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# Interactive Effects between Zinc and Selenium on Mineral Element Accumulation and Fruit Quality of Strawberry

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**Abstract:** In this study, we evaluated zinc (Zn) and selenium (Se) biofortification in strawberry fruits under substrate and soil cultivation, along with their effects on mineral element accumulation and fruit quality. To achieve this, foliar Zn (0.1% and 0.2%) and Se (0.003% and 0.006%) fertilizers were applied separately or in combination at the initial flowering stage. The Zn and Se contents in strawberry fruits increased with the spraying dosage. Compared to the control, the Zn content in the first batch of Zn-treated strawberries increased by 36.9–109% and 27.1–102% under substrate and soil cultivation, respectively, while Se increased by 313–444% and 21.3–53.3%, respectively. However, foliar Zn application could not ensure long-term sustainability as Zn in strawberries gradually decreased in the two subsequent batches, while Se was more stable. Compared to the control, the Se content in the three batches of Se2 (0.006%)-treated strawberries grown in soil increased by 32.9%, 124%, and 109%, respectively. Meanwhile, compared to Se alone, the Zn–Se combined application decreased the Se content in strawberries by 61.2–77.6% and 24.9–45.7% under substrate and soil cultivation, respectively, while low doses of Se promoted Zn enrichment (by 8.62–40.9%) and high doses inhibited it (by 13.2–28.9%) under substrate cultivation. Moreover, the copper content in strawberries under substrate cultivation after the Se1 (0.003%) treatment was significantly higher (by 75.0%) than that in the control. A positive correlation was observed between Cu and Zn contents in strawberries under both substrate and soil cultivation. A consistent positive impact was also observed on fruit quality. The Se2 (0.006%) treatment caused an increase in ascorbic acid content (by 37.2%) in strawberry fruits. The soluble sugar content increased by 36.3% after the Zn1 (0.1%) treatment. The present study provides a practical basis for the biofortification of strawberries with Zn and Se.

**Keywords:** zinc; selenium; strawberry; biofortification; mineral element; fruit quality

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## 1. Introduction

Zinc (Zn) is a micronutrient for plant and human survival. For plant growth and yield, Zn plays an important role in photosynthesis and related enzymes [1]. In the human body, Zn deficiency can cause stunted growth, anorexia, and loss of smell and taste [2]. In general, the distribution of available Zn in farmland soils in China is higher in the south than in the north, and in the east compared to the west [3]. Yang et al. [4] reported that more than 50% of soil in China has a low Zn content (DTPA-extractable Zn $\leq 1.0$ mg kg$^{-1}$).

The trace element selenium (Se) is usually required in minute quantities; nevertheless, it has important physiological functions in both plants and humans during growth and development. Se can improve the activity of antioxidant enzymes in vivo and reduce oxidation [5]. It can also lead to the inhibition of heavy metal uptake by plants and a reduction in their toxicity [6,7]. From a human nutritional point of view, Se is a key component of selenoproteins. Se deficiency can increase the risk of many diseases, such as epilepsy, thyroid dysfunction, Keshan disease, and arthropathies [8,9]. In China, the level of Se in soil shows large variations across regions. Liu et al. [10] measured Se contents ranging from 0.01 to 16.24 mg $kg^{-1}$, with a median of 0.171 mg $kg^{-1}$, in China's pedosphere, which is significantly lower than the global soil Se content of 0.40 mg $kg^{-1}$.

Zn and Se soil contents and species strongly affect the amounts that accumulate in plants, and soil Zn and Se deficiency has a certain impact on human health [11]. In view of the significant differences in soil Se content worldwide and the distribution of Se-poor soils in some densely populated areas, up to one billion people may be affected by Se deficiency globally [12]. Zn and Se deficiency is a threat to people in certain areas of China [13,14], and it is necessary to increase human Zn and Se intake to alleviate related public health issues.

In recent years, several approaches have been developed to enhance Zn and Se levels in crops and animals. According to the World Health Organization (WHO), "fortification" is defined as the intentional addition of nutrients into foods in order to increase the level of one or more nutrients. Biofortification is an economical and effective agricultural process that increases dietary nutrient intake for humans, which has become a key research field [15]. Several studies have been performed aiming at biofortifying crops with Zn and Se [16,17]. Numerous methods have been reported for Zn and Se application, such as seed dressing, seed soaking, and soil and foliar application. Among these, foliar application has been widely used because of its practicability and effectiveness in Zn and Se biofortification. Inorganic selenium is commonly used as an exogenous selenium fertilizer, among which $Na_2SeO_3$ has been widely used in production because of its high concentration of active ingredients and high-water solubility [18]. Absorbed selenite is more easily incorporated into the Se form available to the human body than selenate [19]. For soil application, more than 80% of Se was found to be fixed in soil after Se was added for 2 months, which considerably reduced Se bioavailability [20]. Wang et al. [21] found that both soil and foliar Se applications effectively improved Se content in maize grains (*Zea mays* L.), but foliar Se application is more cost-efficient and effective. Zhu et al. [22] reported that spraying Se fertilizer could increase Se content and lower heavy metals in grapes (*Vitis vinifera* L.). Zahedi et al. [23] also observed that foliar spraying Se nanoparticles improved the growth and yield of strawberry plants. Earlier studies also showed that foliar Zn application significantly increased Zn content in maize and rice grains (*Oryza sativa* L.) [24,25].

Compound Zn–Se foliar application at an appropriate dose may simultaneously fortify Zn and Se. Souza et al. [26] observed an increase in Zn accumulation in rice with a compound Zn and Se application. However, the interactions between Zn and Se remain controversial at present. Nawaz et al. [27] found that foliar spraying Se significantly increased Se content in wheat (*Triticum aestivum* L.), whereas it reduced Zn accumulation by 54% compared to the control. But Boldrin et al. [28] indicated that Se supplementation might have a synergistic effect on Zn in rice grains. In terms of cell physiology, selenium undergoes redox reactions and is incorporated in the form of selenocysteine (SeCys) into selenoproteins [29]. Maret (2000) [30] reported that selenium compounds have the potential to increase zinc concentration in whole cells. Therefore, Zn and Se biofortification may be related to the type of experimental crop, application method, climate, and the dose of Zn and Se, thus deserving further discussion.

Foliar application of Zn and Se may also affect the uptake and accumulation of other mineral nutrients in crops, ultimately affecting plant growth and fruit nutritional quality. Narváez-Ortiz et al. [31] reported that the highest level of Se in leaves was accompanied by increases in iron (Fe), copper (Cu), and manganese (Mn) in strawberry roots, while Longchamp et al. [32] demonstrated that a selenate/selenite mixture tended to decrease Mn accumulation in *Zea mays*. Moreover, the effects of Zn and Se fertilizer application on flavor quality have also been reported. Fruit flavor derives from a combination of aroma and taste. The aroma of a fruit primarily depends on volatiles, whereas the taste of the fruit depends on sugars and organic acids [33]. Quddus et al. [1] noted that Zn application significantly affected the total soluble solid (TSS), vitamin C, and total sugar in strawberries. Previous work on tomato fruits showed that foliar Se application maintained fruit quality by reducing ethylene production [34].

Studies on the application of Zn and Se fertilizers have mainly focused on crops. However, the strawberry fruit (*Fragaria ananassa* Duch) is rich in nutrition and has a high edible value. With a supply for up to half a year, strawberries are widely sought after by consumers and have good business prospects [35]. Zn and Se biofortification has been studied relatively less in strawberries. Therefore, in this study, substrate and soil cultivation experiments were conducted to investigate the effects on strawberries resulting from Zn and Se interaction, in particular (1) Zn and Se accumulation; (2) other mineral nutrient content (Fe, Mn, Cu, and molybdenum [Mo]); and (3) fruit flavor quality (ascorbic acid and soluble sugar contents). We anticipate that the findings of this study will provide a reference for the development of fruits that are rich in Zn and Se.

## 2. Materials and Methods

### 2.1. Experimental Design

The experiments were conducted from October 2020 to March 2021 using two types of protected cultivation (substrate and soil) in a suburb of Beijing, China. The physical and chemical properties of each are shown in Table 1. After air-drying and passing through a 20-mesh nylon sieve, both were shaken with boiled deionized water, and the supernatant was used to determine the pH and electrical conductivity (EC). Organic matter (OM) and total N were determined using the volumetric method. Available P was determined using UV-VIS spectrophotometry. Available Zn was determined using extraction with 0.005 mol $L^{-1}$ diethylenetriamine pentaacetic acid (DTPA) and inductively coupled plasma mass spectrometry (ICP-MS 7700; Agilent Technologies, Santa Clara, CA, USA). Available Se was extracted with 0.10 mol $L^{-1}$ potassium dihydrogen phosphate ($KH_2PO_4$) and determined using ICP-MS (ICP-MS 7700; Agilent Technologies).

**Table 1.** Physical and chemical properties of substrate and soil.

| Type | pH | EC (μs) | OM (g $kg^{-1}$) | Total N (g $kg^{-1}$) | Available P (mg $kg^{-1}$) | Available Zn (mg $kg^{-1}$) | Available Se (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|
| Substrate | 7.34 | 807 | 170 | 6.90 | 1370 | 5.13 | 0.003 |
| Soil | 7.47 | 234 | 12.90 | 0.53 | 18.27 | 1.454 | 0.108 |

In this study, a common cultivar (Hongyan) of strawberry (*Fragaria ananassa* Duch) was cultivated. Zn (as zinc sulfate, $ZnSO_4{\cdot}H_2O$) and Se (as sodium selenite, $Na_2SeO_3$) were foliar applied twice during the initial flowering stage (November 2020). Two doses and application modalities (alone and combined) of Zn and Se were set up to form six treatments, and plants sprayed with deionized water served as the control (Table 2). Forty strawberry plants were cultivated in each plot, and each treatment was replicated in three random plots. Foliar Zn and Se were applied uniformly with a watering can, and the fertilizer solutions were applied at 0.3 L per plot each time. During the experiment, strawberries were conventionally cultivated and managed by local workers. Zinc sulfate and sodium selenite were obtained from Sinopharm (Beijing, PRC).

**Table 2.** Layout for Zn and Se fertilizers in the experiment (%, $m/v$).

| Treatment | CK | Zn1 | Zn2 | Se1 | Se2 | Zn1 + Se1 | Zn2 + Se2 |
|---|---|---|---|---|---|---|---|
| $ZnSO_4{\cdot}H_2O$ | 0 | 0.1 | 0.2 | 0 | 0 | 0.1 | 0.2 |
| $Na_2SeO_3$ | 0 | 0 | 0 | 0.003 | 0.006 | 0.003 | 0.006 |

### 2.2. Sample Collection and Analysis

From December 2020 to February 2021, strawberry fruits were harvested in batches after ripening, with a harvest interval of approximately 20 d between each batch. At harvest, five uniform strawberry fruits were selected from each plot and washed three times with deionized water. After being divided into four parts, two opposite angles were selected and weighed to record the fresh weight. One part of the strawberry sample was immediately used to estimate fruit quality, and the other part was freeze-dried for 48 h and crushed using a grinder before being stored in a refrigerator at −20 °C.

To determine the elemental composition (Zn, Se, Fe, Mn, Cu, and Mo) of the strawberry fruits, crushed strawberry samples (0.25 g) were digested overnight in 8 mL of $HNO_3$ (65%; $v/v$) and microwave-digested (Mars 5, CEM, Cridersville, Ohio, USA). The digestion solution volume was made up to 50 mL by adding distilled water. The microelement content in the digest was determined using ICP-MS (ICP-MS 7700; Agilent Technologies). Blank digestion samples and certified reference material (GSB-27; National Institute of Metrology, Beijing, PRC) were included for quality assurance. The elemental recovery of the reference material was 85–110%.

The ascorbic acid (i.e., vitamin C) content was determined using ultraviolet spectrophotometry [36]. Briefly, 10 g of fresh strawberry samples was homogenized in 10 mL of 2% metaphosphoric acid solution and diluted to 50 mL with 2% metaphosphoric acid solution. Then, 0.2 mL of the dilute solution and 9.8 mL of metaphosphoric acid were mixed, and the absorbance was read using an ultraviolet spectrophotometer at a wavelength of 243 nm. The soluble sugar content was determined using anthrone colorimetry [37]. Briefly, 0.20 g of the strawberry homogenate and 10 mL of distilled water were mixed and heated at 80 °C for 50 min. After cooling, the volume of the solution was made up to 50 mL using distilled water. The filtrate and anthrone were mixed, and the absorbance was determined at 620 nm.

### 2.3. Data Analysis

The data are presented as mean ± standard error (SE). One-way analysis of variance (ANOVA) was used to test the significance of differences among the treatments. Statistical analysis and Tukey's test were carried out using SPSS (v. 26.0), where statistical significance was set at $p < 0.05$. Graphs were plotted using SigmaPlot 12.5 and R 4.1.2.

The percentage of Zn/Se accumulation in strawberry fruits in response to foliar application dose was used to evaluate the utilization efficiency. The Zn/Se recovery of strawberry fruits was calculated as follows:

$$E\% = \frac{(C \times Y) - (C_{CK} \times Y_{CK})}{I} \times 100\%$$

where $E\%$ is the strawberry fruits' Zn/Se recovery, $C$ is the Zn/Se content in strawberries after foliar application (mg kg$^{-1}$), $Y$ is the strawberry yield with foliar application (kg plot$^{-1}$), $C_{CK}$ is the Zn/Se content in the control strawberries (mg kg$^{-1}$), $Y_{CK}$ is the strawberry yield of the control (kg plot$^{-1}$), and $I$ is the Zn/Se foliar application dose (mg plot$^{-1}$).

## 3. Results

### 3.1. Effects of Zn and Se Foliar Spraying on Zn and Se Accumulation in Strawberries

Foliar application of Zn and Se had no significant effect on strawberry yield (data not shown). Individual application of Zn and Se affected the Zn and Se contents in strawberries (Figure 1). Under both substrate and soil cultivation, the Zn content significantly increased after foliar Zn treatment and grew with the spraying dose (Figure 1a,b). In the first batch of fresh strawberries grown in the substrate, the Zn content of strawberries after the Zn1 and Zn2 treatments was 1.4 and 2.1 times that of the control, respectively. Under soil cultivation, the Zn content after the Zn1 and Zn2 treatments was 1.3 and 2.0 times that of the control, respectively. Moreover, compared to the first batch of strawberries under substrate cultivation, the Zn content in the second and third batches gradually decreased with time but was always higher than that of the control: the Zn content in the three batches of strawberries treated with Zn increased by 36.9–109%, 12.5–92.0%, and 34.2–108%, respectively. However, no significant differences were found among the Zn treatments in the second and third batches of soil-cultivated strawberries. Therefore, foliar Zn application can increase Zn content in strawberry fruits in a short time, but it cannot ensure long-term sustainability, especially under soil cultivation. In actual production, topdressing with a Zn fertilizer might be applied to improve Zn enrichment.

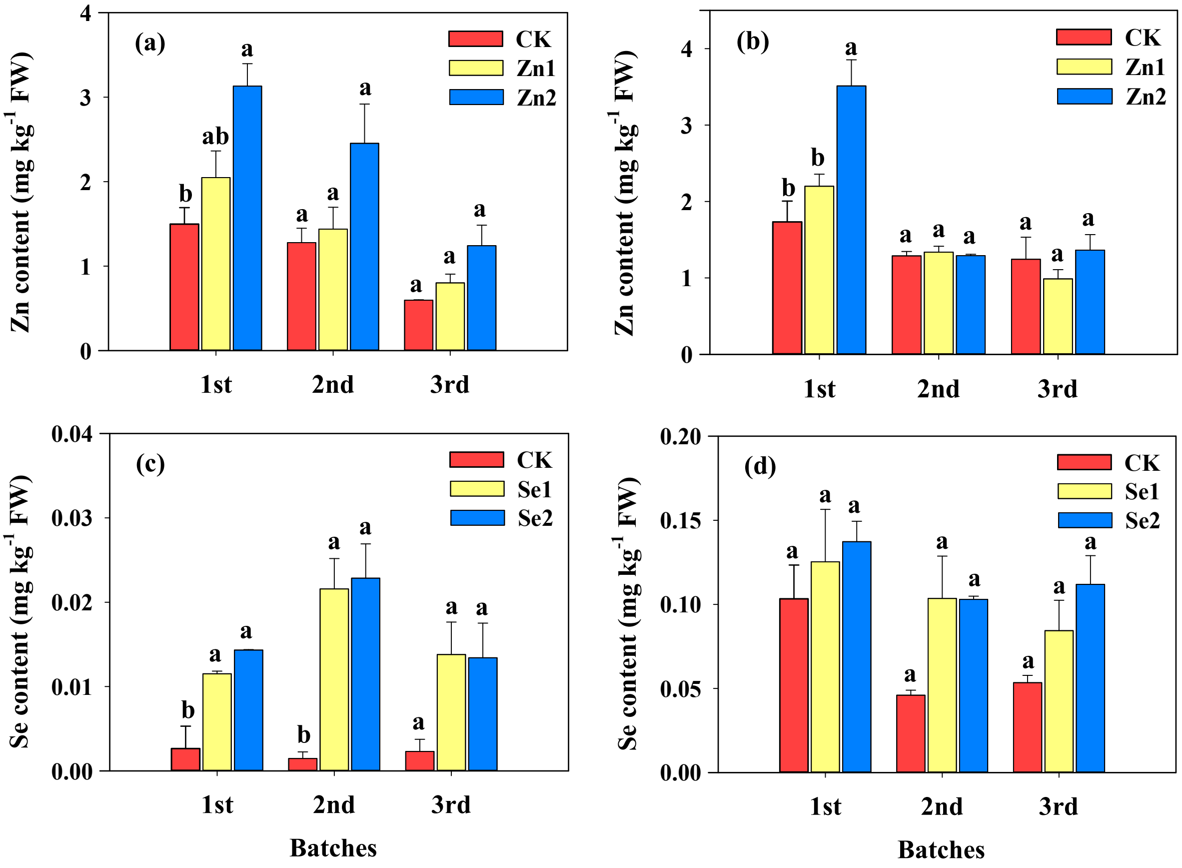

**Figure 1.** Zn and Se contents in different batches of strawberries treated with Zn and Se alone under substrate (**a**,**c**) or soil (**b**,**d**) cultivation. Data are presented as mean + SE ($n = 3$). Different letters above the bars indicate significant differences ($p < 0.05$) among treatments in the same batch.

The Se content in different batches of fresh strawberry fruits grown in substrate and soil was significantly influenced by foliar Se treatment (Figure 1c,d). Foliar selenite application at the initial flowering stage significantly contributed to obtaining a higher Se content in strawberries. Within the experimental dose range, the higher the spraying rate, the higher the content of Se in fresh fruit. In the first batch of substrate-cultivated strawberries, the Se1 and Se2 treatments significantly increased Se content to 0.011 mg kg$^{-1}$ and 0.015 mg kg$^{-1}$, respectively. Under soil cultivation, compared to the control, the Se1 and Se2 treatments increased Se content by 21.3% and 32.9%, respectively. The difference in Se content between the two Se treatments gradually decreased with time. The Se content after the Se1 and Se2 treatments in the third batch of substrate-cultivated strawberries increased to 0.014 mg kg$^{-1}$ and 0.013 mg kg$^{-1}$, respectively. While the Se content in Se-treated strawberries increased and then decreased over time, it was always higher than that of the control. Compared to the control, the Se2 treatment increased Se content in the three batches of substrate-cultivated strawberries by 440%, 1460%, and 482%, respectively, and by 32.9%, 124%, and 109%, respectively, in those grown under soil cultivation. In conclusion, foliar spraying twice with selenite can effectively increase Se content in fresh fruits for a certain period.

According to the Standard for Selenium Content in Selenium-enriched/selenium-containing Foods and Related Products (DB61/T556-2018) [38], fresh fruit with Se content ≥ 0.02 mg $kg^{-1}$ is considered Se-enriched. Under substrate cultivation, the Se content in the second batch of strawberries all reached the Se-rich standard, achieving Se biofortification in strawberries. However, the Se content in the other two batches of strawberries did not reach the level of Se enrichment, requiring a supplementary fertilizer at a later stage. Under soil cultivation, the Se content in the three batches of fresh strawberries all reached the Se-rich standard and achieved healthy Se supplementation, possibly owing to the high availability of Se in the soil.

The Zn content in substrate-grown strawberries treated with combined Zn–Se is shown in Figure 2a. Compared to the Zn1 treatment, in the second and third batches of strawberries, the Zn1 + Se1 treatment increased the Zn content in strawberry fruits by 8.62% and 40.9%, respectively, but the differences were not significant. The Zn content in the three batches of fresh strawberries of the Zn2 + Se2 treatment was 13.2%, 28.9%, and 18.9% lower, respectively, than that in the three Zn2-treated batches. Therefore, low Se application promoted Zn enrichment in fruit over time, while high Se application inhibited Zn enrichment. There were no differences in the Zn content in soil-grown strawberries between the Zn–Se compound and Zn-alone treatments (Figure 2b).

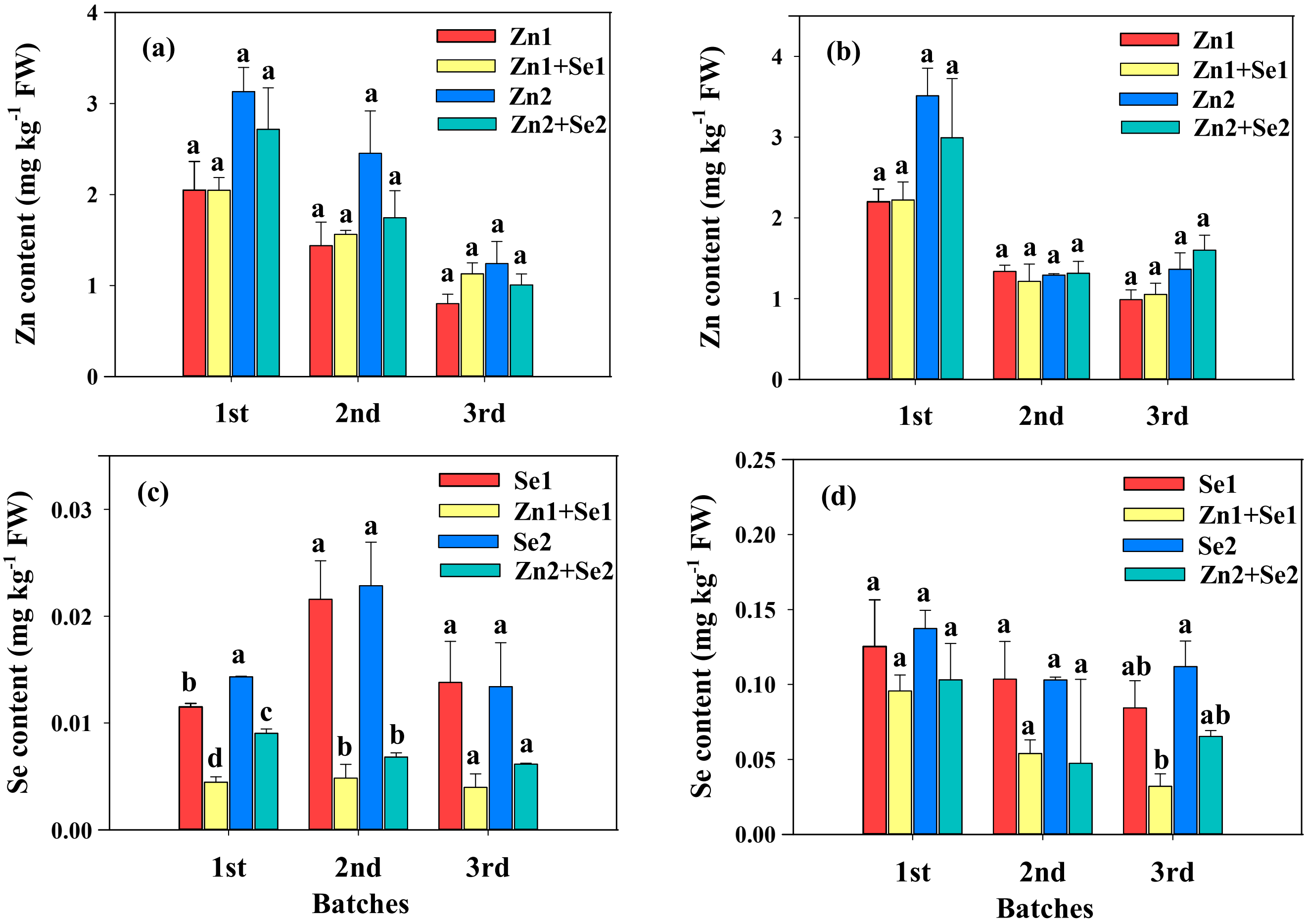

**Figure 2.** Zn and Se contents in different batches of strawberries treated with Zn–Se compound under substrate (**a**,**c**) or soil (**b**,**d**) cultivation. Data are presented as mean + SE ($n$ = 3). Different letters above the bars indicate significant differences ($p$ < 0.05) among treatments in the same batch.

The Se content in strawberries was significantly higher with the Se-alone than with the combined Zn–Se treatments (Figure 2c,d), and Zn had a great impact on Se enrichment in strawberries. In the first batch under substrate cultivation, the Se content after the Zn1 + Se1 and Zn2 + Se2 treatments decreased by 61.2% and 36.9% compared to the Se1 and Se2 treatments, respectively. In the second and third batches, compared to Se alone, additional Zn caused a decrease in Se by 70.2–77.6% and 54.1–71.2%, respectively. Similar results were obtained for strawberries grown under soil cultivation. As shown in Figure 2, the Se content of fresh strawberries treated with Zn–Se compound was 23.7–24.9%, 45.7–47.9%, and 41.6–62.0% lower than that in strawberries treated with Se alone. Thus, compared to the Se-alone treatment, foliar application of Zn–Se compound caused adverse effects on Se biofortification in strawberry fruits; the effect first increased and then decreased over time, showing that Zn may inhibit Se accumulation to a certain extent.

The results of fruit Zn recovery are shown in Table 3. In general, the total Zn recovery in the three batches of strawberries under substrate and soil cultivation ranged from 44.7% to 74.8% and from 10.6% to 18.6%, with an average of 55.7% and 14.4%, respectively. Zn recovery in substrate-cultivated strawberries was higher than that in soil-cultivated strawberries. Regardless of the type of cultivation, the highest Zn recovery was achieved in the first batch of strawberries, compared to the second and third batches. In the first batch of strawberries, Zn recovery was highest with the Zn2 treatment, at 34.1–48.7% and 41.2–89.8% higher than that with other treatments under substrate and soil cultivation, respectively. In addition, under substrate cultivation, Zn recovery in the Zn1 + Se1-treated fruits first decreased and then increased, and was higher in the third batch of strawberries compared to that after the Zn2 treatment. This indicates that Zn–Se compound application with an appropriate dose may improve the sustainability of fruit Zn recovery to a certain extent.

**Table 3.** Fruit Zn recovery as affected by foliar Zn application (%).

| **Treatment** | **Fruit Zn Recovery** | | | | | |
|---|---|---|---|---|---|---|
| | **Substrate Cultivation** | | | **Soil Cultivation** | | |
| | **1st** | **2nd** | **3rd** | **1st** | **2nd** | **3rd** |
| Zn1 | 24.2 ± 0.12 a (A) | 13.1 ± 0.07 a (A) | 8.32 ± 0.04 a (A) | 8.85 ± 0.03 a (A) | 1.72 ± 0.01 a (B) | - |
| Zn2 | 34.2 ± 0.06 a (A) | 27.5 ± 0.11 a (A) | 13.1 ± 0.05 a (A) | 16.8 ± 0.03 a (A) | 0.130 ± 0.00 a (B) | 1.72 ± 0.01 a (B) |
| Zn1 + Se1 | 23.0 ± 0.06 a (A) | 13.3 ± 0.02 a (A) | 21.6 ± 0.05 a (A) | 9.26 ± 0.04 a (A) | 2.63 ± 0.03 a (A) | 0.210 ± 0.00 a (A) |
| Zn2 + Se2 | 25.5 ± 0.10 a (A) | 10.9 ± 0.07 a (A) | 8.33 ± 0.02 a (A) | 11.9 ± 0.07 a (A) | 1.15 ± 0.01 a (A) | 3.14 ± 0.02 a (A) |

Note: Data are presented as mean ± SE ($n$ = 3). Different lower-case letters within the same column indicate significant differences ($p < 0.05$) among treatments in the same batch. Different capital letters indicate significant differences ($p < 0.05$) between different batches under the same treatment.

Table 4 shows Se recovery in different batches of strawberries, as affected by different Se treatments. The total fruit Se recovery in the three batches under substrate and soil cultivation ranged from 10.5% to 60.6% and from 8.32% to 81.1%, with an average of 28.9% and 38.0%, respectively. In contrast to Zn recovery, Se recovery in the second batch was higher than that in the first and third batches. In addition, in the three batches of strawberries, Se recovery was highest in the Se1 treatment, at 5.14–6.52 times and 5.74–10.5 times higher than that in the other treatments under substrate and soil cultivation, respectively. Compared to Se alone, Zn–Se compound application caused a significant decrease in Se recovery. In the second batch of strawberries cultivated in substrate and soil, Se recovery after the Zn2 + Se2 treatment was 75.0% and 82.4% lower, respectively, than that after the Se2 treatment. This indicates that Zn can inhibit effective Se recovery in fruit to a certain extent.

**Table 4.** Fruit Se recovery as affected by foliar Se application (%).

| Treatment | Fruit Se Recovery | | | | | |
|---|---|---|---|---|---|---|
| | Substrate Cultivation | | | Soil Cultivation | | |
| | 1st | 2nd | 3rd | 1st | 2nd | 3rd |
| Se1 | 12.6 ± 0.00 a (A) | 32.1 ± 0.06 a (A) | 15.9 ± 0.05 a (A) | 18.1 ± 0.18 a (A) | 43.8 ± 0.19 a (A) | 19.2 ± 0.10 a (A) |
| Se2 | 8.32 ± 0.00 b (A) | 17.1 ± 0.03 ab (A) | 7.68 ± 0.03 a (A) | 10.9 ± 0.04 a (A) | 21.7 ± 0.01 a (A) | 17.4 ± 0.05 a (A) |
| Zn1 + Se1 | 2.58 ± 0.01 c (A) | 5.40 ± 0.02 b (A) | 2.55 ± 0.02 a (A) | 1.70 ± 0.02 a (A) | 6.62 ± 0.07 a (A) | - |
| Zn2 + Se2 | 4.55 ± 0.00 c (A) | 4.27 ± 0.00 b (A) | 2.66 ± 0.00 a (B) | 5.14 ± 0.05 a (A) | 3.81 ± 0.02 a (A) | 3.53 ± 0.01 a (A) |

Note: Data are presented as mean ± SE ($n$ = 3). Different lower-case letters within the same column indicate significant differences ($p < 0.05$) among treatments in the same batch. Different capital letters indicate significant differences ($p < 0.05$) between different batches under the same treatment.

### 3.2. Effects of Zn and Se Interaction on Mineral Element Accumulation in Strawberries

The trace mineral contents (including Fe, Mn, Cu, and Mo) in the first batch of strawberries are shown in Figure 3. As can be seen from the figure, compared to the control, a reduction in Fe content was observed in all treated strawberries, but the difference was not significant. The Se2 treatment decreased the Fe content by 26.8% in strawberries under substrate cultivation, and the Zn2 treatment decreased it by 30.3% under soil cultivation (Figure 3a,b). In the first batch, treatments had no significant effect on the Mn content. Under soil cultivation, foliar Zn and Se application decreased Mn levels in strawberries compared to the control, while under substrate cultivation, the Se2 and Zn2 + Se2 treatments resulted in an increase in Mn content by 17.9% and 10.7%, respectively (Figure 3c,d). The Cu content in Se1- and Se2-treated strawberries under substrate cultivation was significantly higher than that in the control, with a 75.0% and 66.7% difference, respectively. No significant differences were detected among the other treatments (Figure 3e,f). The Mo content in strawberries did not change significantly with the exogenous application of Zn and Se. Under substrate cultivation, higher Mo content in strawberry fruits was achieved with Zn–Se compound application. Compared to the control, the Zn2 + Se2 treatment increased the Mo content by 38.5%. Under soil cultivation, the Se2 treatment increased the Mo content by 20.0%. (Figure 3g,h).

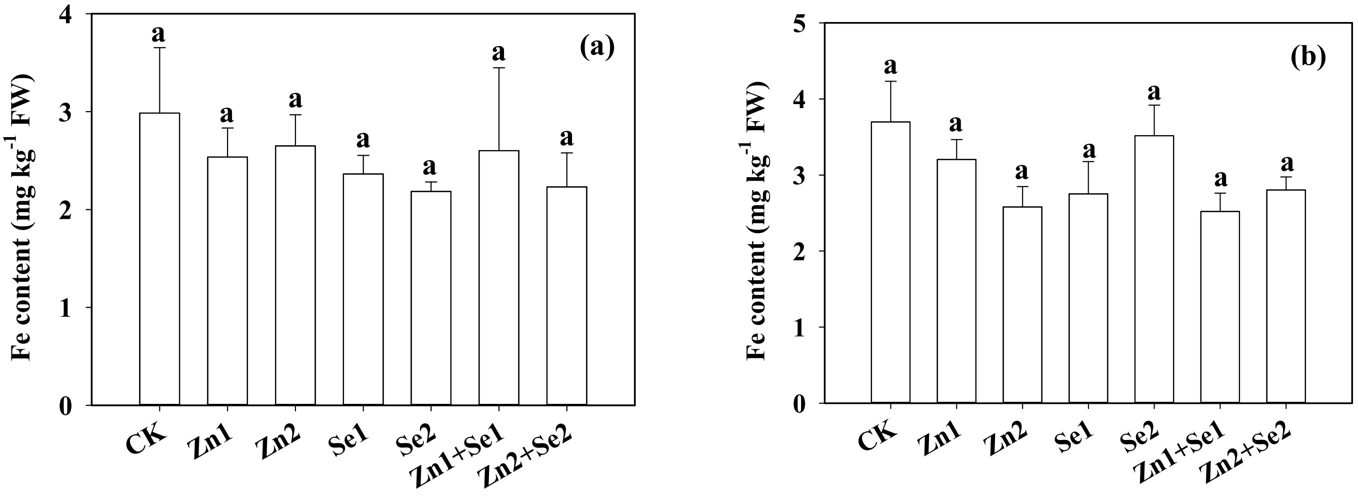

**Figure 3.** *Cont.*

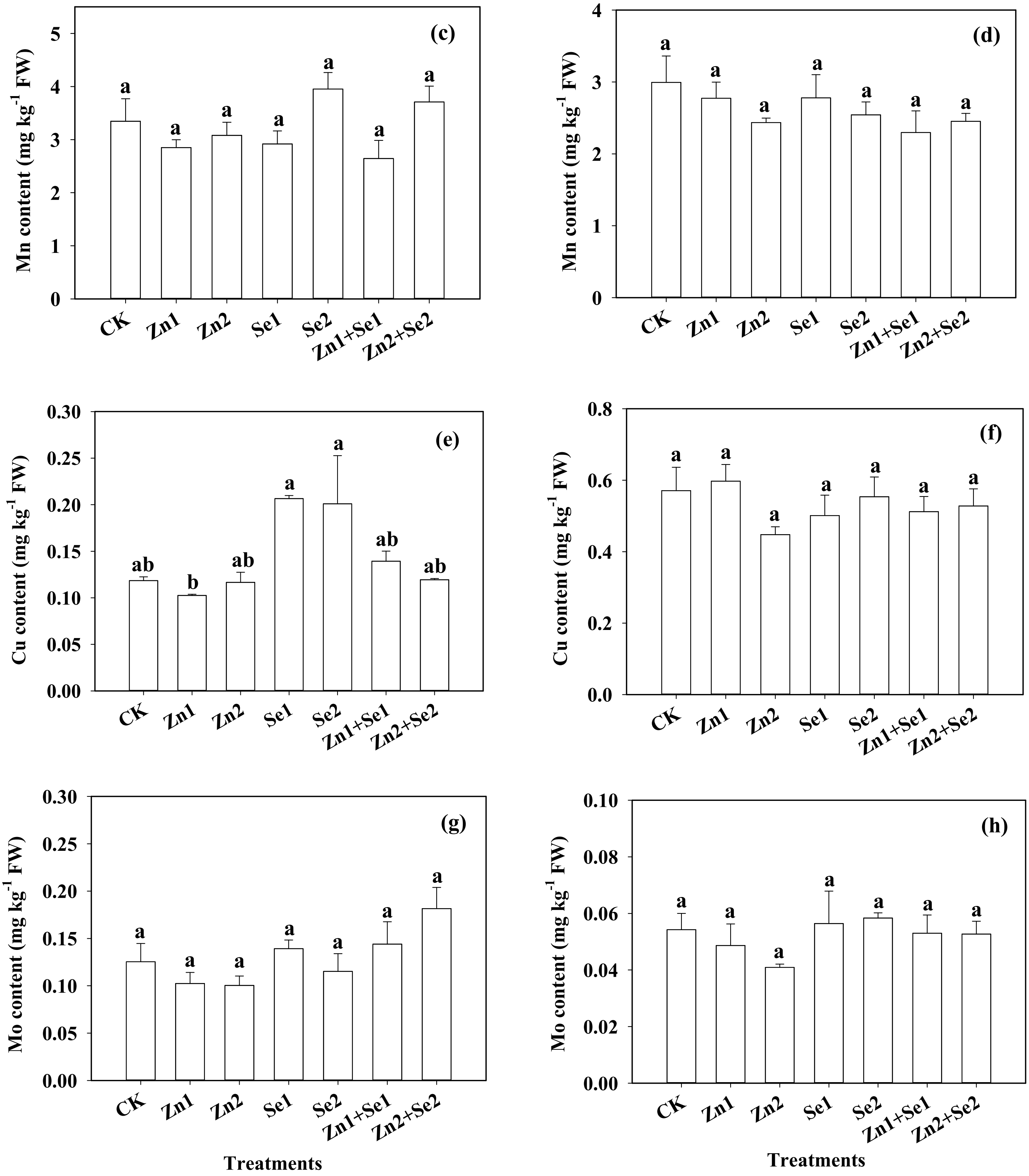

**Figure 3.** Mineral contents in the first batch of strawberries under substrate (**a**,**c**,**e**,**g**) or soil (**b**,**d**,**f**,**h**) cultivation. Data are presented as mean + SE ($n$ = 3). Different letters above the bars indicate significant differences ($p < 0.05$) among treatments.

The results of the correlation analysis indicate that, under substrate cultivation, the Cu content in the second batch of strawberries exhibited a significant positive correlation with Zn and Se contents ($p < 0.05$, Figure 4c). In addition, in the second and third batches, the

Zn content was significantly positively correlated with the Se content ($p < 0.05$, Figure 4c,e). Under soil cultivation, a positive correlation was observed between Cu and Zn contents in the third batch of strawberries ($p < 0.05$, Figure 4f). For other minerals, no clear pattern linked to the effect of Zn and Se was observed ($p < 0.05$, Figure 4a,b,d).

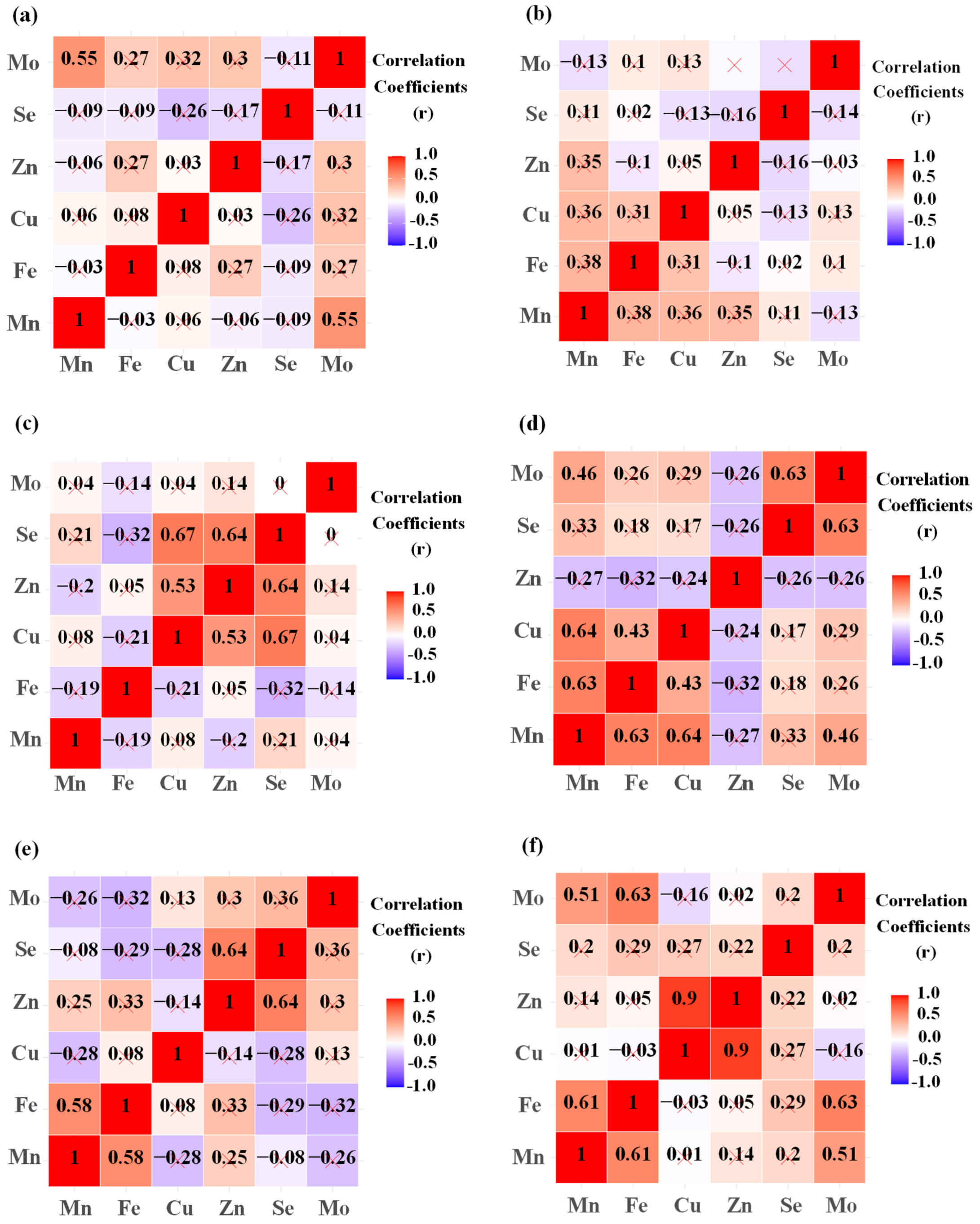

**Figure 4.** Correlation analysis of Zn, Se, and mineral contents in the first, second, and third batches of strawberries under substrate (**a**,**c**,**e**) or soil (**b**,**d**,**f**) cultivation. The red cross indicates the correlation is not significant ($p > 0.05$), $n = 21$.

### 3.3. *Effects of Zn and Se Interaction on Strawberry Fruit Flavor Quality*

The foliar Zn and Se application demonstrated a positive effect on ascorbic acid content in strawberries (Figure 5a,b). Under substrate cultivation, the highest ascorbic acid content in fruits was observed with the Se2 treatment, increasing by 37.2% compared to the control.

Under soil cultivation, the Zn2 + Se2 treatment caused a 23.9% increase in ascorbic acid content, whereas the Zn2 treatment led to a 21.0% decrease compared to the control.

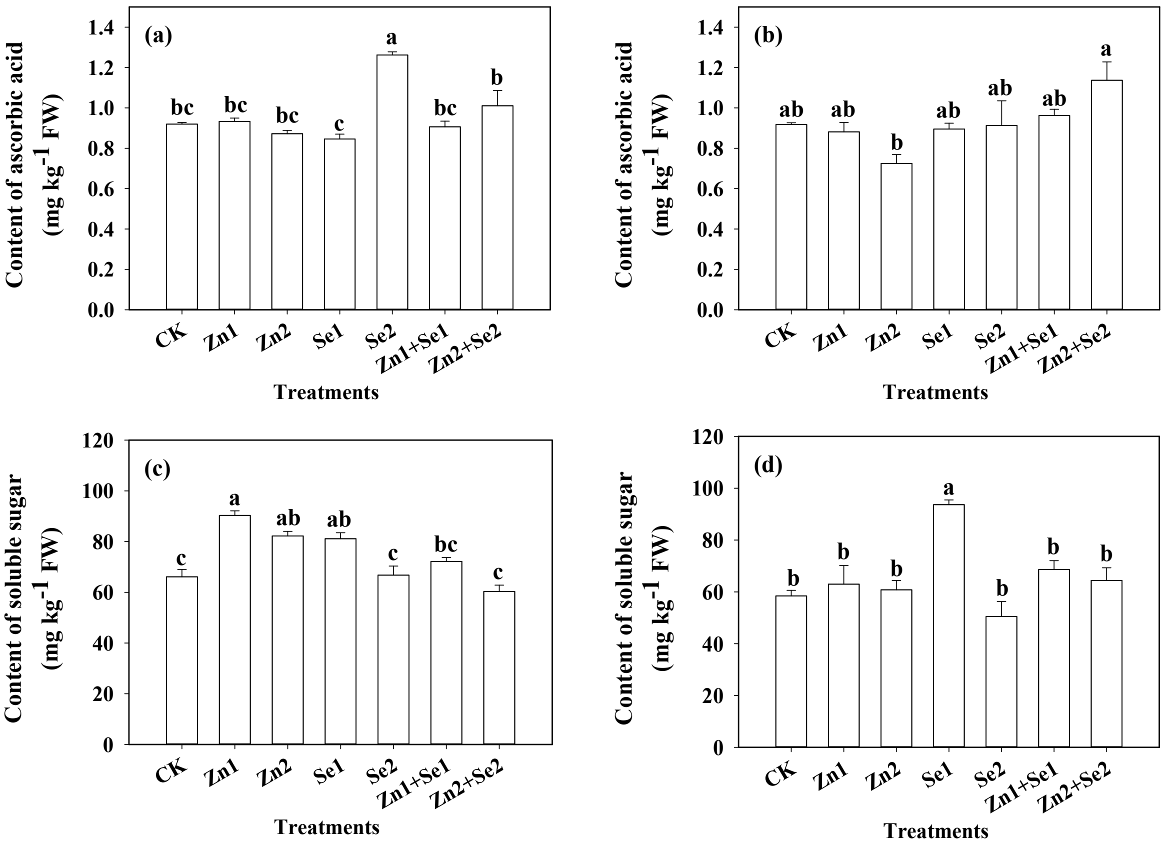

**Figure 5.** Ascorbic acid and soluble sugar contents in the first batch of strawberries under substrate (**a**,**c**) or soil (**b**,**d**) cultivation. Data are presented as mean + SE (*n* = 3). Different letters above the bars indicate significant differences ($p < 0.05$) among treatments.

The foliar Zn and Se application at an appropriate dose contributed significantly to the increase in soluble sugar content in strawberry fruits (Figure 5c,d). Under substrate cultivation, the highest content of soluble sugar was recorded with the Zn1 treatment, increasing by 36.7% compared to the control. The Zn2 and Se1 treatments also contributed to a higher soluble sugar content in strawberries, with a 24.4% and 22.7% increase, respectively. Individual foliar Zn and Se application showed a better effect on increasing the soluble sugar content in strawberry fruits than the Zn–Se compound application. Under soil cultivation, the highest content of soluble sugar was found with the Se1 treatment, which was a significant 60.1% increase compared to the control. No significant differences were observed among the other treatments.

## 4. Discussion

In the present study, foliar application of Zn and Se rapidly and significantly increased Zn and Se contents in strawberry fruits (Figure 1). Similar results were reported by Sharifan et al. [6] and Xia et al. [25], who demonstrated that foliar Zn application increased the Zn content and bioavailability in rice and maize grains. Numerous studies also confirmed that foliar application of Se effectively increased the Se content in the edible part of crops, such as in grape berry [22] and tomato fruit [34]. However, in the present study, foliar Zn application could not ensure long-term sustainability, which is partly supported by Saini et al. [39]. In their study, $ZnSO_4$ showed a shorter persistence of available Zn supply on strawberries than nano-ZnO, owing to its high solubility and low retention period in plant systems. Thus, in practical production, subsequent topdressing with a Zn fertilizer can be considered to achieve long-term sustainability of Zn supply. In addition, the second batch of substrate-cultivated strawberries treated with Se can be considered Se-rich fruits (DB61/T556-2018). Thus, foliar Se application achieves Se-biofortified strawberry, which can be regarded as a Se supplement for humans. Within the experimental dose range, a higher spraying dose tended to increase the Se content in fresh fruits. However, some studies have reported that elevated Se levels can have toxic effects on plant growth and development [40,41]. Deng et al. [42] also reported that fruit growth and appearance of pears (*Pyrus communis* L.) were adversely affected by high Se doses. Thus, Se has a dual effect on plants and its application requires attention in practical production.

In our study, compared to individual Se treatment, the Zn–Se combined application hindered Se biofortification in strawberries (Figure 2). In addition, low doses of applied Se promoted Zn enrichment in strawberries, which was instead inhibited by the application of high Se doses. These results are in agreement with those of Ning et al. [43], who reported that foliar application of a Zn–Se compound reduced Se density compared to Se alone, reflecting an antagonistic effect of Zn on Se biofortification in wheat. However, Germ et al. [44] showed that foliar Zn–Se compound application increases Se content in wheat grains more effectively than Se alone. Different crop types and Zn–Se application forms and rates may result in inconsistent results. Since Se and sulfur (S) have chemical and physical similarities with regard to plant uptake, Zn might influence the expression of sulfate transporters in treated plants and ultimately affect Se absorption [45]. Further studies are needed to explain the effects of Zn–Se compound application on Zn and Se biofortification in strawberries.

Fruit Zn and Se recovery was used to evaluate biofortification efficiency and fertilizer utilization rate. In the present study, the highest Zn recovery was achieved with the Zn2 treatment in the first batch, and the highest Se recovery was achieved with the Se1 treatment in the second batch. The average Zn recovery under substrate cultivation (55.7%) was higher than that under soil cultivation (14.4%). However, the average Se recovery under substrate cultivation (28.9%) was lower than that under soil cultivation (38.0%), showing the opposite trend. These results indicate that Zn and Se absorption in strawberries was significantly affected by the properties of the growth medium. One reason for the low Se recovery may be that sodium selenite is difficult to be absorbed by plants [46]. Thus, crop species, method of application, dosage, and soil conditions all affect Zn and Se recovery [21].

Micronutrients play a crucial role in plant and human health and participate in life-sustaining processes. Our work mainly highlighted the effect of foliar Zn and Se application on Fe, Mn, Cu, and Mo contents in strawberries. Fe is involved in respiration and photosynthesis [47], and Mo is necessary for nitrogen and carbon assimilation [48] and might also contribute to the production of volatile metabolites [33]. Moreover, Cu plays a central role in respiration, photosynthesis, and antioxidant activity [49]. In the present study, the Fe and Mn contents in strawberries tended to decrease with foliar Zn application, but the difference was not significant (Figure 3). Zhao et al. [50] reported that Zn supplementation reduced the Fe content in wheat by an average of 8% but had no significant effect on that of Cu and Mn. Niyigaba et al. [51] also found that high quantities of a Zn spray fertilizer were not ideal for increasing wheat grain Fe. The antagonism between Zn and Fe, Mn, and Cu might be partly due to the competition for transport proteins. It is thought that ZRT-, IRT-like proteins (ZIPs), and/or yellow stripe-like proteins (YSLs) are responsible for transporting not only Zn but also Fe, Mn, and Cu [52]. Further research is needed to understand the interactions at the physiological and molecular levels. In the present study, the Cu content in substrate-cultivated strawberries treated with Se1 and Se2 was significantly higher than that in the control (Figure 3e). In addition, under soil cultivation, a higher Mo content in strawberries was achieved through Se application (Figure 3h), but the difference was not significant. The metabolic pathway of Se modification is cellular redox balance variation involved in antioxidant synthesis [53]. Depending on the Se content, an antioxidant response can be induced, thereby modifying the activity of superoxide dismutase enzymes, which could relate to the variability in the content of mineral elements [54].

Mineral nutrients may also be linked to the Zn and Se tolerance of different plant species, which needs to be investigated in further research.

The quality of fruit flavor mainly depends on sugars, acids, and other aromatic compounds [55]. Ascorbic acid is closely correlated with the antioxidant power of fruits [35]. The content of ascorbic acid increases as strawberries ripen [56]. Soluble sugar is an important index of fruit quality [57], and sugars also influence flavor characteristics, including pigments and aromatic compounds [34]. In the present study, proper application of Se tended to increase the ascorbic acid content in strawberries, whereas foliar Zn application had no significant effect. Low Zn and Se doses promoted the content of soluble sugar in strawberries, whereas excessive Zn and Se inhibited it. Al-Obeed et al. [58] reported that the ascorbic acid content in mandarin fruit was significantly influenced by foliar application of Zn. Moreover, Zhu et al. [34] found that foliar Se application increased the contents of soluble sugar and ascorbic acid in pink tomato fruit. In actual production, attention should be paid to the dosage of Zn and Se to ensure that plant growth is not hindered.

## 5. Conclusions

Biofortification is an effective agricultural process to increase Zn and Se intake for humans. In the present study, individual Zn foliar application increased the Zn content in strawberries in a short time, but it could not ensure long-term sustainability. Individual Se application also contributed significantly to obtaining a high Se content in strawberries. The second batch of substrate-cultivated strawberries treated with Se can be considered Se-rich fruits (DB61/T556-2018). However, the combined Zn–Se application caused adverse effects on Se biofortification in strawberries compared to Se alone. Additionally, a low dose of Se application promoted Zn enrichment in fruits over time, whereas a high dose of Se application inhibited it. In terms of fruit nutritional quality, the Cu content in Se-treated strawberries under substrate cultivation was significantly higher than that in the control, and a positive correlation was observed between Cu and Zn contents in strawberries. In addition, Zn and Se application at an appropriate dose contributed significantly to an increase in ascorbic acid and soluble sugar contents in strawberry fruits. Thus, foliar application achieves Zn and Se enrichment in strawberries and can be regarded as a method for supplementing Zn and Se for humans.

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