometric indophenol method on a spectrophotometer; phosphorus was determined by the photometric method on a spectrophotometer; potassium was determined by the flame photometric method; calcium was determined by the complexometric method.

2.5. Statistical Analysis

For the statistical analysis, we used one-factor analysis of variance (ANOVA) and Duncan's multiple range test to determine the significance of differences between the mean values. The number of repeats for each characteristic is shown in the tables and figures. The mean ± SE values presented in the tables and figures were calculated using MS Excel 2016 and v.12.0 software (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. The Influence of Fullerenol $C_{60}(OH)_{22–24}$ Introduction into the Soil on the Cucumber Plant and the Content of the Main Macro- and Microelements in the Soil and Plants

Under the controlled conditions of the AFI agricultural biopolygon, experimental studies were carried out to assess the effect of introducing C_{60} fullerenol into the soil at various concentrations (1 mg/kg, 10 mg/kg, and 100 mg/kg) on the trace element content in the soil as well as in the roots, stems, and leaves of the test cucumber plants (hybrid F_1 Neva) and on the indicators of their growth and development.

For a more pronounced manifestation of the effect of C_{60} fullerenol on the transport of microelements in the soil–plant system, chalk was previously introduced into the soddy-podzolic sandy loamy soil (arable layer) at a previously established dose (2.5 g/kg of soil). Chalk at this dose in soil does not adversely affect the plants' growth and development, but provides, in particular, a decrease in the mobility of zinc and manganese compounds in soils, and, accordingly, their digestibility by plants.

It has been established that when C_{60} fullerenol was introduced into the soil at initial concentrations of 1 mg/kg, 10 mg/kg, and 100 mg/kg, a decrease in the content of ammonium nitrogen and an increase in the content of nitrate nitrogen were observed (Tables 3 and S1).

Table 3. The influence of C_{60} fullerenol on some soil agrochemical indicators and element content by introduction of its solutions in various concentrations into the soil.

Soils Indicators	Concentrations of Applied Fullerenol, mg/kg of Soil			
	0 (Control)	1	10	100
pH $_{KCl}$, units pH	5.10 ^{a}	5.10 ^{a}	5.10 ^{a}	5.10 ^{a}
Ammonium nitrogen, mg/kg	6.84 ± 0.34 ^{a}	5.09 ± 0.25 ^{b}	6.52 ± 0.33 ^{a}	5.01 ± 0.25 ^{b}
Nitrate nitrogen, mg/kg	0.53 ± 0.03 ^{c}	0.50 ± 0.03 ^{c}	0.72 ± 0.04 ^{b}	0.84 ± 0.04 ^{a}
Phosphorus: mobile in terms of P_2O_5, mg/kg	130.00 ± 6.50 ^{b}	121.00 ± 6.05 ^{b}	125.00 ± 6.25 ^{b}	174.00 ± 8.70 ^{a}
Potassium: mobile in terms of K_2O, mg/kg	48.00 ± 2.40 ^{a}	42.00 ± 2.10 ^{b}	48.00 ± 2.40 ^{a}	52.00 ± 2.60 ^{a}
Calcium: exchangeable, mmol/100 g	5.00 ± 0.25 ^{a}	4.38 ± 0.22 ^{b}	4.63 ± 0.23 ^{ab}	4.88 ± 0.24 ^{a}
Magnesium: exchangeable, mmol/100 g	<0.10 ± 0.005 ^{c}	<0.10 ± 0.005 ^{c}	1.25 ± 0.063 ^{b}	1.50 ± 0.075 ^{a}
Iron: mobile, mg/kg	12.00 ± 0.60 ^{b}	12.60 ± 0.63 ^{b}	13.80 ± 0.69 ^{a}	11.40 ± 0.57 ^{b}
Copper: mobile, mg/kg	0.18 ± 0.009 ^{c}	0.22 ± 0.011 ^{b}	0.25 ± 0.013 ^{a}	0.23 ± 0.012 ^{ab}
Zinc: mobile, mg/kg	2.40 ± 0.12 ^{c}	2.17 ± 0.11 ^{c}	3.20 ± 0.16 ^{b}	4.49 ± 0.22 ^{a}
Manganese: mobile, mg/kg	7.31 ± 0.37 ^{a}	7.39 ± 0.37 ^{a}	7.77 ± 0.39 ^{a}	6.43 ± 0.32 ^{b}

Note: values in rows followed by different letters $^{(a–c)}$ are significantly different at $p \leq 0.05$, as determined by Duncan's multiple range test.

At the same time, the proportion of mobile forms of phosphorus, potassium (tendency), and exchangeable magnesium, as well as the content of iron, copper, and zinc, increased predominantly in variants with higher initial concentrations of C_{60} fullerenol (10 mg/kg and 100 mg/kg). The content of manganese did not differ from the control values in the variants with the introduction of fullerenol into the soil at concentrations of 1.0 mg/kg and 10.0 mg/kg and, conversely, decreased at a concentration of 100 mg/kg. This indirectly indicates the ability of C_{60} fullerenol, in certain concentrations, to increase the availability of macro- and microelements in the soil for plants. This assumption is confirmed by the data from the chemical elemental analysis of the plants (Figures 2 and 3). It was found that raw ash in roots and leaves, and, consequently, the content of mineral elements, significantly increased in the variants with the introduction of C_{60} fullerenol into the soil in various concentrations, but there were no differences in raw ash values in the stems (Figure 2). This indirectly indicates the activation of the functioning of two absorption centers: the roots and the leaves.

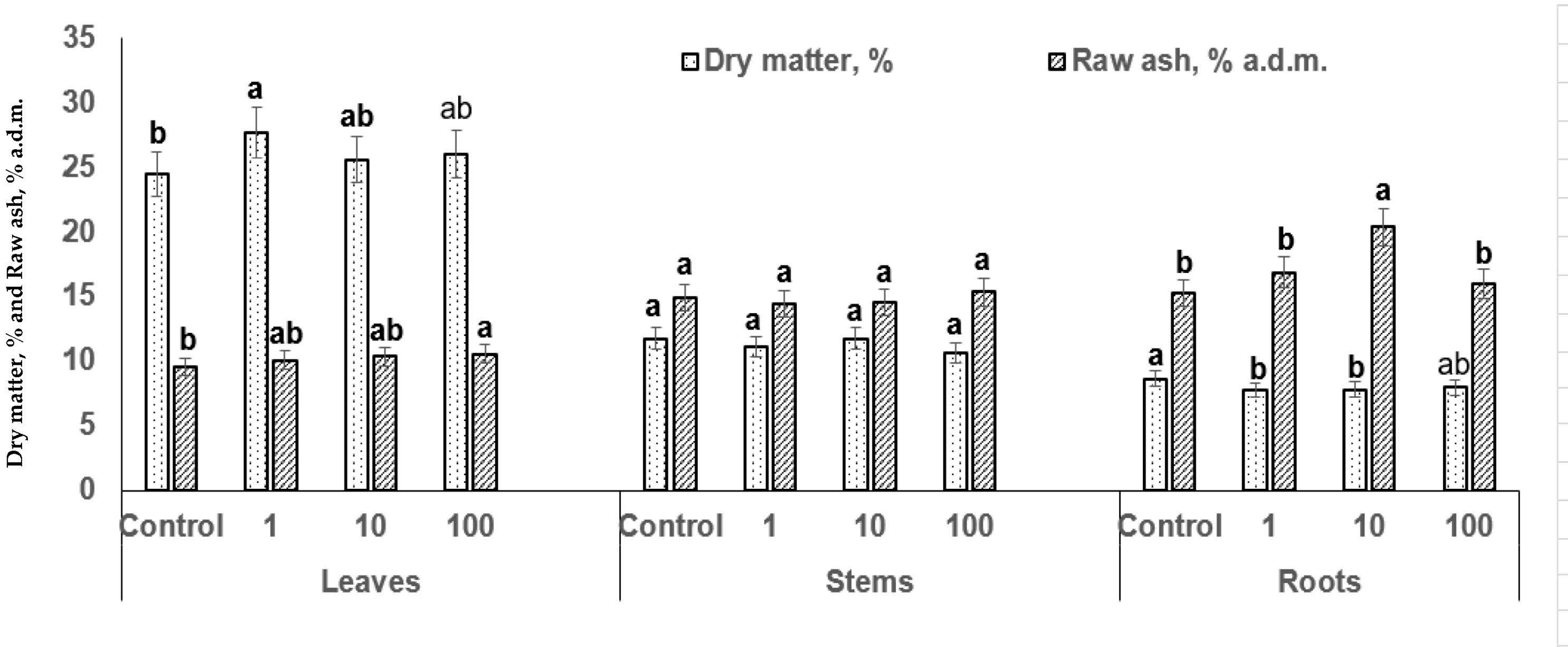


Figure 2. Influence of the introduction of C_{60} fullerenol solutions at various concentrations in the soil on dry matter and raw ash in the roots, stems, and leaves of cucumber plants (hybrid F_1 Neva). Bars with different letters are significantly different at $p \leq 0.05$, as determined by Duncan's multiple range test. Note: a.d.m.—absolutely dry matter.

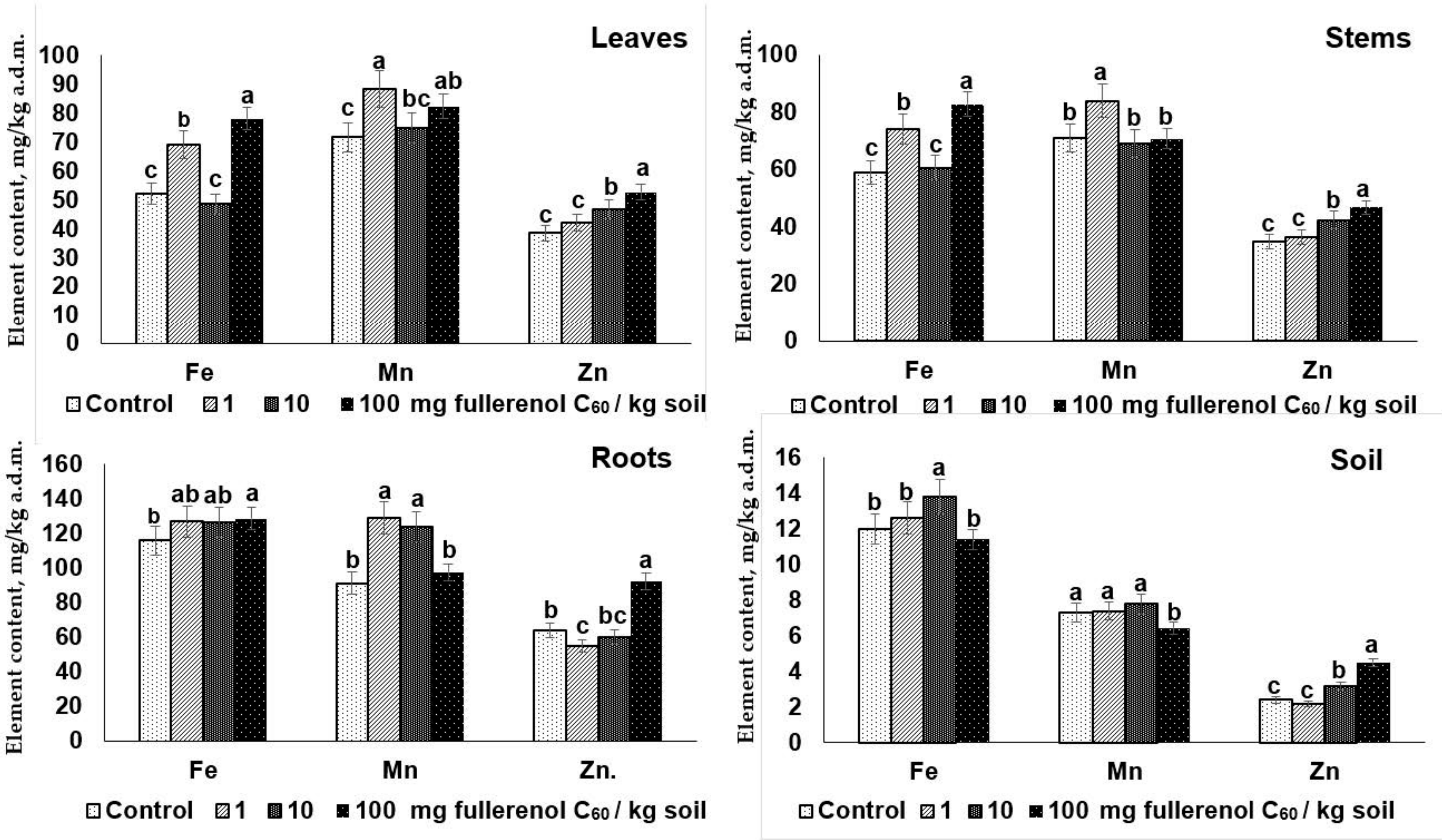


Figure 3. Influence of the introduction of C_{60} fullerenol solutions at various concentrations in the soil on the content of the studied microelements in it and in cucumber plants' (hybrid F_1 Neva) leaves, stems, and roots. Bars with different letters are significantly different at $p \leq 0.05$, as determined by Duncan's multiple range test. Note: a.d.m.—absolutely dry matter.

The most significant effects of the presence of fullerenol in the soil on the absorption and enrichment of plant organs by microelements should be emphasized: zinc increased by 5–44% (with maximum values in the variants with the highest concentration of fullerenol), manganese by 7–41% (with maximum values at a concentration of 1 mg/kg of fullerenol in the soil), and iron by 10–50% (with maxima in leaves and stems at fullerenol concentrations in the soil of 100 and 1 mg/kg and no significant differences in the roots) (Figure 3).

The trends observed at the same time of a lower content of dry matter in the roots, an unreliable change in the stems, and an increase in dry matter content in the leaves in the variants with the introduction of C_{60} fullerenol into the soil indirectly indicate the activation of the transport of nutrients into the leaves from the roots and stems and, possibly, of the synthesis of substances in the leaves.

The noted effects of changes in dry matter on plant organs are more pronounced in the variant with the introduction of C_{60} fullerenol into the soil at a concentration of 1 mg/kg. This, along with the most pronounced plants' absorption of iron and manganese (Figure 3)—key microelements in photosynthetic and other physiological processes in plants—is one of the reasons for the higher values of photosynthetic pigment content in this variant (Tables 4 and S2) by area, fresh mass of leaves, fresh mass of roots, and cross-sectional area of the stem (Tables 5 and S2).

Table 4. The influence of the introduction of C_{60} fullerenol solutions at various concentrations in the soil on photosynthetic pigment content of cucumber plants' leaves (hybrid F_1 Neva) grown under controlled conditions.

Concentrations of C_{60} Fullerenol Introduced into the Soil, mg/kg of Soil	Chlorophyll a, µg 100 g^{-1} FM	Chlorophyll b, µg 100 g^{-1} FM	Total Chlorophyll, µg 100 g^{-1} FM	Carotenoid, µg 100 g^{-1} FM
Control	70.82 ± 1.43^{b}	25.24 ± 1.16^{c}	96.06 ± 4.44^{c}	21.68 ± 1.00^{b}
1	80.83 ± 3.73^{a}	30.35 ± 1.40^{a}	111.18 ± 5.14^{a}	24.74 ± 1.14^{a}
10	70.88 ± 1.42^{b}	27.38 ± 1.27^{bc}	98.26 ± 4.54^{bc}	22.42 ± 1.04^{b}
100	78.14 ± 1.76^{a}	28.41 ± 1.31^{ab}	106.55 ± 4.92^{ab}	24.79 ± 1.15^{a}

Note: FM—fresh mass of leaves. Values in columns followed by different letters $^{(a–c)}$ are significantly different at $p \leq 0.05$, as determined by Duncan's multiple range test.

Plants in the variant with the introduction of fullerenol into the soil at a concentration of 10 mg/kg did not differ from the control ones in the content of photosynthetic pigments (Table 4 and Table S2) or in morphometric and weight indicators of plant growth (Tables 5 and S2). At the same time, in plants in the variant with the introduction of fullerenol into the soil at a concentration of 100 mg/kg, the content of photosynthetic pigments was significantly higher than in the control (Tables 4 and S2). But, morphometric (number of leaves and stem cross-sectional area) and weight characteristics (raw and dry mass of leaves, stems, and roots) of their growth did not differ significantly from those in the control (Tables 5 and S2). It should be noted that there is a trend towards lower values of leaf area in this variant, as well as significantly higher values of dry matter content in leaves and stems and lower values in roots.

Increasing the provision of plants with essential macro- and microelements in variants with the introduction of fullerenol into the soil also positively affects the functioning of antioxidant systems, namely:

- It mainly contributes in the form of a trend or a significant decrease (maximum change of 47%) in the activity of the oxidative enzyme peroxidase in leaves and roots. The exception is the roots in the variant with the introduction of fullerenol into the soil at a concentration of 100 mg/kg, where the activity of peroxidase was higher than that in the control by 14%;
- A significant decrease (maximum change of 41%) in the intensity of lipid peroxidation;
- A significant increase (maximum change of 647%) in the activity of the catalase enzyme (Table 6 and Table S2).

Table 5. The influence of the introduction of C_{60} fullerenol solutions at various concentrations in the soil on biometric indicators of cucumber plants (hybrid F_1 Neva) grown under controlled conditions.

Concentrations of C_{60} Fullerenol Introduced into the Soil, mg/kg of Soil	Number of Leaves, pcs.	Leaves' Area, cm^2	Stem Cross-Sectional Area cm^2	Leaves			Stems			Roots		
				Raw Mass, g/Plant	Dry Mass, g/Plant	% Dry Matter	Raw Mass, g/Plant	Dry Mass, g/Plant	% Dry Matter	Raw Mass, g/Plant	Dry Mass, g/Plant	% Dry Matter
Control	4.4 ± 0.3 [a]	240.1 ± 9.3 [bc]	0.218 ± 0.014 [b]	5.04 ± 0.25 [b]	1.23 ± 0.07 [a]	24.4 ± 0.2 [c]	3.92 ± 0.25 [a]	0.43 ± 0.05 [ab]	11.0 ± 0.2 [b]	2.28 ± 0.34 [b]	1.96 ± 0.03 [b]	8.6 ± 0.2 [a]
1	4.9 ± 0.3 [a]	259.4 ± 10.3 [a]	0.242 ± 0.014 [a]	5.45 ± 0.17 [a]	1.33 ± 0.11 [a]	24.4 ± 0.2 [c]	4.12 ± 0.28 [a]	0.40 ± 0.05 [b]	9.7 ± 0.2 [c]	2.67 ± 0.48 [a]	2.06 ± 0.03 [a]	7.7 ± 0.2 [b]
10	4.5 ± 0.3 [a]	245.2 ± 11.7 [b]	0.238 ± 0.014 [a]	5.14 ± 0.32 [b]	1.31 ± 0.09 [a]	25.5 ± 0.3[b]	4.00 ± 0.22 [a]	0.47 ± 0.05 [a]	11.7 ± 0.3 [a]	2.48 ± 0.40 [ab]	1.94 ± 0.05 [b]	7.8 ± 0.2 [b]
100	4.4 ± 0.3 [a]	230.2 ± 11.1 [c]	0.231 ± 0.014 [ab]	5.10 ± 0.35 [b]	1.36 ± 0.08 [a]	26.7 ± 0.3 [a]	3.93 ± 0.26 [a]	0.46 ± 0.03 [a]	11.7 ± 0.2 [a]	2.33 ± 0.32 [b]	1.85 ± 0.04 [c]	7.9 ± 0.2 [b]

Note: values in columns followed by different letters [(a–c)] are significantly different at $p \leq 0.05$, as determined by Duncan's multiple range test.

Table 6. The influence of the introduction of C_{60} fullerenol solutions at various concentrations in the soil on the work of antioxidant systems in the roots and leaves of cucumber plants (hybrid F_1 Neva) grown under controlled conditions.

Concentrations of C_{60} Fullerenol Introduced into the Soil, mg/kg of Soil	POX, $U\ s^{-1}\ g^{-1}$	CAT, $\mu M\ H_2O_2\ mg^{-1}$ Protein min^{-1}	LPO, $\mu M\ g^{-1}$
		Leaves	
Control	19.46 ± 0.90 ^{a}	269.73 ± 12.46 ^{c}	0.0157 ± 0.0007 ^{a}
1	18.18 ± 0.84 ^{a}	330.55 ± 15.27 ^{b}	0.0092 ± 0.0004 ^{c}
10	19.45 ± 0.91 ^{a}	373.56 ± 17.26 ^{a}	0.0101 ± 0.0005 ^{bc}
100	15.94 ± 0.74 ^{b}	293.56 ± 13.57 ^{c}	0.0108 ± 0.0007 ^{b}
		Roots	
Control	49.32 ± 2.28 ^{b}	6.61 ± 0.31 ^{d}	0.0150 ± 0.0007 ^{a}
1	37.94 ± 1.75 ^{c}	44.83 ± 2.07 ^{b}	0.0132 ± 0.0006 ^{b}
10	26.18 ± 1.21 ^{d}	16.96 ± 0.78 ^{c}	0.0117 ± 0.0005 ^{c}
100	55.99 ± 2.59 ^{a}	49.35 ± 2.28 ^{a}	0.0121 ± 0.0006b ^{c}

Note: values in columns followed by different letters $^{(a–d)}$ are significantly different at $p \leq 0.05$, as determined by Duncan's multiple range test; POX—the activity of peroxidase; CAT—the activity of catalase; LPO—the intensity of lipid peroxidation.

To enhance the described effect of fullerene derivatives and to increase the controllability of the process of plant enrichment with micronutrients, a number of exploratory experimental studies were carried out to assess the effect of compositions of fullerene derivatives with a number of minor but important microelements for humans.

Based on the analysis of the complex results obtained [47–53,80,81], the most effective carbon (fullerenols $C_{60}(OH)_{20–24}$ and $C_{70}(OH)_{12–14}$, amino acid derivatives of fullerene C_{60}-*L*-Gly or C_{60}-*L*-Arg) were selected for the purposes of biofortification of plant production.

To enrich plants with positively charged cations—trace metals, for example, Zn^{2+}, Mn^{2+}, Fe^{3+}, etc., as the fullerene derivatives have negatively charged functional groups—OH^- (polyhydroxylated fullerene $C_{60}(OH)_{20–24}$ and $C_{70}(OH)_{12–14}$) were used. For plant enrichment with negatively charged anions, for example, SeO_4^{2-}, J^-. $Si0_3^{2-}$ and others—amino acid derivatives of fullerenes have positively charged functional groups—NH_2^+- or -NH_3^+ in their adducts (fullerene with histidine, arginine, or lysine) or fullerene derivatives with low molecular mass neutral amino acids containing positive and negative groups in equal proportions (fullerene with glycine, etc.) were used.

3.2. Enrichment of Plants with Selenium Anions

In the example of selenium anions, the potential ability of amino acid derivatives of fullerene (C_{60}-*L*-Gly or C_{60}-*L*-Arg) with positively charged or neutral functional groups to increase their content in the obtained plant production was studied. At the same time, the solutions of these fullerenes with sodium selenate were introduced in the root environment or through a foliar treatment of vegetative plants.

3.2.1. Influence of the C_{60}-*L*-Gly or C_{60}-*L*-Arg and Na_2SeO_4 Compositions Introduced into the Nutrient Solution on Chinese Cabbage and the Selenium Content in the Plants

The introduction of sodium selenate and amino acid derivatives of C_{60} fullerene with arginine or with glycine into the nutrient solution supplied to the root systems of Chinese cabbage cv. Daqingkou in the rhizotron contributed to the enhancement of selenium transport into plants (Tables 7 and S3).

Table 7. The influence of the introduction into the nutrient solution of mineral selenium-containing substances and their compositions with amino acid derivatives of C_{60} fullerene on biomass of edible parts of Chinese cabbage plants cv. Daqingkou and on the selenium content in aerial parts and roots by growing in the rhizotron under favorable controlled conditions.

Experience Variant	Raw Mass, g	Selenium Content mg/kg a.d.m.
Aboveground part		
Control (NS)	35.1 ± 2.0^{c}	49.0 ± 2.8^{c}
NS + 0.0001% Na_2SeO_4	4.3 ± 0.3^{d}	51.0 ± 2.9^{c}
NS + 0.0001% Na_2SeO_4+0,0001% C_{60}-*L*-Arg	85.8 ± 4.9^{b}	92.5 ± 5.2^{a}
NS + 0.0001% Na_2SeO_4+0,0001% C_{60}-*L*-Gly	119.4 ± 6.8^{a}	64.4 ± 3.6^{b}
Roots		
Control (NS)	10.4 ± 0.6^{c}	58.6 ± 3.3^{c}
NS + 0,0001% Na_2SeO_4	1.8 ± 0.1^{d}	69.3 ± 3.9^{b}
NS + 0.0001% Na_2SeO_4+0.0001% C_{60}-*L*-Arg	18.7 ± 1.1^{b}	101.5 ± 5.7^{a}
NS + 0.0001% Na_2SeO_4+0.0001% C_{60}-*L*-Gly	34.4 ± 1.9^{a}	72.2 ± 4.1^{b}

Note: NS—nutrient solution; a.d.m.—absolutely dry mass; C_{60}-*L*-Arg—fullerene with arginine solution; C_{60}-*L*-Gly—fullerene with glycine solution. Values in columns followed by different letters (a–d) are significantly different at $p \leq 0.05$, as determined by Duncan's multiple range test.

It should be emphasized that the content of selenium in the roots was somewhat higher than in the leaves. At the same time, the nutrient solution with the amino acid derivative of C_{60} fullerene with arginine and sodium selenate had an increase in the selenium content in the roots and aerial parts of Chinese cabbage by 73% and 89%, respectively, relative to the control and by 47% and 81% relative to the variant with sodium selenate; the nutrient solution with the amino acid derivative of C_{60} fullerene with glycine and sodium selenate increased by 23% and 31% relative to the control and by 4% and 26% relative to the variant with sodium selenate (Table 7 and Table S3).

It should be noted that the presence of sodium selenate at a concentration of 1 mg/L in the nutrient solution adversely affected the physiological state of Chinese cabbage plants and, ultimately, the mass of their aerial parts and roots (lower than that in the control by 88% and 83%). At the same time, plants grown in a nutrient solution with sodium selenate and amino acid derivatives of C_{60} fullerene with arginine or glycine were 80–240% higher in terms of the mass of roots and aerial parts than control plants (Table 7 and Table S3).

3.2.2. Influence of Foliar Treatment with C_{60}-*L*-Gly or C_{60}-*L*-Arg and Na_2SeO_4 Compositions on Tomato and the Selenium Content in Its Production

The above hypothesis of the increased transport of selenium into plants with the help of certain amino acid derivatives of fullerene C_{60} was confirmed in a series of vegetative experiments with three rounds of foliar treatment on tomato plants from flowering to the beginning of fruiting with solutions of amino acid derivatives of 0.0001% C_{60} fullerene with arginine + 0.0001% Na_2SeO_4 and 0.0001% C_{60} fullerene with glycine + 0.0001% Na_2SeO_4, as well as a solution of 0.0001% Na_2SeO_4.

Fullerene amino acid derivatives containing COOH, CO, or NH_2 NH functional groups are capable of ensuring the supply of selenium to plants by establishing coordination, hydrogen, or covalent bonds with the selenate ion. It has been shown that the treatment of tomato plants with a mixed solution of the amino acid derivative of C_{60} fullerene with arginine and sodium selenate increases the selenium content by 33.7% relative to that in the control and by 10.2% relative to the treatment of plants with a mono-solution of 0.0001% sodium selenate. The treatment of plants with a solution of the amino acid derivative of C_{60} fullerene with glycine and sodium selenate increased the selenium content by 42.2% and 17.2%, respectively (Figure 4). It should be noted that foliar treatment with these substances does not lead to significant changes in plant productivity per unit area. However, there is a pronounced tendency to reduce the yield of tomato fruits in the variant that had a foliar treatment with a solution of 0.0001% Na_2SeO_4 (by 12%) relative to the control values and to level this negative effect in the case of using mixed solutions with amino acid derivatives of C_{60} fullerene, especially C_{60} with glycine.

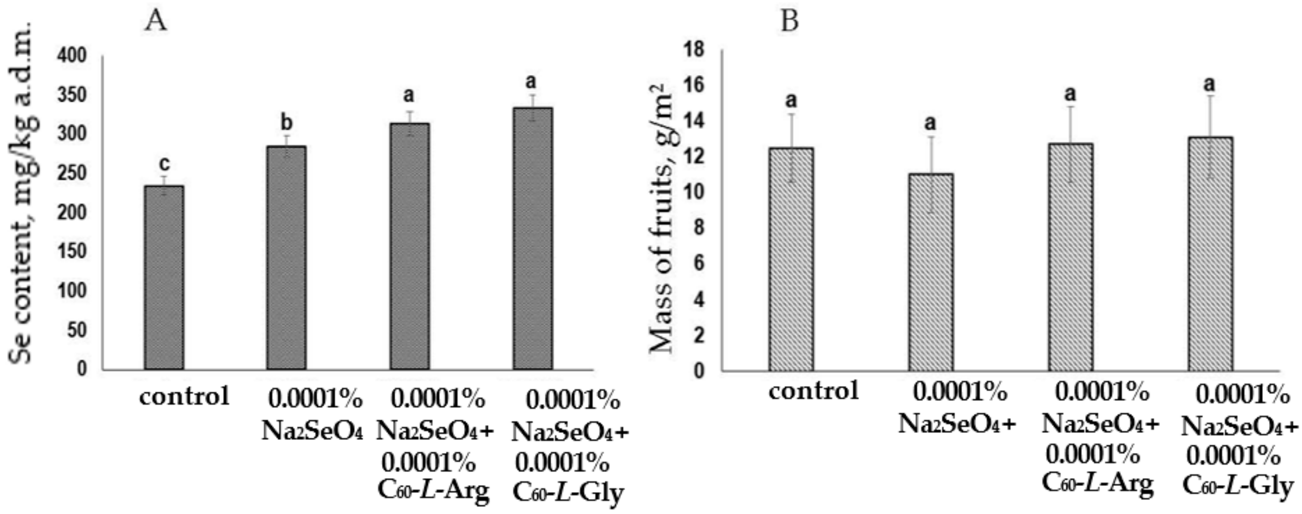


Figure 4. The effect of foliar treatment of tomato plants cv. Natasha during the period of budding–flowering with mono- and mixed solutions of C_{60} fullerene amino acid derivatives (C_{60}-*L*-Gly or C_{60}-*L*-Arg) and/or selenium nitrate (N_2SeO_4) on the content of selenium (**A**) in tomato fruits and tomato fruit mass (**B**). Bars with different letters are significantly different at $p \leq 0.05$, as determined by Duncan's multiple range test. Note: a.d.m.—absolutely dry matter.

At the same time, foliar treatment with mono- and mixed solutions of sodium selenate and amino acid derivatives significantly improves the quality of tomato fruits: the content of sugars, carotene, and vitamin C increases, while a very low content of nitrates can be observed (Tables 8 and S4).

Table 8. Influence of foliar treatment with solutions of selenium-containing substances and compositions on fruit quality of tomato cv. Natasha grown under controlled conditions.

Indicators	Foliar Treatment with Mono- and Mixed Solutions			
	Control (Water)	**0.0001% Na_2SeO_4**	**Na_2SeO_4 + 0.0001% C_{60}-*L*-Arg**	**0,0001% Na_2SeO_4 + 0.0001% C_{60}-*L*-Gly**
% Dry matter	5.6 ± 0.3^{a}	6.2 ± 0.4^{a}	6.0 ± 0.3^{a}	5.8 ± 0.3^{a}
Total saccharide % a.d.m	35.6 ± 2.0^{c}	42.8 ± 2.4^{ab}	39.8 ± 2.3^{b}	45.0 ± 2.6^{a}
Monosaccharide, % a.d.m	34.3 ± 1.9^{b}	41.7 ± 2.4^{a}	35.9 ± 2.0^{b}	43.6 ± 2.5^{a}
Disaccharide, % a.d.m.	1.3 ± 0.1^{bc}	1.1 ± 0.1^{c}	3.9 ± 0.2^{a}	1.4 ± 0.1^{b}
Vitamin C, mg/100 g r.m.	18.9 ± 1.1^{c}	24.6 ± 1.4^{a}	22.0 ± 1.2^{b}	25.3 ± 1.4^{a}
Nitrates, mg/kg r.m.	$<29.7 \pm 1.5^{a}$	$<29.7 \pm 1.5^{a}$	$<29.7 \pm 1.5^{a}$	$<29.7 \pm 1.5^{a}$
Carotene, mg/kg r.m.	50.3 ± 2.8^{c}	85.3 ± 4.8^{a}	59.7 ± 3.4^{b}	65.1 ± 3.7^{b}

Note: C_{60}-*L*-Arg—C_{60} fullerene with arginine; C_{60}-*L*-Gly—C_{60} fullerene with glycine; a.d.m.—absolutely dry matter; r.m.—raw mass. Values in rows followed by different letters $^{(a–c)}$ are significantly different at $p \leq 0.05$, as determined by Duncan's multiple range test.

The obtained data for agrobiological tests of the developed methods of treating plants with solutions of C_{60} fullerene amino acid derivatives and selenium nitrate indicate their effectiveness in ensuring an increase in the content of a minor microelement in the consumed part of plant production while also improving other qualitative characteristics of tomato. The solutions that we propose will minimize the risks associated with the safety of plant products due to increased bioavailability and activity of substances in the composition of nanoforms and will achieve the required positive effects on plants at low concentrations.

3.2.3. Influence of Foliar Treatment with the Compositions of Fullerenol $C_{60}(OH)_{22–24}$ or $C_{70}(OH)_{12–14}$ and $ZnSO_4$ on Cucumber and the Zinc Content in its Production

As is known, zinc, which is essential for plants, belongs to the group of elements with an intermediate availability and ability to penetrate through the cell walls and membranes of plant cells [82,83]. Due to its fixation in the soil organo-mineral complex or the action of other edaphic and anthropogenic factors that reduce its availability to plants, the latter may experience a deficiency in the content of this element [84–86]. To intensify the supply of positively charged zinc ions to fruit and vegetable crops, polyhydroxylated fullerenes with negatively charged OH^- functional groups (fullerenols $C_{60}(OH)_{22–24}$ or $C_{70}(OH)_{12–14}$) and the foliar treatment method were used as the most effective for the purposes of biofortification, according to researchers [87,88].

Foliar treatment with mono- and mixed solutions of these fullerenols with previously established concentrations favorable for plants (1 mg/L and 0.1 mg/L) and zinc sulfate (160 mg/L) provided a significant increase in the zinc content in fruits. Thus, in the variant with treatment of zinc sulfate, the content of this element in the fruits of cucumber (hybrid F_1 Neva) was higher than in control plants by 29%, while in the variant with treatment with mixed solutions of zinc sulfate and $C_{60}(OH)_{22–24}$ or $C_{70}(OH)_{12-14}$ the zinc content was higher by 59% and 42%, respectively, and compared to zinc sulfate, by 23% and 10% (Figure 5A).

At the same time, the content of zinc in 37.3–45.9 mg/kg a.d.m. is not toxic to humans or animals. Interestingly, in the variants with a foliar treatment of mono-solutions of C_{60} or C_{70} fullerenols, there was a tendency to increase the zinc content in fruits that was more pronounced in the variant with $C_{60}(OH)_{22–24}$, which corresponds to our data about the ability of water-soluble derivatives of C_{60} and C_{70} fullerenes to activate the supply of elemental nutrition in plants [51,53].

The plants' foliar intake of zinc sulfate in the form of a mono-solution contributed to the manifestation of a tendency to reduce the yield of cucumber (hybrid F_1 Neva) fruits relatively to the control values, while this effect in mixed solutions was leveled and significantly higher values were observed for the yield of fruits: by 22% in the variant with a mixed solution of C_{70} fullerenol and zinc sulfate and by 43% in the variant with a mixed solution of C_{60} fullerenol and zinc sulfate (Figure 5B).

It should be noted that foliar treatment with mono- and mixed solutions of zinc sulfate and fullerenols C_{60} or C_{70} contributed to an increase in the values of raw ash in cucumber fruits and hence the content of minerals. It was noted that the treatment with mono- and mixed solutions of C_{70}, as well as with a C_{60} solution in combination with a zinc sulfate solution, was more pronounced (Table 9 and Table S5).

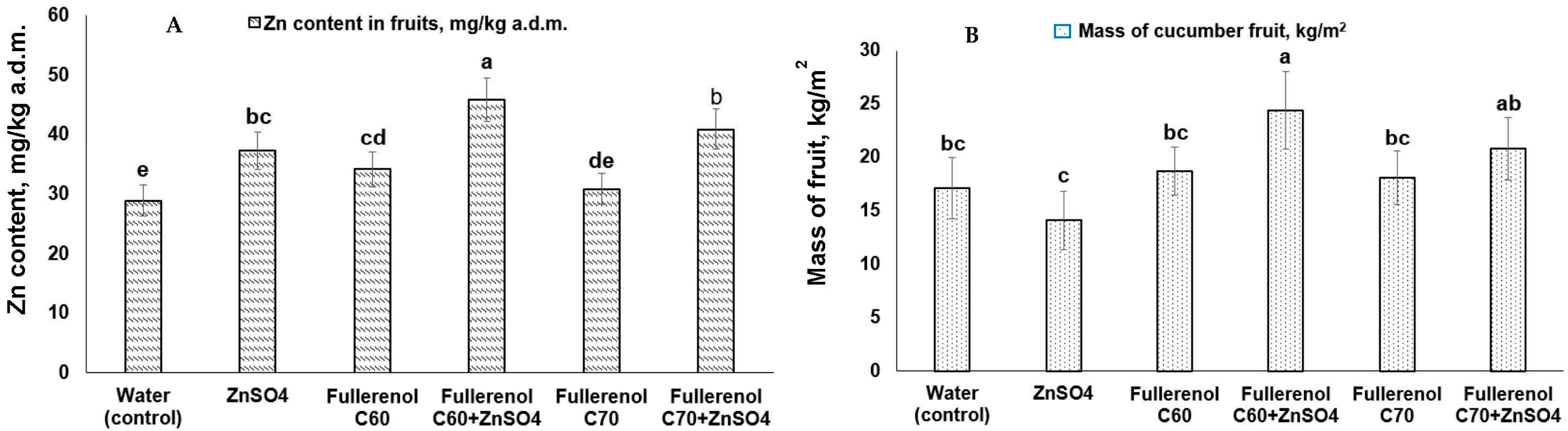


Figure 5. Zinc content (**A**) in fruits of cucumber (hybrid F_1Neva) and (**B**) its fruit yield after foliar treatment of plants during the vegetative period of their development with mono- and mixed solutions of C_{60} or C_{70} fullerenol and zinc sulfate when growing plants under controlled conditions of intensive light culture. Bars with different letters are significantly different at $p \leq 0.05$, as determined by Duncan's multiple range test. Note: a.d.m.—absolutely dry matter.

Table 9. Some indicators of the quality and safety of fruits in cucumber plants (hybrid F_1 Neva) after foliar treatment during the vegetative period of their development with mono- and mixed solutions of fullerenol C_{60} (1 mg/L) or C_{70} (0.1 mg/L) and zinc sulfate (160.0 mg/L) under controlled conditions.

Indicator	Foliar Treatment with Mono- and Mixed Solutions					
	Control	$ZnSO_4$	Fullerenol C_{60}	Fullerenol C_{60} + $ZnSO_4$	Fullerenol C_{70}	Fullerenol C_{70} +$ZnSO_4$
Raw ash, % a.d.m.	12.36 ± 0.46 c	12.80 ± 0.36 bc	12.84 ± 0.34 abc	13.40 ± 0.38 ab	13.49 ± 0.34 a	13.45 ± 0.46 ab
N, % a.d.m.	4.60 ± 0.54 a	4.98 ± 0.41 a	4.97 ± 0.43 a	4.70 ± 0.36 a	4.80 ± 0.45 a	5.05 ± 0.48 a
P, % a.d.m.	1.08 ± 0.23 a	1.07 ± 0.20 a	1.02 ± 0.18 a	1.07 ± 0.16 a	1.05 ± 0.16 a	1.10 ± 0.20 a
K, % a.d.m.	5.03 ± 0.35 b	5.67 ± 0.41 ab	5.20 ± 0.38 ab	5.37 ± 0.40 ab	5.74 ± 0.35 a	5.54 ± 0.43 ab
Ca, % a.d.m.	1.10 ± 0.09 ab	1.09 ± 0.07 abc	1.18 ± 0.06 a	1.06 ± 0.06 bcd	1.00 ± 0.05 cd	0.97 ± 0.07 d
Mg, % a.d.m.	0.29 ± 0.02 b	0.30 ± 0.05 ab	0.30 ± 0.03 ab	0.31 ± 0.03 ab	0.34 ± 0.03 a	0.31 ± 0.05 ab
Fe, mg/kg a.d.m.	51.70 ± 3.62 c	54.50 ± 5.43 c	52.20 ± 4.30 c	97.10 ± 5.66 a	74.20 ± 4.75 b	57.60 ± 5.88 a
Mn, mg/kg a.d.m.	40.0 ± 3.28 c	48.00 ± 3.73 a	45.30 ± 3.17 abc	41.40 ± 3.39 bc	42.90 ± 3.17 abc	45.90 ± 3.62 ab
Cu, mg/kg a.d.m.	21.9 ± 0.45 b	10.40 ± 0.52 d	10.90 ± 0.38 d	9.32 ± 0.34 e	12.40 ± 0.48 c	25.70 ± 0.68 a
Zn, mg/kg a.d.m.	28.90 ± 0.50 f	37.30 ± 0.45 c	34.10 ± 0.41 d	45.90 ± 0.43 a	30.80 ± 0.34 e	40.90 ± 0.46
Dry matter, %	3.20 ± 0.2 a	2.80 ± 0.3 a	2.80 ± 0.2 a	3.00 ± 0.3 a	3.20 ± 0.2 a	3.00 ± 0.2 a
Vitamin C, mg/100 g r.m.	9.40 ± 0.7 a	8.10 ± 0.7 b	8.80 ± 0.5 ab	8.60 ± 0.7 ab	8.70 ± 0.6 ab	8.50 ± 0.7 ab
Nitrates, mg/kg r.m.	159.0 ± 10.6 c	151.80 ± 10.9 c	141.70 ± 10.0 c	219.50 ± 11.8 a	117.90 ± 10.4 d	195.60 ± 11.9 b
Total saccharide, % a.d.m.	29.10 ± 3.2 ab	28.30 ± 2.9 ab	28.70 ± 2.6 ab	31.10 ± 2.9 a	25.60 ± 2.5 b	26.70 ± 2.8 ab
Monosaccharide, % a.d.m.	28.60 ± 3.4 ab	27.50 ± 2.9 abc	27.80 ± 2.5 abc	30.60 ± 2.9 a	23.30 ± 2.7 c	25.60 ± 2.7 bc
Disaccharide, % a.d.m.	0.50 ± 0.03 a	0.80 ± 0.05 d	0.90 ± 0.04 c	0.50 ± 0.03 e	2.40 ± 0.02 a	1.10 ± 0.03 b

Note: a.d.m.—absolutely dry mass; r.m.—raw mass. Values in rows followed by different letters (a–d) are significantly different at $p \leq 0.05$, as determined by Duncan's multiple range test.

In accordance with the changes in the data options on the ash content in fruits, the content of nitrogen, potassium, magnesium, iron, and manganese in them increased in the form of a trend or significantly. Only the copper content had predominantly significantly lower values than in the control. Probably, the latter is associated with the known antagonistic interactions of zinc and copper upon entering plants [89,90].

An exception was the treatment of plants with a mixed solution of C_{70} fullerenol and zinc sulfate, where an increase in the copper content was observed compared to that of the control. It is interesting to note a significant increase in the iron content in the variants with foliar treatment with a mixed solution of C_{60} fullerenol and zinc sulfate, as well as a single solution of C_{70} fullerenol, by 88% and 44% of the control.

According to the content of nitrates, all the products of the cucumber plants meet the sanitary and hygienic requirements of Russia and, even more so, foreign standards, and are significantly lower than the maximum permissible concentrations (MPC) for nitrates [91–93]. An interesting phenomenon should be noted: the foliar treatment of plants with mono-solutions of C_{60} and C_{70} fullerenols and zinc sulfate contributed to a significant reduction or a tendency to reduce the content of nitrates in the fruits relative to the control, while their mixed solutions provided a significant increase in the values of this safety indicator relative to control values (Table 9 and Table S5). The experimental variants did not differ significantly relative to the control or from each other according to the values of other evaluated indicators, in particular the content of dry matter and the total amount of saccharides, monosaccharides, and vitamin C (an exception is the variant with a foliar treatment with a solution of zinc sulfate, which caused a significant decrease in the vitamin C content). However, in relation to the content of disaccharides, there is a predominantly significant increase relative to the control. The latter is most pronounced in the variants of foliar treatment with mono- and mixed solutions of C_{70} fullerenol, which indirectly indicate the activation of secondary metabolism processes.

The increase in the yield of cucumber fruits in the variants with a foliar treatment of mono- and mixed solutions of C_{60} and C_{70} fullerenols, judging by the data presented in Figure 5 and Tables 9 and 10, is due to:

- The intensification of the supply of vital plant nutrients to the fruits of the cucumber plants (Tables 9 and S5), which may also be associated with an increase in the stem cross-sectional area (Tables 10 and S6);
- An increase in the leaves' area and, consequently, the assimilation surface for absorbing light and undergoing photosynthetic reactions with the formation and accumulation of plastic photosynthetic products, which is indirectly confirmed by an increase (tendency) in the values of the leaves' raw and dry mass (Tables 10 and S6);
- Interconnected with the above-mentioned increase in the mass of the fruit, the number, and, as a result, the mass of fruits per plant.

Thus, the foliar treatment of cucumber plants in the vegetative period of their development with mono- and mixed solutions of C_{60} or C_{70} fullerenols and zinc sulfate contributed to the enrichment of cucumber fruits with zinc compounds as well as improving their overall quality by increasing the content of a number of macro- and microelements vital for humans and plastic photosynthetic products, which include disaccharides. All of this combined had a positive effect on the plants' growth and productivity.

Table 10. Growth indicators of cucumber plant (hybrid F_1 Neva) fruits after foliar treatment during the vegetative period of their development with mono- and mixed solutions of fullerenol C_{60} (1 mg/L) or C_{70} (0.1 mg/L) and zinc sulfate (160.0 mg/L) under controlled conditions.

Experience Variant	Stem Cross-Sectional Area cm^2	Leaves' Area, cm^2	Leaves			Stems		
			Raw Mass, g/Plant	Dry Mass, g/Plant	% Dry Matter	Raw Mass, g/Plant	Dry Mass, g/Plant	% Dry Matter
Control (water)	1183.0 ± 158.4^{d}	2412.0 ± 656.3^{c}	338.0 ± 91.7^{a}	33.6 ± 8.9^{a}	10.0 ± 0.8^{ab}	320.0 ± 58.5^{a}	18.1 ± 4.3^{a}	5.7 ± 0.7^{a}
$ZnSO_4$	1398.0 ± 158.4^{abc}	3149.0 ± 339.5^{a}	361.0 ± 37.3^{a}	34.5 ± 2.0^{a}	9.6 ± 0.2^{b}	297.0 ± 20.1^{a}	13.5 ± 2.4^{a}	4.6 ± 0.5^{b}
Fullerenol C_{60}	1224.0 ± 237.6^{cd}	2418.0 ± 80.3^{c}	348.0 ± 101.8^{a}	36.9 ± 5.0^{a}	10.6 ± 0.3^{a}	299.5 ± 27.0^{a}	15.9 ± 2.8^{a}	5.3 ± 0.2^{a}
Fullerenol C_{60}+ $ZnSO_4$	1575.0 ± 181.1^{a}	3053.0 ± 56.6^{a}	360.0 ± 37.3^{a}	37.3 ± 1.9^{a}	10.4 ± 0.2^{a}	327.0 ± 21.6^{a}	18.1 ± 2.3^{a}	5.5 ± 0.2^{a}
Fullerenol C_{70}	1360.0 ± 69.0^{bcd}	2618.0 ± 339.5^{bc}	343.5 ± 48.7^{a}	36.5 ± 5.5^{a}	10.6 ± 0.3^{a}	318.5 ± 39.0^{a}	17.9 ± 2.6^{a}	5.6 ± 0.2^{a}
Fullerenol C_{70} + $ZnSO_4$	1441.0 ± 66.8^{ab}	2843.0 ± 509.2^{ab}	354.0 ± 65.6^{a}	37.9 ± 5.9^{a}	10.7 ± 0.4^{a}	320.0 ± 62.4^{a}	18.0 ± 4.1^{a}	5.6 ± 0.4^{a}

Note: values in columns followed by different letters $^{(a–d)}$ are significantly different at $p \leq 0.05$, as determined by Duncan's multiple range test.

4. Discussion

The biological properties and effects on agricultural plants of various water-soluble polyhydroxylated, carboxylated, and amino acid derivatives of C_{60} fullerenes synthesized by a group of chemists led by Professor Semenov K.N. [39,59–61] were investigated by us from 2012 to the present. The ability of these substances to intensify the macro- and trace elements' entry into the aerial parts of plants (cereal spring crops like barley and wheat as well as leaf lettuce and cabbage vegetable crops) and a pronounced trend or a significant increase in their content in plants was shown [51,53].

This effect was observed in plants both after pre-sowing treatments of the seeds and after foliar treatments of vegetative plants during their periods of intensive growth. We have also previously shown in the literature that under conditions of deficiency of manganese, zinc, or iron in an aerated liquid root-inhabited hydroponic medium, foliar treatment of plants with solutions of C_{60} fullerenol in various concentrations provides a decrease in the negative impact of deficiency factors on cucumber plants and a tendency to increase the content of deficient nutrients. It was especially pronounced in the variants of foliar treatments with mixed solutions of C_{60} fullerenol and zinc, manganese, or iron compounds [51,80,81].

The indirect effect of C_{60} solutions on agricultural plants after their introduction into the soil has not previously been practically studied. The analysis of our research data shows that the introduction of C_{60} fullerenol into the soil at concentrations of 1 mg/kg, 10 mg/kg, and 100 mg/kg activates the processes of nitrogen transformation in the soil. In particular, it enhances the process of nitrification, judging by the decrease in the content of ammonium nitrogen and the increase in the content of nitrate nitrogen (Table 3 and Table S1).

Along with this, an increase in the content of mobile forms of phosphorus, potassium, magnesium, and microelements (iron, copper, and zinc) in the soil and a decrease in the content of manganese indirectly indicate an increase in the pool of nutrients available to the plants. And, judging by the analysis of the elemental composition of plants, their active absorption and transportation to the leaves. An increase in the leaves of key elements involved in photosynthesis and other metabolic processes probably contributed to the increase in the content of photosynthetic pigments and, as a result, contributed to the increase in a number of morphometric and weight indicators of plant growth. We have previously shown the ability of C_{60} fullerenol to penetrate into plants and be found in greater quantities in the organs that were directly treated with these compounds [50,53]. Also, we have previously established and demonstrated the antioxidant properties of fullerenol C_{60} and a number of amino acid derivatives of fullerene C_{60} and their positive ability to neutralize free radicals. Thus, it can be assumed that the presence of fullerenols in cucumber plants and the probable decrease in the number of free radicals in plant cells apparently contribute to such significant changes in enzyme activity, namely, a predominant decrease in peroxidase activity in the leaves and roots, the intensity of lipid peroxidation, and such a strong increase in catalase activity. The confirmation of the possible mechanism of action of fullerene derivatives, which are capable of binding free radicals and significantly reducing their amount, on the antioxidant system of plants is provided in the literature: that the activation of antioxidant enzymes (including, obviously, catalase) in plant cells, under the influence of the TiO_2 nanoparticles, helps to reduce the pool of free radicals, including peroxidases, and, as a result, the oxidative process is reduced, including a decrease in the intensity of lipid peroxidation and peroxidase activity [94,95].

The increase in the activity of the catalase with a decrease in the activity of the peroxidase enzyme is apparently due to the participation of catalase in the metabolic processes for the transformation of nitrogen's nitrate and nitrite forms, the content of which increases in the soil and, apparently, in plants after the entry of C_{60} fullerenol into the soil. All of the above indicates an improvement in the physiological state of plants, which also ensures the stability of their growth (Table 5 and Table S2).

It should be noted that there were no significant changes in growth rates in cucumber plants with a higher content of photosynthetic pigments relative to the control in the variant with the maximum concentration of fullerenol in the soil. At the same time, the percentage of dry matter in plant organs in this variant had significantly higher values compared to the control ones. Probably, the concentration of C_{60} fullerenol in 100 mg/kg of soil is in the range of threshold values, and with a further increase in its values, the plant will experience a stress state. This is confirmed by the higher activity of peroxidase in the plant roots in this variant, which are the first to directly interact with fullerenol molecules.

Thus, the water-soluble fullerene derivative compounds provide an increase in the content of some macro- and microelements in plants by their direct effect on plants (treatment with seed solutions and foliar treatment of vegetative plants) and by their indirect effect when they enter root-inhabited media (soil and soil substitutes). This ultimately has a positive effect on the plants' physiological state and the realization of their productive potential.

Further evaluation of the effect of fullerene derivatives on the enrichment of plant production with zinc and selenium, obtained in a series of vegetation experiments under controlled conditions of intensive light culture, indicates their potentially high efficiency. The data obtained confirm the hypothesis about the ability of the studied fullerene derivatives (amino acid derivatives of C_{60} fullerene with arginine or glycine, $C_{60}(OH)_{22–24}$ or $C_{70}(OH)_{12–14}$ fullerenols) to make selenium, zinc, and other trace elements more available and assimilable for plants (for example, Chinese cabbage, tomato, and cucumber) when there is the direct effect of derivatives of C_{60} fullerene on plants (foliar treatment of vegetative plants) and their indirect effect by entering the root-inhabited media. It should be noted that, for all methods on the impact on the soil (soil substitute) and plant system by the tested nanocomposites, the content of the studied trace elements in the obtained plant production does not exceed the recommended levels of their consumption in the composition of food products according to the methodological recommendations of the Federal Center for Sanitary and Epidemiological Surveillance of the Ministry of Health of Russia [96] and abroad [11,97,98].

As a result of the selection of compositions of fullerenes and compounds of microelements, which, for example, differ in the charge of molecules, it is possible, at low concentrations, to enrich plant products with the required micronutrients in a targeted, more efficient, and safer manner compared to the action of mineral salts of microelements, which, along with other useful functions of water-soluble fullerenes, ultimately, contribute to the improvement of the physiological state of plants and favorably affect their growth and productivity, as well as the quality of plant production. Along with this, the stable composition of used preparations, which are synthesized molecules of substances, eliminates the risks of inconstancy in the manifestation of their positive effects.

5. Conclusions

The study demonstrated the ability of water-soluble polyhydroxylated and amino acid derivatives of fullerenes in certain concentrations to enrich plants and form plant products with selenium, zinc, and other useful microelements while having a positive effect on the physiological state of plants. It was revealed for the first time that the introduction of solutions of C_{60} fullerenol in various concentrations into soddy-podzolic soil contributed to the activation of the processes of nitrogen transformation in the soil, in particular, enhancing the process of nitrification and increasing the content of mobile forms of some macro- and microelements in the soil. This provided the observed increase in the content of the latter in plant organs, for example, cucumber plants, especially in their leaves.

The compositions of solutions of amino acid fullerenes and sodium selenate, as well as C_{60} or C_{70} fullerenols and zinc sulfate, selected on the basis of different charges of molecules or functional groups of fullerene derivatives, showed higher efficiency in enriching plant products in Chinese cabbage, tomato, and cucumber with selenium and zinc, respectively, compared with mineral salts of the indicated elements and with control. At the same time, the quantitative characteristics of growth, productivity, and/or quality of the obtained products increased and improved accordingly. To increase the controllability of the impact on plants, a prospect of further research is an in-depth investigation into the mechanisms of the compositions of fullerene derivatives and various compounds of trace elements' influence on the plants, as well as the synthesis and study of the various exo- and endo-derivatives of fullerenes' properties, including C_{60} complex compounds with transition metals and fullerenes containing their carbon network atoms of various chemical elements, such as lanthanum and others.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9070828/s1, Table S1: Analysis of variance (ANOVA) of the effect of C_{60} fullerenol in various concentrations into the soil on the agrochemical indicators of the state of the soil; Table S2: Analysis of variance (ANOVA) of the effect of C_{60} fullerenol in various concentrations into the soil on the studied traits of cucumber plants (hybrid F_1 Neva) grown under controlled conditions; Table S3: Analysis of variance (ANOVA) of the effect of the introduction to the nutrient solution of mineral selenium-containing substances and their compositions with amino acid derivatives of C_{60} fullereneon on biomass of edible part of Chinese cabbage plants cv. Daqingkou and on the selenium content in aerial parts and roots; Table S4: Analysis of variance (ANOVA) of the effect for foliar treatment with solutions of selenium-containing substances and compositions on fruit quality of tomato cv. Natasha; Table S5: Analysis of variance (ANOVA) of the effect of mono- and mixed solutions of fullerenol C_{60} (1 mg/L) or C_{70} (0.1 mg/L) and of zinc sulfate (160.0 mg/L) on some indicators of the quality of fruits in cucumber plants (hybrid F_1 Neva) fruits; Table S6: Analysis of variance (ANOVA) of the effect of mono- and mixed solutions of fullerenol C_{60} (1 mg/L) or C_{70} (0.1 mg/L) and of zinc sulfate (160.0 mg/L) on growth indicators of cucumber plants (hybrid F_1 Neva) fruits.

Author Contributions: Conceptualization, G.G.P. and O.R.U.; Methodology, G.G.P., O.R.U. and Y.V.K.; Software, G.G.P. and G.V.M.; Validation, G.G.P., K.N.S. and A.S.Z.; Formal Analysis, G.V.M. and A.S.Z.; Investigation, A.S.Z., E.N.V., N.R.I., O.R.U., V.I.D., G.G.P. and Y.V.K.; Resources, G.G.P. and A.S.Z.; Data Curation, G.G.P., O.R.U., Y.V.K., G.V.M. and A.M.A.; Writing—Original Draft Preparation, G.G.P. and A.S.Z.; Writing—Review and Editing, A.S.Z., E.N.V., A.M.A., Y.V.K., K.N.S. and G.G.P.; Visualization, A.S.Z., K.N.S., G.V.M. and N.R.I.; Supervision, G.G.P. and O.R.U.; Project Administration, O.R.U. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Agrophysical Research Institute, scientific and technical state task № FGEG-2022-0005 in the theoretical part and in ensuring the conduct of vegetation experiments, and by the Russian Science Foundation (project No. 22-26-00267) https://rscf.ru/project/22-26-00267/ (accessed on 18 July 2023) in the chapter "Foliar treatment of vegetative plants with mono- and mixed solutions of zinc-containing substances and C_{60} or C_{70} fullerenols".

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: We express our deep gratitude to the direction of the AFI for the opportunity to implement the research project, to the directions of the VIR and of the Federal Scientific Center for Vegetable Growing for the provided vegetable seeds, as well as to the curators of vegetable crops from these institutes: Kurina A.B. and Balashova I.T.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Vergnaud, A.-C.; Romaguera, D.; Peeters, P.H.; van Gils, C.H.; Chan, D.S.; Romieu, I.; Freisling, H.; Ferrari, P.; Clavel-Chapelon, F.; Fagherazzi, G.; et al. Adherence to the World Cancer Research Fund/American Institute for Cancer Research guidelines and risk of death in Europe: Results from the European Prospective Investigation into Nutrition and Cancer cohort study. *Am. J. Clin. Nutr.* **2013**, *97*, 1107–1120. [CrossRef] [PubMed]
2. Granato, D.; Barba, F.J.; Kovačević, D.B.; Lorenzo, J.M.; Cruz, A.G.; Putnik, P. Functional Foods: Product Development, Technological Trends, Efficacy Testing, and Safety. *Annu. Rev. Food Sci. Technol.* **2020**, *11*, 93–118. [CrossRef] [PubMed]
3. Huang, S.; Wang, P.; Yamaji, N.; Ma, J.F. Plant nutrition for human nutrition: Hints from rice research and future perspectives. *Mol. Plant* **2020**, *13*, 825–835. [CrossRef] [PubMed]
4. Puzin, S.N.; Pogozheva, A.V.; Potapov, V.N. Optimizing nutrition of older people as a mean of preventing premature aging. *Probl. Nutr.* **2018**, *87*, 69–77. [CrossRef]

5. Hurst, R.; Hooper, L.; Norat, T.; Lau, R.; Aune, D.; Greenwood, D.C.; Vieira, R.; Collings, R.; Harvey, L.J.; Sterne, J.A.; et al. Selenium and prostate cancer: Systematic review and meta-analysis. *Am. J. Clin. Nutr.* **2012**, *96*, 111–122. [CrossRef]
6. Ibrahim, S.A.Z.; Kerkadi, A.; Agouni, A. Selenium and Health: An Update on the Situation in the Middle East and North Africa. *Nutrients* **2019**, *11*, 1457. [CrossRef] [PubMed]
7. Costello, L.C.; Franklin, R.B. Zinc is decreased in prostate cancer: An established relationship of prostate cancer! *J. Biol. Inorg. Chem.* **2011**, *16*, 3–8. [CrossRef]
8. Ho, E.; Song, Y. Zinc and prostatic cancer. *Curr. Opin. Clin. Nutr. Metab. Care* **2009**, *12*, 640–645. [CrossRef]
9. Kieliszek, M.; Bła'zejak, S. Current Knowledge on the Importance of Selenium in Food for Living Organisms: A Review. *Molecules* **2016**, *21*, 609. [CrossRef]
10. World Health Organization. *Trace Elements in Human Nutrition and Health*; World Health Organization: Geneva, Switzerland, 1996; 360p.
11. *Vitamin and Mineral Requirements in Human Nutrition: Report of a Joint FAO/WHO Expert Consultation. Bangkok, Thailand, 21–30 September 1998*; World Health Organization and Food and Agriculture Organization of the United Nations: Geneva, Switzerland, 2004; 362p.
12. Scherz, H.; Kirchhoff, E. Trace elements in foods: Zinc contents of raw foods—A comparison of data originating from different geographical regions of the world. *J. Food Compos. Anal.* **2006**, *19*, 420–433. [CrossRef]
13. Praharaj, S.; Skalicky, M.; Maitra, S.; Bhadra, P.; Shankar, T.; Brestic, M.; Hossain, A. Zinc biofortification in food crops could alleviate the zinc malnutrition in human health. *Molecules* **2021**, *26*, 3509. [CrossRef] [PubMed]
14. Ros, G.H.; van Rotterdam, A.M.D.; Bussink, D.W.; Bindraban, P.S. Selenium fertilization strategies for bio-fortification of food: An agro-ecosystem approach. *Plant Soil* **2016**, *404*, 99–112. [CrossRef]
15. Franchini, A.; Quattrini, E.; Giuffrida, F.; Ferrante, A. Biofortification of fresh leafy vegetables using a nutrient solution containing selenium. *J. Sci. Food Agric.* **2023**, *9*, 5472–5480. [CrossRef]
16. Perminova, I.V. From green chemistry and nature-like technologies towards ecoadaptive chemistry and technology. *Pure Appl. Chem.* **2019**, *91*, 851–864. [CrossRef]
17. Folmanis, G.E.; Fedotov, M.A. Dispersion methods of preparation of nanosized biological agents for vegetable crops. *J. Phys. Conf. Ser.* **2020**, *1*, 012061. [CrossRef]
18. Churilov, G.I.; Polischuk, S.D.; Kuznetsov, D.; Borychev, S.N.; Byshov, N.V.; Churilov, D.G. Agro ecological grounding for the application of metal nanopowders in agriculture. *Int. J. Nanotechnol.* **2018**, *15*, 258–279. [CrossRef]
19. Zhou, H.; Yang, J.; Kronzuker, H.J.; Shi, W. Selenium biofortification and interactions with other elements in plants: A review. *Front. Plant Sci.* **2020**, *11*, 586421. [CrossRef]
20. Alfthan, G. Effect of selenium fertilization on the human selenium status and the environment. *Norw. J. Agric. Sci.* **1993**, *11*, 175–181.
21. Ermakov, V.V. Biogeochemistry of selenium and its importance in the prevention of endemic human diseases. In *Bulletin of the Department of Earth Sciences*; RAS: Moscow, Russia, 2004; Volume 1, pp. 1–17. (In Russian)
22. Ekholm, P.; Reinivuo, H.; Mattila, P.; Pakkala, H.; Koronen, J.; Happonen, A.; Hellstrom, J.; Pvaskainen, M.L. Changes in the mineral and trace element contents of cereals, fruits and vegetables in Finland. *J. Food. Comp. Anal.* **2007**, *20*, 487–495. [CrossRef]
23. Golubkina, N.; Moldovan, A.; Kekina, H.; Kharchenko, V.; Sekara, A.; Vasileva, V.; Caruso, G. Joint biofortification of plants with selenium and iodine: New field of discoveries. *Plants* **2021**, *10*, 1352. [CrossRef]
24. Koivistoinnen, J.K.; Huttenen, J.K. Selenium in food and nutrition in Finland. An overview on research and action. *Ann. Clin. Res.* **1986**, *18*, 296–298.
25. Varo, P.; Alftan, G.; Huttenen, J.; Aro, A. Nationwide selenium supplementation in Finland-effects on diet, blood and tissue levels, and health. In *Selenium in Biology and Human Health*; Burk, R.F., Ed.; Springer: New York, NY, USA, 1994; pp. 198–218, ISBN 978-1-4612-2592-8.
26. Torshin, S.P.; Udelnova, T.M.; Yagodin, B.A. Biogeochemistry and agrochemistry of selenium and methods for eliminating selenium deficiency in food and feedstuffs. *Agrochemistry* **1996**, *8*, 127–145. (In Russian)
27. Rak, M.V.; Safronovskaya, G.M.; Barashkova, E.N.; Tikhonovich, Z.N.; Mukovozchik, V.A. Method for Dressing Clover with Meadow Selenium. Russian Federation Patent 14128 C1, 28 February 2011. (In Russian).
28. Pushkarev, A. Enrichment of broccoli with selenium using apiones. *Veg. Farming Green Housekeep.* **2012**, *4*, 4–7. (In Russian)
29. Oliveira, V.C.D.; Faquin, V.; Andrade, F.R.; Carneiro, J.P.; da Silva Júnior, E.C.; de Souza, K.R.D.; Guilherme, L.R.G. Physiological and physicochemical responses of potato to selenium biofortification in tropical soil. *Potato Res.* **2019**, *62*, 315–331. [CrossRef]
30. Thavaraja, D.; Abare, A.; Mapa, I.; Koyne, S.J.; Thavaraja, K.S. Lentil accession selection for global selenium biofortification. *Plants* **2017**, *3*, 34. [CrossRef]
31. Oliveira, V.C.D.; Faquin, V.; Guimarães, K.C.; Andrade, F.R.; Pereira, J.; Guilherme, L.R.G. Agronomic biofortification of carrot with selenium. *Cienc. Agrotecnol.* **2018**, *42*, 138–147. [CrossRef]
32. Germ, M.; Stibilj, V.; Šircelj, H.; Jerše, A.; Kroflič, A.; Golob, A.; Maršić, N.K. Biofortification of common buckwheat microgreens and seeds with different forms of selenium and iodine. *J. Sci. Food Agric.* **2019**, *99*, 4353–4362. [CrossRef]
33. Ducsay, L.; Ložek, O.; Marček, M.; Varényiová, M.; Hozlár, P.; Lošák, T. Possibility of selenium biofortification of winter wheat grain. *Plant Soil Environ.* **2016**, *62*, 379–383. [CrossRef]

34. Hawrylak-Nowak, B. Comparative effects of selenite and selenate on growth and selenium accumulation in lettuce plants under hydroponic conditions. *Plant Growth Regul.* **2013**, *70*, 149–157. [CrossRef]
35. Li, X.; Wu, Y.; Li, B.; Yang, Y.; Yang, Y. Selenium accumulation characteristics and biofortification potentiality in turnip (*Brassica rapa* var. *rapa*) supplied with selenite or selenite. *Front. Plant Sci.* **2018**, *8*, 2207. [CrossRef]
36. Lidon, F.C.; Oliveira, K.; Galhano, C.; Guerra, M.; Ribeiro, M.M.; Pelica, J.; Reboredo, F.H. Selenium biofortification of rice through foliar application with selenite and selenite. *Exp. Agric.* **2019**, *55*, 528–542. [CrossRef]
37. Finley, J.W. Selenium accumulation in plant foods. *Nutr. Rev.* **2005**, *63*, 196–202. [CrossRef] [PubMed]
38. Dumpis, M.A.; Nikolayev, D.N.; Litasova, E.V.; Iljin, V.V.; Brusina, M.A.; Piotrovsky, L.B. Biological activity of fullerenes-reality and prospects. *Rev. Clin. Pharmacol. Drug Ther.* **2018**, *16*, 4–20. [CrossRef]
39. Semenov, K.N.; Charykov, N.A.; Keskinov, V.A. Fullerenol synthesis and identification. *Properties of fullerenol water solutions. J. Chem. Eng. Data* **2011**, *56*, 230–239. [CrossRef]
40. Andreev, I.; Petrukhina, A.; Garmanova, A.; Babakhin, A.; Andreev, S.; Romanova, V.; Troshin, P.; Troshina, O.; Du Buske, L. Penetration of fullerene C_{60} derivatives through biological membranes. *Fuller. Nanotub. Carbon Nanostructures* **2008**, *16*, 89–102. [CrossRef]
41. Gao, J.; Wang, Y.; Folta, K.M.; Krishna, V.; Bai, W.; Indeglia, P.; Georgieva, A.; Nakamura, H.; Koopman, B.; Moudgi, B. Polyhydroxy fullerenes (fullerols or fullerenols): Beneficial effects on growth and lifespan in diverse biological models. *PLoS ONE* **2011**, *6*, e19976. [CrossRef]
42. Chen, R.; Ratnikova, T.A.; Stone, M.B.; Lin, S.; Lard, M.; Huang, G.; Hudson, J.S.; Ke, P.C. Differential uptake of carbon nanoparticles by plant and mammalian cells. *Small* **2010**, *6*, 612–617. [CrossRef]
43. Kole, C.; Kole, P.; Randunu, K.M.; Choudhary, P.; Podila, R.; Ke, P.C.; Rao, A.M.; Marcus, R.K. Nanobiotechnology can boost crop production and quality: First evidence from increased plant biomass, fruit yield and phytomedicine content in bitter melon (*Momordica charantia*). *BMC Biotechnol.* **2013**, *13*, 37–58. [CrossRef]
44. Lin, S.; Reppert, J.; Hu, Q.; Hudson, J.S.; Reid, M.L.; Ratnikova, T.A.; Rao, A.M.; Luo, H.; Ke, P.C. Uptake, translocation, and transmission of carbon nanomaterials in rice plants. *Small* **2009**, *5*, 1128–1132. [CrossRef]
45. Avanasi, R.; Jackson, W.A.; Sherwin, B.; Mudge, J.F.; Anderson, T.A. C_{60} fullerene soil sorption, biodegradation, and plant uptake. *Environ. Sci. Technol.* **2014**, *48*, 2792–2797. [CrossRef]
46. Liang, C.; Xiao, H.; Hu, Z.; Zhang, X.; Hu, J. Uptake, transportation, and accumulation of C_{60} fullerene and heavy metal ions (Cd, Cu, and Pb) in rice plants grown in an agricultural soil. *Environ. Pollut.* **2018**, *235*, 330–338. [CrossRef] [PubMed]
47. Panova, G.G.; Ktitorova, I.N.; Skobeleva, O.V.; Sinjavina, N.G.; Charykov, N.A.; Semenov, K.N. Impact of polyhydroxy fullerene (fullerol or fullerenol) on growth and biophysical characteristics of barley seedlings in favourable and stressful conditions. *Plant Growth Regul.* **2016**, *79*, 309–317. [CrossRef]
48. Semenov, K.N.; Meshcheriakov, A.A.; Charykov, N.A.; Dmitrenko, M.E.; Keskinov, V.A.; Murin, I.V.; Panova, G.G.; Sharoyko, V.V.; Kanash, E.V.; Khomyakov, Y.V. Physico-chemical and biological properties of C 60-l-hydroxyproline water solutions. *RSC Adv.* **2017**, *7*, 15189–15200. [CrossRef]
49. Panova, G.G.; Kanash, E.V.; Khomyakov, Y.V.; Shpanev, A.M.; Serebryakov, E.B.; Semenov, K.N.; Shemchuk, O.S.; Andrusenko, E.V.; Podolsky, N.E.; Sharoyko, V.V.; et al. Bioactivity study of the C60-L-threonine derivative for potential application in agriculture. *J. Nanomater.* **2019**, *2306518*, 13. [CrossRef]
50. Panova, G.G.; Zhuravleva, A.S.; Khomyakov, Y.V.; Vertebnyi, V.E.; Ageev, S.V.; Sharoyko, V.V.; Semenov, K.N.; Petrov, A.V.; Podolsky, N.E.; Morozova, E.I. Plant impact properties of carboxylated fullerene C60 [C (COOH)2]3. *J. Mol. Struct.* **2021**, *1235*, 130163. [CrossRef]
51. Bityutskii, N.P.; Yakkonen, K.L.; Lukina, K.A.; Semenov, K.N.; Panova, G.G. Fullerenol can ameliorate iron deficiency in cucumber grown hydroponically. *J. Plant Growth Regul.* **2020**, *40*, 1017–1031. [CrossRef]
52. Shpanev, A.M.; Denisyuk, E.S.; Shilova, O.A.; Semenov, K.N.; Panova, G.G. Carbon and silica nanostructures in the protection of spring barley from diseases in the North-West Russia. Bioelectrochemical systems based on the electroactivity of plants and microorganisms in the root environment (review). *Sel'skokhozyaistvennaya Biol.* **2022**, *57*, 441–459. [CrossRef]
53. Panova, G.G.; Semenov, K.N.; Artemyeva, A.M.; Rogozhin, E.A.; Barashkova, A.S.; Kornyukhin, D.L.; Khomyakov, Y.V.; Balashov, E.V.; Galushko, A.S.; Vertebny, V.E.; et al. Influence of nanocompositions based on derivatives of light fullerenes on cultivated plants in favorable and stressful conditions of their habitat. *J. Tech. Phys.* **2022**, *92*, 1045–1059. [CrossRef]
54. Samadi, S.; Lajayer, B.A.; Moghiseh, E.; Rodríguez-Couto, S. Effect of carbon nanomaterials on cell toxicity, biomass production, nutritional and active compound accumulation in plants. *Environ. Technol. Innov.* **2021**, *21*, 101323. [CrossRef]
55. Kovac, T.; Marcek, T.; Šarkanj, B.; Borišev, I.; Ižakovic, M.; Jukic, K.; Krska, R.F. C60 (OH)24 Nanoparticles and drought impact on wheat (*Triticum aestivum* L.) during growth and infection with *Aspergillus flavus*. *J. Fungi* **2021**, *7*, 236. [CrossRef]
56. Tai, F.; Wang, S.; Liang, B.; Li, Y.; Wu, J.; Fan, C.; Wang, W. Quaternary ammonium iminofullerenes improve root growth of oxidative-stress maize through ASA-GSH cycle modulating redox homeostasis of roots and ROS-mediated root-hair elongation. *J. Nanobiotechnol.* **2022**, *20*, 1–15. [CrossRef] [PubMed]
57. Xiong, J.L.; Li, J.; Wang, H.C.; Zhang, C.L.; Naeem, M.S. Fullerol improves seed germination, biomass accumulation, photosynthesis and antioxidant system in *Brassica napus* L. under water stress. *Plant Physiol. Biochem.* **2018**, *129*, 130–140. [CrossRef] [PubMed]

58. Wang, C.; Zhang, H.; Ruan, L.; Chen, L.; Li, H.; Chang, X.L.; Yang, S.T. Bioaccumulation of 13 C-fullerenol nanomaterials in wheat. *Environ. Sci. Nano* **2016**, *3*, 799–805. [CrossRef]
59. Semenov, K.N.; Charykov, N.A.; Namazbaev, V.I.; Keskinov, V.A. Method of producing mixture of fullerenols. RU 2495821 C2, 20 October 2013. (In Russian). Available online: https://pubchem.ncbi.nlm.nih.gov/patent/RU-2495821-C2 (accessed on 28 May 2023).
60. Semenov, K.N.; Charykov, N.A.; Pronskikh, A.E.; Keskinov, V.A. Fullerenol-70-d: Synthesis, identification, polythermal solubility and density of water solutions. *Nanosyst. Phys. Chem. Math.* **2012**, *3*, 146–156.
61. Shestopalova, A.A.; Semenov, K.N.; Charykov, N.A.; Postnov, V.N.; Ivanova, N.M.; Sharoyko, V.V.; Keskinov, V.A.; Letenko, D.G.; Nikitin, V.A.; Klepikov, V.V.; et al. Physico-chemical properties of the C_{60}-arginine water solutions. *J. Mol. Liq.* **2015**, *211*, 301–307. [CrossRef]
62. Panova, G.G.; Chernousov, I.N.; Udalova, O.R.; Alexandrov, A.V.; Karmanov, I.V.; Anikina, L.M.; Sudakov, V.L.; Yakushev, V.P. Scientific basis for large year-round yields of high-quality crop products under artificial lighting. *Russ. Agric. Sci.* **2015**, *41*, 335–339. [CrossRef]
63. Panova, G.G.; Udalova, O.R.; Kanash, E.V.; Galushko, A.S.; Kochetov, A.A.; Priyatkin, N.S.; Arkhypov, M.V.; Chernousov, I.N. Fundamentals of physical modeling of "ideal" agroecosystems. *Tech. Phys.* **2020**, *65*, 1563–1569. [CrossRef]
64. State Standard of Russian Federation. *Products of Fruits and Vegetables. Processing. Methods for Determination of Vitamin C*; Standartinform Publishing: Moscow, Russia, 2003; pp. 24556–24589. (In Russian)
65. Guidelines for the determination of nitrates and nitrites in crop production MU N. In *USSR Minist. Health USSR State Agroprom*; Publishing: Moscow, Russia, 1989; pp. 5048–5089. 52p. (In Russian)
66. Interstate Standard GOST EN 14084–2014; Foodstuffs. Definition of trace elements. Determination of lead, cadmium, zinc, copper and iron content by atomic absorption spectrometry (AAS) after microwave digestion. In *Eurasian Council for Standardization, Metrology and Certification*; National Institute of Standards: Minsk, Belarus, 2014; 17p. (In Russian)
67. Technical Regulation of the Customs Union "On Food Safety" (TR TS 021/2011) dated 09.12.2011 N021/2011 (as amended on 14 July 2021), Official Website of the Customs Union Commission. 173p. Available online: www.tsouz.ru (accessed on 15 December 2021).
68. Assessment of the quality of food products and assessment of the population's access to domestic food products, contributing to the elimination of macro- and micronutrient deficiencies: Guidelines. MP 2.3.7.0168-20. *Bull. Regul. Methodol. Doc. State Sanit. Epidemiol. Superv. Russ. Fed.* **2020**, *2*, 43–106. (In Russian)
69. *GOST R 53182–2008*; Foodstuffs. Determination of Trace Elements. Determination of Total Arsenic and Selenium by Hydride Generation Atomic Absorption Spectrometry (HGAAS) Method after Pressure Digestion. Standartinform Publishing: Moscow, Russia, 2010; p. 12. (In Russian)
70. *ISO 10390*; Soil, Treated Biowaste and Sludg–Determination of pH. ISO: Genewa, Switzerland, 2021.
71. *RF State Standard 26489-85*; Soils. Determination of Exchangeable Ammonium by CINAO Method. Standards Publishing House: Moscow, Russia,, 1985. (In Russian)
72. *RF State Standard 26951-86*; Soils. Determination of Nitrates by Ionometric Method. Standards Publishing House: Moscow, Russia, 1986. (In Russian)
73. *RF State Standard R 54650-2011*; Soils. Determination of Mobile Compounds of Phosphorus and Potassium According to the Kirsanov Method in the Modification of CINAO. Federal Agency for Technical Regulation and Metrology on the Internet. Standartinform Publishing: Moscow, Russia, 2011. (In Russian)
74. *RF State Standard 26487-85*; Soils. Determination of Exchangeable Calcium and Exchangeable (Mobile) Magnesium by CINAO Methods. Standards Publishing House: Moscow, Russia, 1985. (In Russian)
75. Nichiporovich, A.A. Photosynthesis and the productive process. *M Sci.* **1988**, 276. (In Russian)
76. Trineeva, O.V.; Safonova, E.F.; Slivkin, A.I.; Voropaeva, S.V. Method of Spectrophotometric Quantitative Determination in the Leaves of Stinging Nettle in the Joint Presence of Chlorophyll, Carotenoids and Hydroxycinnamic Acids. Russian Federation Patent 2531940, 27 October 2014. (In Russian).
77. Kumar, G.N.M.; Knowles, N.R. Changes in lipid peroxidation and lipolytic and freeradical scavenging enzyme activities during aging and sprouting of potato (*Solanum tuberosum*) seed-tubers. *Plant. Physiol.* **1993**, *102*, 115–124. [CrossRef]
78. Lukatkin, A.S. Contribution of oxidative stress to development of cold-induced damage on leaves of chilling-sensitive plants: 1. Reactive oxygen species formation during plant during plant chilling. *Russ. J. Plant Physiol.* **2002**, *49*, 622–627. [CrossRef]
79. Pochinok, H.N. *Methods of Biochemical Analysis of Plants*; Naukovo Dumka: Kyiv, Ukraine, 1976; 334p. (In Russian)
80. Bityutskii, N.P.; Yakkonen, K.L.; Semenov, K.N. Zinc deficiency in cucumber plants can be alleviated by fullerenol. *J. Plant Nutr.* **2023**, *46*, 1504–1518. [CrossRef]
81. Bityutskii, N.P.; Yakkonen, K.L.; Lukina, K.A.; Semenov, K.N. Fullerenol increases effectiveness of foliar iron fertilization in iron deficient cucumber. *PLoS ONE* **2020**, *15*, e0232765. [CrossRef]
82. Kabata-Pendias, A.; Pendias, H. Trace elements in soils and plants. In *Biogeochemistry of Trace Elements*; 3rd ed.; CRC Press: Boca Raton, FL, USA; London, UK; New York, NY, USA; Washington, DC, USA, 2001; 403p.
83. Marschner, H. *Mineral Nutrition of Higher Plants*, 3rd ed.; Academic Press: London, UK, 2012; 651p, ISBN 978-0-12-384905-2. [CrossRef]
84. Tsonev, T.; Lidon, F.J.C. Zinc in plants-an overview. *Emir. J. Food Agric. (EJFA)* **2012**, *24*, 322–333.

85. Hafeez, B.M.; Khanif, Y.M.; Saleem, M. Role of zinc in plant nutrition-a review. *Am. J. Exp. Agric.* **2013**, *3*, 374–391. [CrossRef]
86. Shams Tabrez, K.; Malik, A. (Eds.) *Microbial Biofertilizers and Micronutrient Availability: The Role of Zinc in Agriculture and Human Health*; Springer Nature: Cham, Switzerland, 2021. [CrossRef]
87. Sharma, A.; Kumar, V.; Shahzad, B.; Ramakrishnan, M.; Singh Sidhu, G.P.; Bali, A.S.; Handa, N.; Kapoor, D.; Yadav, P.; Khanna, K.; et al. Photosynthetic Response of Plants Under Different Abiotic Stresses: A Review. *J. Plant Growth Regul.* **2019**, *39*, 509–531. [CrossRef]
88. Buturi, C.V.; Mauro, R.P.; Fogliano, V.; Leonardi, C.; Giuffrida, F. Mineral Biofortification of Vegetables as a Tool to Improve Human Diet. *Foods* **2021**, *10*, 223. [CrossRef]
89. Chaudhry, F.M.; Sharif, M.; Latif, A.; Qureshi, R.H. Zinc-copper antagonism in the nutrition of rice (*Oryza sativa* L.). *Plant Soil* **1973**, *38*, 573–580. [CrossRef]
90. Poudel, P.; Di Gioia, F.; Lambert, J.D.; and Connolly, E.L. Zinc biofortification through seed nutri-priming using alternative zinc sources and concentration levels in pea and sunflower microgreens. *Front. Plant Sci.* **2023**, *14*, 1177844. [CrossRef]
91. Hygienic Requirements for the Safety and Nutritional Value of Food: Sanitary and Epidemiological Rules and Regulations SanPiN 2.3.2.1078-01. approved by the Chief State Sanitary Doctor of the Russian Federation 06.11.2001, Moskow, Russian Federation, 44p. (In Russian). Available online: https://www.russiangost.com/p-149772-sanpin-2321078-01.aspx (accessed on 28 May 2023).
92. Commission Regulation (EU) No 1258/2011. Amending Regulation (EC) No 1881/2006 as regards maximum levels for nitrates in foodstuffs. *Off. J. Eur. Union* **2011**, *320*, 15–17. Available online: http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:320:0015:0017:EN:PDF (accessed on 28 May 2023).
93. Gorenjak, A.H.; Cencič, A. Nitrate in vegetables and their impact on human health (a review). *Acta Aliment.* **2013**, *42*, 158–172. [CrossRef]
94. Xu, J.; Yang, J.; Duan, X.; Jiang, Y.; Zhang, P. Increased expression of native cytosolic Cu/Zn superoxide dismutase and ascorbate peroxidase improves tolerance to oxidative and chilling stresses in cassava (*Manihot esculenta* Crantz). *BMC Plant Biol.* **2014**, *14*, 208. [CrossRef] [PubMed]
95. Ali, S.; Mehmood, A.; Khan, N. Uptake, translocation, and consequences of nanomaterials on plant growth and stress adaptation. *J. Nanomater.* **2021**, 6677616. [CrossRef]
96. *MR 2.3.1.1915-04*; Recommended Levels of Food Intake and Biologically Active Substances: Guidelines. Federal Center for State Sanitary and Epidemiological Surveillance of the Ministry of Health of Russia: Moskow, Russia, 2004; 46p.
97. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Manganese, Mohbdenium, Nikel, Silicon, Vanadium and Zinc Food and Nutrition Board (FNB), Institute of Medicine (IOM)*; Copyright by National Academy of Sciences; The National Academies Press: Washington, DC, USA, 2002; 773p. [CrossRef]
98. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*; National Academy of Sciences: Washington, DC, USA, 2000; 529p. [CrossRef]