Impact of the use of essential trace elements (zinc, copper) on the chemical composition of perennial medicinal plants in southern Western Siberia

N N Zharkova¹, V V Suhotskaya² and Yu I Ermohin²

¹ Department of Ecology, Nature Management and Biology, Omsk State Agrarian University named after P.A. Stolypin, 1, Institutskaya square, Omsk, 644008, Russia
² Department of Agrochemistry and Soil Science, Omsk State Agrarian University named after P.A. Stolypin, 1, Institutskaya square, Omsk, 644008, Russia

E-mail: nn.zharkova@omgau.org

Abstract. The studies were conducted in 2012-2018 on meadow chernozem soil in the conditions of the south of Western Siberia. The purpose of the study was to study the effect of zinc and copper fertilizers on the chemical composition of perennial medicinal plants in the zonal soil-climatic conditions of Western Siberia. The objects of study were Achillea millefolium L., Tanacetum vulgare L. and Echinacea purpurea L. Zinc and copper fertilizers were applied in MAC fractions: 0.25, 0.50, 0.75, and 1.0 on the grounds N135P45K45 (A. millefolium, T. vulgare) and N125 (E. purpurea). The use of zinc and copper fertilizers in the main application for the studied medicinal crops contributed to an increase in the accumulation of mobile forms of zinc and copper in the medicinal raw materials. On average, over the years of research in experiments with A. millefolium, T. vulgare, E. purpurea, each kg of Zn fertilizers increased the Zn content in plants by 0.30 and 0.38, Cu by 0.07, 0.05 and 0.03 mg / kg; while 1 kg of fertilizer added by Cu increased the Cu content by 0.47, 0.07 and 0.23, Zn by 1.40, 2.32 and 1.26 mg / kg, respectively. A synergistic relationship has been established between the elements of Zn↔Cu and Cu↔Zn.

1. Introduction

Recently, a comprehensive approach has been taken to the problem of plant nutrition, which includes determining the need of plants for nutrition, setting doses of fertilizers, timing and methods of their application in specific soil-climatic conditions. All the above cannot be solved only by soil analysis, so an important addition to it is the method of plant diagnostics [1-3].

Chemical analysis of plants is used to diagnose mineral nutrition by the phases of crop development. Plants in different periods of ontogenesis impose certain requirements on the supply of nutrients involved in the synthesis of dry matter, therefore, each species is characterized by certain levels of optimal nutrition and the ratio of substances [4].

In recent years, about 80% of all pharmaceutical preparations produced have been obtained directly or indirectly from medicinal plants [5], in connection with this it is necessary to study their chemical
composition from physiological and biochemical positions, including not only quality indicators, but also trace elements.

Data on the effect of zinc and copper on the elemental composition of medicinal plants (*Achillea millefolium* L., *Tanacetum vulgare* L., *Echinacea purpurea* L.) are practically single [6-7], so these cultures were selected as subjects of research.

The work presents for the first time the results confirming the role of copper and zinc fertilizers in the biological enrichment of medicinal raw materials, as well as the optimal levels and ratios of elements in nutrition of medicinal plants.

The aim of the study is to investigate, on the basis of experimental data and expert methods, the influence of zinc and copper fertilizers on the chemical composition of perennial medicinal plants (thousand-year-old common, pyjma common, echinacea purple) in the zonal soil-climatic conditions of Western Siberia.

### 2. Materials and methods

The field experiments of 2012-2018 (Omsk, the Omsk Region) studied influence of zinc and copper fertilizers on the maintenance of individual minerals (Zn, Cu) in plants *Achillea millefolium* (L.), *Tanacetum vulgare* (L.), *Echinacea purpurea* (L.).

The objects of research were *Achillea millefolium* variety White Beauty, *Tanacetum vulgare* variety Luck, *Echinacea purpurea* variety Znahar and meadow-black soil.

Microfield experiments with perennial medicinal cultures with *Achillea millefolium* (experiment 1), *Tanacetum vulgare* (experiment 2), *Echinacea purpurea* (experiment 3) were laid down according to the schemes shown in Table 1.

| Experiment | Area (m²) | Fertilizer | Zinc (mg/kg) | Copper (mg/kg) |
|------------|-----------|------------|--------------|----------------|
| 1          | 200       | Zn, Cu     | 20-30        | 1-2            |
| 2          | 50        | Zn, Cu     | 0.5          | 0.1            |
| 3          | 10        | Zn, Cu     | 0.05         | 0.005          |

Due to the insufficient content of macronutrients in the soil, micronutrients were introduced taking into account the optimization of nutrition according to N-P-K on the grounds of N_{135}P_{37}K_{15} and N_{125}. Doses of zinc and copper fertilizers were calculated based on Zn MAC (23 mg/kg) and Cu (3 mg/kg) and their pre-planting content. Micro-samples were introduced in doses (kg/ha) in MAC fractions: 0.25; 0.5; 0.75; 1.0.

Replication - 4-fold, sequence of variants - systematic in one tier (experiments 1-2) and several tiers (experiment 3). The area of the divisions was 10 m². Zinc and copper acetate (CH₃COO)₂Zn 29.7%; (CH₃COO)₂Cu – 32%), ammonium nitrate (N – 34.4%), double superphosphate (P₂O₅ – 37%) and potassium chloride (K – 60%) were added to the ground [8].

Agricultural machinery in the experiment was that common for this zone, Medicinal plants were planted in May 2012 and 2016. Fertilizers were introduced into the soil manually in rows (experiments 1-2) and in a scattered manner with attachment to a depth of 10-15 cm before planting the culture (experiment 3).

Plant samples were taken from the test variants in the mass flowering phase. The content of mobile zinc and copper forms in samples ground to powder state was determined by atomic absorption method on a Varian AA-140 spectrometer as per GOST 30178-96.

Statistical processing of experimental data included calculation of mean (M) and standard mean (± SEM) errors, correlation and regression analysis. The validity of the differences was estimated from the smallest significant difference at a 5% significance level. Correlation coefficients (r) were calculated to determine the relationships between the indicators studied. Values of correlation coefficients at p < 0.05 were considered valid. Standard Microsoft Office Excel 2007 and STATISTICA 6.0 software packages were used for statistical data processing.
3. Results and discussion

Table 1 shows the average multi-year data of the content of trace elements (zinc and copper) in the flowering phase in plants of yarrow, common pyjma and purple echinacea.

**Table 1.** Content of copper and zinc in medicinal plants in mass flowering phase, mg/kg (M±SEM)

| Variant                      | Copper | Zinc  |
|------------------------------|--------|-------|
| **Achillea millefolium (2012-2015)** |        |       |
| Control                      | 2.5±0.15 | 9.9±0.69 |
| N_{132}P_{45}K_{45} (Ground) | 2.2±0.15 | 11.3±0.69* |
| Ground + Zn_{20} (0.25 MAC)  | 4.8±0.08* | 22.3±0.11* |
| Ground + Zn_{40} (0.5 MAC)   | 5.4±0.26* | 26.5±2.49* |
| Ground + Zn_{60} (0.75 MAC)  | 6.4±0.83* | 29.8±4.36 |
| Ground + Zn_{80} (1.0 MAC)   | 8.3±1.90  | 32.8±6.05 |
| Ground+ Cu_{2.3} (0.25 MAC)  | 2.9±0.57* | 22.7±1.64* |
| Ground + Cu_{4.8} (0.5 MAC)  | 3.9±0.001*| 23.3±1.98* |
| Ground + Cu_{7.0} (0.75 MAC) | 5.1±0.68* | 25.3±3.11* |
| Ground + Cu_{9.2} (1.0 MAC)  | 6.8±0.64* | 26.3±3.68* |
| **Tanacetum vulgare (2012-2015)** |        |       |
| Control                      | 4.23±0.06 | 17.28±0.12 |
| N_{132}P_{45}K_{45} (Ground) | 4.35±0.06* | 17.03±0.12 |
| Ground + Zn_{20} (0.25 MAC)  | 5.67±0.26* | 25.80±2.00* |
| Ground + Zn_{40} (0.5 MAC)   | 6.5±0.20*  | 28.33±0.58* |
| Ground + Zn_{60} (0.75 MAC)  | 7.73±0.90  | 39.65±5.80 |
| Ground + Zn_{80} (1.0 MAC)   | 8.33±1.24  | 48.00±10.00 |
| Ground + Cu_{2.4} (0.25 MAC) | 4.28±0.24* | 25.33±1.07* |
| Ground + Cu_{4.9} (0.5 MAC)  | 4.48±0.13* | 29.33±1.19* |
| Ground + Cu_{7.8} (0.75 MAC) | 4.88±0.10* | 33.67±3.65* |
| Ground + Cu_{10.0} (1.0 MAC)| 6.00±0.73* | 40.70±7.63 |
| **Echinacea purpurea (2016-2018)** |        |       |
| Control                      | 2.1±0.41  | 4.4±0.09 |
| N_{125} (Ground)             | 2.1±0.41  | 4.6±0.09* |
| Ground + Zn_{10.7} (0.25 MAC)| 2.1±0.41  | 11.1±0.39* |
| Ground + Zn_{21.4} (0.5 MAC) | 2.7±0.07* | 12.7±1.29* |
| Ground + Zn_{32.4} (0.75 MAC)| 3.5±0.39* | 13.9±1.97 |
| Ground + Zn_{42.8} (1.0 MAC) | 4.4±0.89  | 15.8±3.04 |
| Ground + Cu_{3.3} (0.25 MAC) | 2.6±0.17* | 9.9±0.42* |
| Ground + Cu_{4.7} (0.5 MAC)  | 2.8±0.06* | 13.3±1.50* |
| Ground + Cu_{7.0} (0.75 MAC) | 3.5±0.34* | 14.6±2.23* |
| Ground + Cu_{9.4} (1.0 MAC)  | 4.3±0.79  | 17.1±3.65 |
| MAL                          | 50      | 30    |

* differences between experimental and control and background variants are statistically significant at p <0.05.

According to the table 1, fig. 1 introduction of doses of Zn_{20.80} for medicinal crops Achillea and Tanacetum vulgare on average for 2012-2015 leads to an increase in the zinc content in plants compared to the background version by 11.0...21.5 mg / kg (Achillea) and 8.77...30.97 mg / kg (Tanacetum). When the calculated doses of Cu_{2.3-10.0} (0.25-1.0 MAC) were introduced, its concentration in plants increased to 6.8 mg / kg or 4.6 mg / kg (Achillea) and to 6.0 mg / kg or 1.65 mg / kg (Tanacetum).
A similar situation arises in research with echinacea purple. When the doses of zinc and copper fertilizers are increased, the content of zinc in plants increases by 11.2 mg/kg (Zn\textsubscript{10.7-42.8}), copper by 2.2 mg/kg (Cu\textsubscript{2.3-9.4}).

Evaluation of medicinal raw materials of studied cultures from ecological positions made it possible to determine the absence of excess of maximum-permissible level (MAL) in terms of Zn and Cu content (table 1).

According to the agrochemical and biogeochemical criteria [9], the zinc content in *Achillea millefolium* and *Tanacetum vulgare* when applying Zn fertilizers to the soil is estimated as normal (limit of 21-60 content), as insufficient in *Echinacea purpurea* (limit of content < 20). At introduction of copper, its content in studied plants is characterized as insufficient (limit of content < 5) and normal (limit of content of 6-12).

According to the results of field experiments carried out in the soil-climatic conditions of the Omsk region, it was revealed that *T. vulgare* is a concentrator of Zn and Cu among studied medicinal crops.

Studies have led to the construction of a number of medicinal crops on their ability to accumulate trace elements: Zn/Cu: *Tanacetum vulgare > Achillea millefolium > Echinacea purpurea*.

Our research has established that *Tanacetum vulgare* is a natural ecological bioindicator of trace elements accumulation, in particular zinc and copper, which is confirm by other researchers [10-11].

The data of table 1 revealed the relationships between Zn and Cu in the nutrition of medicinal plants when microelements were introduced into the soil (table 2).

It was established that medicinal plants are responsive to the introduction of trace elements, which is reflected in the identified relationships in the system "dose - chemical composition of plants" (r = 0.85-0.99). Each kg of zinc fertilizers ("b"\textsubscript{xy}) increased the content of mobile zinc in the medicinal crops by 0.30 ... 0.38 mg / kg, and 1 kg of copper by 0.07 ... 0.23 ... 0.49 mg / kg (table 2).

In addition, the obtained action intensity coefficients ("bov") made it possible to establish not only direct influence, but also feedbacks between Zn → Cu. In a series of field experiments with perennial medicinal plants, it was revealed that copper fertilizers increase the zinc content to a greater extent than even when zinc fertilizers were added. The intensity coefficient of the action “b” of the introduced copper on the zinc content in plants varied from 1.29 (*Echinacea purpurea*), 1.40 (*Achillea millefolium*) to 2.32 (*Tanacetum vulgare*). Zinc fertilizers to a lesser extent led to an increase in the copper content in plants - 0.03-0.07 mg / kg. From which it follows that copper fertilizers increase not only the copper content in plants, but also zinc, which helps to reduce the anthropogenic load on the soil when fertilizing.
Table 2. Relationships between microelement doses and zinc and copper in plants

| Regression equation | The coefficient of intensity of action "b" | Correlation coefficient (r) |
|---------------------|------------------------------------------|-----------------------------|
| **Achillea millefolium (2012-2015)** | | |
| y(Zn) = 0.30 Zn + 13.5; | (1) | 0.30 | r = 0.96 |
| y(Zn) = 1.40 Cu + 15.3; | (2) | 1.40 | r = 0.85 |
| y(Cu) = 0.48 Cu + 1.9; | (3) | 0.48 | r = 0.99 |
| y(Cu) = 0.07 Zn + 2.7; | (4) | 0.07 | r = 0.95 |
| **Tanacetum vulgare (2012-2015)** | | |
| y(Zn) = 0.38 Zn + 16.6; | (5) | 0.38 | r = 0.99 |
| y(Zn) = 2.32 Cu + 18.1; | (6) | 2.32 | r = 0.99 |
| y(Cu) = 0.07 Cu + 4.0; | (7) | 0.07 | r = 0.88 |
| y(Cu) = 0.05 Zn + 4.5; | (8) | 0.05 | r = 0.99 |
| **Echinacea purpurea (2016-2018)** | | |
| y(Zn) = 0.38 Zn + 5.4; | (9) | 0.38 | r = 0.94 |
| y(Zn) = 1.26 Cu + 6.0; | (10) | 1.26 | r = 0.97 |
| y(Cu) = 0.23 Cu + 2.0; | (11) | 0.23 | r = 0.99 |
| y(Cu) = 0.03 Zn + 2.0; | (12) | 0.03 | r = 0.87 |

The obtained regression equations (equations 1-12 in Table 2) and the action intensity factors “b” allow us to calculate the optimal content of Zn and Cu in medicinal plants in the flowering phase according to equation 13:

\[ \text{Copt} = Cn + D \cdot "b" \]  

(13)

where: \( Cn \) is the actual content of trace elements in plants of the background variant, mg / kg; \( D \) is the optimal microelement dose in experiments with fertilizers, kg / ha; \( "b" \) is the coefficient of intensity of action mg / kg.

Using mathematical equation 1, we predict the optimal level of Zn and Cu in *Achillea millefolium, Tanacetum vulgare* and *Echinacea purpurea* plants during the flowering phase (table 3).

The obtained calculated optimal levels of zinc and copper in plants were close to the actual content in plants on the best experimental options. Thus, equations 1-2 and the coefficients \( "b" \) allow us to determine the optimal concentration and balanced balance of nutrients in medicinal plants during the flowering phase (table 3).

Table 3. Optimum balanced zinc and copper content in medicinal plants during the flowering phase

| Culture             | Element | Content, mg / kg | Forecast Error, % | Balanced ratio Zn/Cu |
|---------------------|---------|-----------------|-------------------|----------------------|
| *Achillea millefolium* | Zn      | 29.8            | -1.7              | Zn ≈ 4.3 Cu          |
|                     | Cu      | 6.8             | 6.8               | Zn ≈ 4.3 Cu          |
| *Tanacetum vulgare*  | Zn      | 39.7            | +0.3              | Cu ≈ 8.1 Cu          |
|                     | Cu      | 4.9             | 4.9               | Zn ≈ 8.1 Cu          |
| *Echinacea purpurea* | Zn      | 12.7            | -                 | Zn ≈ 3.0 Cu          |
|                     | Cu      | 4.3             | 4.3               | Zn ≈ 3.0 Cu          |

With unbalanced plant nutrition or an excess of applied fertilizer doses, balanced nutrition equations play an important role in nutrition diagnostics and calculation of fertilizer doses.

The results of the chemical analysis of plants allow us to calculate the doses of zinc and copper in the soil as the main application for medicinal crops, using the formula (14), converted from formula (13):

\[ D = (\varnothing - \varnothing) / b, \text{ kg / ha} \]  

(14)
where: $\mathcal{E}p$ is the predicted content in plants, mg / kg; $\mathcal{E}o$ is the actual content in plants, mg / kg; $b$ is a coefficient of intensity of action, mg / kg.

With balanced macroelement nutrition of medicinal plants, when zinc and copper are introduced into the soil, the highest yield is observed on the ground + $Zn_{60}$ variants; ground + $Cu_{9.7}$ (A. millefolium, T. vulgare) and ground + $Zn_{31.4}$, ground + $Cu_{9.4}$ (E. purpurea). Doses of zinc and copper, calculated taking into account the optimal content of trace elements, as well as the content on the background (table 1) are shown in table 4.

Table 4. Estimated doses of microfertilizers for medicinal herbs

| Culture                | The optimal content in plants, mg / kg | The content on the background, mg / kg | Intensity factor «b» | Dose, kg / ha (equation 16) |
|------------------------|---------------------------------------|---------------------------------------|----------------------|-----------------------------|
|                        | Zn          | Cu          | Zn          | Cu          | Zn          | Cu          |                          |
| Achillea millefolium   | 29.3        | 6.8         | 11.3        | 2.2         | 0.30        | 0.49        | 60                      | 9.4                        |
| Tanacetum vulgare      | 39.8        | 4.9         | 17.0        | 4.4         | 0.38        | 0.07        | 60                      | 7.8                        |
| Echinacea purpurea     | 12.7        | 4.3         | 4.6         | 2.1         | 0.38        | 0.23        | 21.3                   | 9.6                        |

The obtained calculated doses of zinc and copper fertilizers correspond to those actually entered in the field experiments.

4. **Conclusion**

Summing up the obtained data, we can say that the results of plant analysis allow us to obtain with high accuracy the normative characteristics used to calculate the doses of fertilizers in the main application.

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