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ACCUMULATION OF BIOACTIVE SUBSTANCES AND CHEMICAL ELEMENTS IN *Echinacea purpurea* (L.) Moench MEDICINAL HERB AS INFLUENCED BY SOIL APPLICATION OF COPPER, AN ESSENTIAL MICROELEMENT

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Abstract

Copper, an essential element in human metabolism, is a trace element for plants and animals. It plays a significant role in physiological processes (i.e. in photosynthesis, respiration, carbohydrate and protein metabolism), increases productivity, improves plant quality characteristics and increases resistance to adverse factors. A particular concern is the need to apply copper fertilizers in the biogeochemical provinces with Cu deficit in the soil. *Echinacea purpurea* (L.) Moench is one of the best-selling plant-based medicines in many developed countries around the world. It has been widely used in medicine and veterinary medicine for immunocorrection. However, publications on the effect of trace elements on the yield and content of biologically active substances in the medicinal raw materials of the *E. purpurea* are very few. Our study presents the results confirming the role of copper fertilizers in increasing the content of biologically active substances in the medicinal raw materials of *E. purpurea*, as well as in its enrichment with certain trace elements. The work aimed to assess the influence of essential microelement (Cu) on the accumulation of certain biologically active substances (tanning substances, carotene, vitamin C) and chemical elements (zinc and copper) in the medicinal raw material of *E. purpurea* variety Znakhar. The plot tests were run in the conditions of the southern forest-steppe of Western Siberia (an experimental field of Omsk Stolypin State Agrarian University, Omsk, May-September 2016-2018). The experiment design was as follows: absolute control (without fertilizers), N_{125} (N-based fertilizer), N_{125} + 0.25MAC Cu (the maximum allowed concentration) (2.3 kg a.m./ha), N_{125} + 0.5MAC Cu (4.7 kg a.m./ha), N_{125} + 0.75MAC Cu (7.0 kg a.m./ha), N_{125} + 1MAC Cu (9.4 kg a.m./ha). The soil of the test site was meadow chernozem, low-power, low humus, medium loamy with a 5.2 % humus content, 10.0 mg/kg nitrate nitrogen, 394 mg/kg mobile phosphorus, 749 mg/kg exchange potassium; pH 6.5-6.8. The mobile copper level in the soil was 0.3 mg/kg. The experiment was arranged in four replicates, plots were systematically located in several tiers, with a plot size of 10 m^2. Copper acetate $(CH_3COO)_2Cu$ (32 %) was used as Cu fertilizer, ammonium nitrate (34.4%) was an N-based fertilizer. The crop was planted at the end of May 2016, 24 plants per plot, with 70×60 cm spacing. Fertilizers were manually incorporated into the soil to a 10-15 cm depth before planting (at tilling), and uniformly distributed throughout the entire plot area. Plants were collected in September, during *E. purpurea* mass flowering phase. Tannings, carotene, and ascorbic acid were quantified in the medicinal raw material of *E. purpurea*. The concentration of trace elements (copper, zinc) in powdered herb samples was determined by atomic absorption spectroscopy. We have found out that a single application of copper fertilizers contributed to accumulation of tanning substances, ascorbic acid and carotene in the herb raw material. On average, over three years of our research, the level of bioactive substances in the raw material (grass) increased reliably ($p < 0.05$), to 16.6 mg% for tanning, to 5.8 mg% for ascorbic acid, and to 51.2 mg/kg for carotene. A copper acetate dose of 9.4 kg a.m./ha resulted in maximum concentration of the bioactive substances. Each 1 kg of Cu fertilizer increased the content of tannins by 0.51 mg%, ascorbic acid by 0.29 mg%, and carotene by 2.56 mg/kg ($p < 0.05$). Thus, the use of $(CH_3COO)_2Cu$ contributes to an improved medicinal value of *E. purpurea*. The copper fertilizer dosages correlated tightly with the level of mobile zinc and copper in medicinal raw materials (r = 0.98, $p < 0.05$). Each 1 kg of copper added with N_{125} increased the content of mobile copper and zinc in *E. purpurea* raw material by 0.23 and 1.15 mg/kg, respectively. These values are b coefficients that evaluate the effects of copper fertilizers and, together with the regression equations we obtained, allow practitioners to draw up protocols for applying micronutrient fertilizers in meadow chernozem soils at an early stage of *E. purpurea* plant development in specific growing conditions.

Keywords: *Echinacea purpurea* (L.) Moench, meadow chernozem soil, essential microelements, copper, zinc, bioactive substances, southern forest-steppe, Western Siberia.

The World Health Organization recognizes herbal medicines as a significant part of the pharmaceutical industry market. More than 80% of the world's population is increasingly using medicinal plants as the main source of medical care. This is not only the growing population of China and India, but also many developed countries [1]. About 80% of all modern pharmaceuticals are obtained directly or indirectly from plant sources [2, 3]. According to forecasts, in the next 10 years, the use of herbal remedies in the world market will grow and reach 60% [4]. Herbal medicinal products have a number of advantages: no side effects, low toxicity, availability, complex effects on the body (anti-inflammatory, antimicrobial, antispasmodic, analgesic, antitoxic) [5].

In the main agricultural regions of the Russian Federation, the soil area with a low levels of mobile trace elements (copper, zinc, etc.) reaches 50-90% of the surveyed territory (6, 7). The critical amount of trace elements in soils leads to significant losses in crop production. Bioenrichment of agricultural plants [8, 9], including medicinal crops, with various bioelements, for example, copper, may be a convenient solution.

Copper is an essential chemical element along with iron, zinc, iodine, and selenium [10]. Cu acts as a cofactor for many enzymes [11]. Copper and its compounds play a significant role in physiological processes in plant cells, being involved into respiration, photosynthesis, carbohydrate and phosphorus metabolism, protein synthesis, nitrogen fixation and reduction [12, 13]. It has a positive effect on the water regime of plants, their drought and frost resistance, increases plant resistance to various diseases. Copper fertilizers increase the content of sugars, fat, ascorbic acid, vitamin A, and group B vitamins in crop production [14, 15].

Application of essential trace elements activates enzymatic processes in medicinal plants, which leads to biosynthesis and the accumulation of biologically active substances, improving the valuable qualities of medicinal raw materials [16]. Microelements in plants are in an accessible, organically bound form, which enable their better assimilation. In the plant organism, essential trace elements bind to biologically active substances, which enhances the pharmacological effects [17].

Echinacea purpurea (L.) Moench is one of the best-selling herbal medicines in many developed countries [18]. It is widely used in medicine and veterinary medicine as an immunocorrector. All plant organs contain biologically active substances — vitamins A and C, tannins, flavonoids, essential oils, anthocyanins and alcamides [19-21]. Fundamental and clinical studies have proven the antiviral, anti-inflammatory, antioxidant, antibacterial and antimycotic effects of *Echinacea purpurea* [22]. However, scant data are available on the effect of microelements on the yield and the content of biologically active substances in the medicinal raw material of *E. purpurea* [23, 24].

This paper provides the first confirmation of the role of copper fertilizers in increasing the content of biologically active substances in the medicinal raw material of *Echinacea purpurea* and its enrichment with microelements.

Our objective was to assess the effect of an essential trace element (Cu) on the accumulation of some biologically active substances (tannins, carotene, vitamin C) and chemical elements (zinc and copper) in the medicinal raw material of *Echinacea purpurea*.

Materials and methods. Echinacea purpurea (L.) Moench variety Znakhar was grown in the conditions of the southern forest-steppe of Western Siberia was carried out in May-September 2016-2018 (Stolypin Omsk GAU, Omsk). The soil of the experimental site is meadow chernozem, shallow low-humus, medium loamy. Agrochemical analysis was performed by conventional methods [25], the 0-30 cm layer contained 5.2% humus (according to Tyurin), 10.0 mg/kg nitrate nitrogen (according to Kochergin), 394 mg/kg mobile phosphorus and 749 mg/kg exchangeable potassium (according to Chirikov), absorption capacity of 25.2-28.2 mg-eq/100 g, 0.3 mg/kg mobile copper; pH values of aqueous extracts ranged within 6.5-6.8.

The plots were put in test for the following treatments: control without fertilizers; N_{125}; N_{125} + 0.25MPC (maximum permissible concentration) Cu (2.3 kg Cu/ha); N_{125} + 0.5MPC Cu (4,7 kg Cu/ha); N_{125} + 0.75MPC Cu (7.0 kg Cu/ha); N_{125} + MPC Cu (9.4 kg Cu/ha). Ammonium nitrate (34.4%) was referred to as a background, and copper acetate $(CH_3COO)_2Cu$ (32%) was considered as fertilizer. The N_{125} dosage was chosen as a baseline due to the low $N\text{-}NO_3$ concentration in the soil, as only a balanced macronutrient composition allows the micronutrients to take full effect. Doses of copper fertilizers were calculated based on MPC Cu (3 mg/kg) and copper content before planting. The experiment was repeated four times, the sequence of variants was systematic, in several tiers, using 10 m^2 plots.

The approved zonal agrotechnology was applied. *E. purpurea* planting was carried out at the end of May 2016, 24 plants per plot with 70×60 cm plant spacing (70 cm between rows and 60 cm within the row) in order to create an optimal density of 24 thousand plants per hectare, providing a 0.4 m^2 space per plant. Fertilizers were manually incorporated to a depth of 10-15 cm when digging prior to planting and evenly distributed over each plot.

Plant samples were collected according to the variants in September in the phase of mass flowering of the culture. The medicinal raw material (grass) was dried in the shade in well-ventilated rooms. The contents of bioactive substances were measured and the values were converted to absolutely dry matter basis. Tannins were measured as per GOST 24027.2-80 [26], carotene as per GOST 13496.17-95 [27], ascorbic acid as per Murry [28[. Trace elements (copper, zinc) were measured according to GOST 30178-96 [29] by the atomic absorption method (a Varian AA-140 spectrometer, Akvilon, Russia) in the samples ground to a powder state.

For statistical processing, we used standard software packages Microsoft Office Excel 2007 and STATISTICA 6.0 (StatSoft, Inc., USA). Mean values (M*)* and standard errors of means (±SEM) were calculated, and regression and correlation were assessed. Differences were considered statistically significant at $p < 0.05$. To determine the relationship between the studied parameters, the correlation coefficients (*r*) were calculated. The values of the correlation coefficients were considered significant at $p < 0.05$

Results. Microelements can increase the content of vitamins and other biologically active substances in medicinal plants. In particular, there are evidences of their positive effect on the synthesis of vitamins C, A, and $B_{1\text{-}12}$ [30, 31]. In medicinal raw materials of *E. purpurea* enriched with copper, we determined the content of tannins, ascorbic acid, and carotene (Table 1).

Tannins (tannic acids) are important plant secondary metabolites in the plant organism. They provide astringent, antibacterial, antienzymatic, anti-inflammatory effects, and also protect plants from mammals and insects [32]. According to Zaprometov [33], the leaves of *E. purpurea* contain up to 7.2-10.2% tannins. In our experiments, tannins increased from 13.07 to 16.58 mg% on average over the study years depending on the doses of copper acetate. The maximum concentration of tannins (16.58 mg%) occurred when applying 9.4 kg Cu/ha. Differences in the amount of tannins for all treatments were significant at $p < 0.05$ as compared to both the control and the N_{125} when applied separately.

1. Concentration of bioactive compounds in medicinal raw materials of *Echinacea purpurea* (L.) Moench at flowering as influenced by Cu fertilizer dosage ($n = 24$, M±SEM, Omsk, 2016-2018)

Treatments	Tannins, mg%	Ascorbic acid, mg%	Carotene, mg/kg
Control (without fertilizers)	10.34±1.82	2.11±1.12	25.69±6.48
Background (N_{125})	11.76±1.02	3.07±0.58	27.65±5.38
N_{125} + 0.25MPC Cu (2.3 kg Cu/haa)	13.07±0.28	3.85±0.14	32.31±2.74
N_{125} + 0.5 ПДК Cu (4,7 кг д.в/га)	14.23±0.38	4.54±0.25	40.65±1.97
N_{125} + 0.75ПДК Cu (7,0 кг д.в/га)	15.37±1.02	5.18±0.61	45.45±4.69
N_{125} + ПДК Cu (9,4 кг д.в/га)	16.58±1.71	5.84±0.99	51.21±7.95

N o t e. All treatments differ form control and N_{125} background at a statistically significant level ($p < 0.05$).

Andrade et al. [34] note the ability of tannins to chelate copper, which determines their antioxidant activity. The positive effect of copper is also due to its role of a key component of many biological compounds and involvement in biosynthesis of secondary metabolites, including tannins [35]. Our findings are consistent with reports about direct dependence of the concentration of tannins on the amount of nutrients, in particular Cu and Mn, which serve as cofactors of enzymes involved in phenol decomposition and lignin biosynthesis. Copper deficiency impairs lignification in plants [36].

2. Regression and correlation between concentration of bioactive compounds in medicinal raw materials of *Echinacea purpurea* (L.) Moench and the dosage fertilizers (Omsk, 2016-2018)

Content of compounds (x)	Regression	Correlation r
Tannins	$y = 0.51x + 11.82$	0.99 ($p < 0.05$)
Ascorbic acid	$y = 0.29x + 3.13$	0.99 ($p < 0.05$)
Carotene	$y = 2.56x + 27.45$	0.99 ($p < 0.05$)

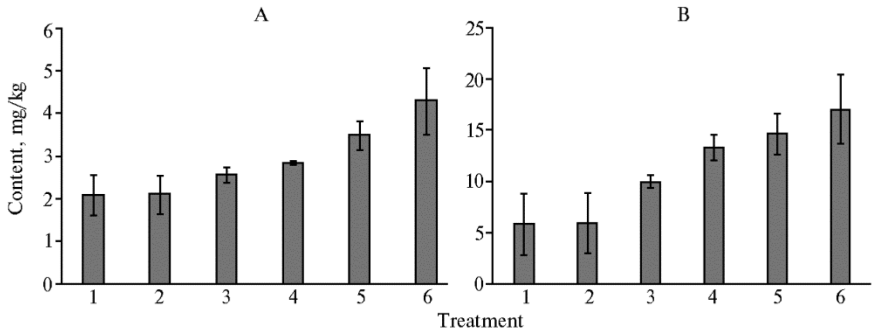
We revealed a high relationship ($r = 0.99$ at $p < 0.05$) between the doses of copper fertilizers and the content of tannins (Table 2). The regression coefficient shows that copper acetate applied at 1 kg Cu/ha provides a 0.51 mg% increase in tannins.

Ascorbic acid (vitamin C) is a micronutrient and an essential chemical compound that participates in many metabolic processes, performs antitoxic and antioxidant functions, increases resistance and protective properties of the body. To compensate for the deficiency of vitamin C, artificial enrichment of plant products and foodstuffs is currently widely used [37]. Carotene which plays photoprotective, light-harvesting, and structural role in plant photosynthesis, participates in the process of cell growth and division, promotes activation of immune system, helps the normal functioning of the gonads, affects growth, participates in the process of visual perception, and it also is an anticarcinogen. The intake of provitamin A into the body occurs mainly with plant foods, which are the main primary source of carotenoids [38].

Ascorbic acid was found in the leaves of *E. purpurea* [39]. In our research, the calculated doses of copper had a positive effect on the accumulation of vitamins in medicinal raw materials (see Table 1). The content of ascorbic acid and carotene were becoming significantly higher ($p < 0.05$) as the copper acetate dosage increased. The amount of vitamin C varied upon copper application from 3.85 to 5.84 mg%, carotene from 32.31 to 51.21 mg/kg. The maximum content of vitamins was noted for N_{125} + MPC Cu. According to Zagumennikov et al. [40], *Echinacea purpurea* accumulates small amounts of ascorbic acid, while plant nutrition and age have an insignificant effect on the content of vitamin C.

Each 1 kg of the copper fertilizer increased the concentration of ascorbic acid by 0.29 mg%, carotene by 2.56 mg/kg (see Table 2). Correlation analysis indicated a very strong correlation between the content of vitamins and the copper doses ($r = 0.99$ at $p < 0.05$).

Copper interacts with various chemical elements, including zinc, iron, molybdenum, sulfur, and selenium [41]. Therefore, we compared the content of two microelements, zinc and copper, in the medicinal raw materials of *E. purpurea* (Fig.).



Content of mobile forms of Cu (A) **and Zi** (B) **in medicinal raw materials of *Echinacea purpurea* (L.) Moench at flowering as influenced by Cu fertilizer dosage:** 1 — control (without fertilizers), 2 — N_{125} (a background), 3 — N_{125} + 0,25MPC Cu (2.3 kg Cu/ha), 4 — N_{125} + 0.5MPCCu (4.7 kg Cu/ha), 5 — N_{125} + 0.75MPC Cu (7.0 kg Cu/ha), 5 — N_{125} + MPC Cu (9.4 kg Cu/ha) (n = 24, M±SEM, Omsk, 2016-2018).

Many chemical elements can inhibit or enhance the intake of other elements, that is, their action may be either synergistic or antagonistic [42]. Therefore, we investigated the effect of copper fertilizers in the soil—plant system.

The copper and zinc concentration in the aboveground mass of *E. purpurea* had a clearly pronounced linear dependence on the doses of copper fertilizers applied. The relationships between different doses of copper acetate and the amount of Cu and Zn in plants during the flowering period were characterized by the equations:

$$y = 0.23Cu + 1.98 \ (r = 0.98 \text{ at } p < 0.05),$$
$$y = 1.15Zn + 6.78 \ (r = 0.98 \text{ at } p < 0.05).$$

In 2016, the coefficient (b) characterizing the copper effect on the content of mobile Cu and Zn in the medicinal raw materials was 0.23 and 1.15 mg/kg, respectively, for each kilogram of copper fertilizers in doses of 2.3, 4.7, 7.0 and 9.4 kg/ha (with N_{125} as a background). To increase the Cu content in medicinal raw materials by 1 mg/kg, 4.3 kg of copper fertilizers are required ($r = 0.98$ at $p < 0.05$). The obtained normative b valued (0.23 and 1.15 mg/kg) can be used to predict how the amount of available nutrients in medicinal raw materials will increase when micronutrient fertilizers are applied. Many researchers also noted that the accumulation of copper in plants increased with an increase in the dose of applied copper fertilizers [43-45].

Thus, incorporation of copper acetate into the soil, together with macrofertilizers, allows for an increase in the content of biologically active substances (tannins, vitamins C and A) in the medicinal raw materials of *Echinacea purpurea* (L.) Moench. Our findings provide a significant increase ($p < 0.05$) in tannins to

16.58 mg%, in vitamin C up to 5.84 mg%, and in carotene up to 51.21 mg/kg. The Cu micro-fertilizer also contributes to the enrichment of raw materials with microelements (copper and zinc). The concentration of copper in the raw material increased by 2.19 mg/kg as compared to N_{125} applied separately (a background) and amounted to 4.27 mg/kg ($p < 0.05$). The highest observed zinc concentration was 17.06 mg/kg, which is 11.17 mg/kg higher than the value for N_{125} as a background. The obtained linear regression equations and coefficients b of the copper effect enable development of practical application of microelements on meadow chernozem soils in the conditions of the southern forest-steppe of Western Siberia. Our results also expand the databases on the biochemical composition of medicinal raw materials grown with the use of copper fertilizers.

REFERENCES

1. Jamshidi-Kia F., Lorigooini Z., Amini-Khoei H. Medicinal plants: Past history and future perspective. *Journal of Herbmed Pharmacology,* 2018, 7(1): 1-7 (doi: 10.15171/jhp.2018.01).
2. Hariharan P., Subburaju T. Medicinal plants and its standardization — a global and industrial overview. *Global Journal of Medicinal Plant Research*, 2012, 1(1): 10-13.
3. Nirmal S.A., Pal S.C., Otimenyin S.O., Aye T., Elachouri M., Kundu S.K., Thandavarayan R.A., Mandal S.C. Contribution of herbal products global market. *The Pharma Review*, 2013: 95-104.
4. Bogoyavlenskii A.P., Aleksyuk P.G., Turmagambetova A.S., Berezin V.E. *Farmatsevticheskie nauki*, 2013, 6(5): 1184-1187 (in Russ.).
5. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 2013, 4: 177 (doi: 10.3389/fphar.2013.00177).
6. Kabata-Pendias A., Pendias H. *Trace elements in soils and plants.* CRC Press, Boca Raton, London, New York, Washington, D.C., 2001.
7. Aristarkhov A.N., Bushuev N.N., Safronova K.G. *Agrokhimiya*, 2012, 9: 26-40 (in Russ.).
8. Dimpka C.O., Bindraban P. Fortification on micronutrients for efficient agronomic production: a review. *Agronomy for Sustainable Development*, 2016, 36: 1-26 (doi: 10.1007/s13593-015-0346-6).
9. Valença A.W., Bake A., Brouwer I.D., Giller K.E. Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa author links open overlay panel. *Global Food Security*, 2017, 12: 8-14 (doi: 10.1016/j.gfs.2016.12.001).
10. Vavoulidou E.E., Avramides E.J., Papadopoulos P., Dimirkou A.T., Charoulis A., Konstantinidou-Doltsinis S. Copper content in agricultural soils related to cropping systems in different regions of Greece. *Communications in Soil Science and Plant Analysis*, 2005, 36(4-6): 759-773 (doi: 10.1081/CSS-200043367).
11. Pavelkova M., Vysloužil J., Kubova K., Vetchy D. Biological role of copper as an essential trace element in the human organism. *Czech and Slovak Pharmacy*, 2018, 67(4): 143-153.
12. Broadley M., Brown P., Buerkert A., Camak I., Cooper J., Eichert T., Engels C., Fernandez V., Kirkby T., Eckhard G., Hawkesford M., Horst W., Huber D., Kichey T., Ludwi B., Jian Feng Ma., Marschner P., Neumann E., Neumann G., Schlecht H., Rengel Z., Romheld V., Schjoerring J., Moller I.S., Weinmann M., White P., Wiesler F., Zhao F. *Marschner's mineral nutrition of higher plants.* Elsevier Ltd., 2012 (doi: 10.1016/B978-0-12-384905-2.00025-X).
13. Braz J. Copper in plants. *Brazilian Journal of Plant Physiology*, 2005, 17(1): 145-156 (doi: 10.1590/S1677-04202005000100012).
14. Burkhead J.L., Gogolin Reynolds K.A., Abdel-Ghany S.E., Cohu C.M., Pilon M. Copper homeostasis. *New Phytologist,* 2009, 182(4): 799-816 (doi: 10.1111/j.1469-8137.2009.02846.x).
15. Printz B., Lutts S., Hausman J.-F., Sergeant K. Copper trafficking in plants and its implication on cell wall dynamics. *Frontiers in Plant Science*, 2016, 7: 601 (doi: 10.3389/fpls.2016.00601).
16. Makarova V.G., Laksaeva E.A., Martynov E.G. *Rossiiskii mediko-biologicheskii vestnik imeni akademika I.P. Pavlova*, 2006, 3: 29-35 (in Russ.).
17. Krasnov E.A., Savel'eva E.E., Ryzhakova N.K., Reshetov Ya.E., Gataullina A.R. *Khimiya rastitel'nogo syr'ya*, 2017, 4: 145-151 (doi: 10.14258/jcprm.2017041934) (in Russ.).
18. Nonvitamin and nonmineral nutritional supplements. S.M. Nabavi, A.S. Silva (eds.). *Academic Press*, 2019 (doi: 10.1016/C2016-0-03546-5).
19. Sharifi-Rad M., Mnayer D., Morais-Braga M.F.B., Carneiro J.N.P., Bezerra C.F., Coutinho H.D.M., Salehi B., Martorell M., del Mar Contreras M., Soltani-Nejad A., Uribe Y.A.H., Yousaf Z., Iriti M., Sharifi-Rad J. Echinacea plants as antioxidant and antibacterial agents: From traditional medicine to biotechnological applications. *Phytotherapy Research*, 2018, 32(9): 1653-1663 (doi: 10.1002/ptr.6101).
20. Kumar R.M., Ramaiah S. Pharmacological importance of *Echinacea purpurea*. *International Journal of Pharma and Bio Sciences*, 2011, 2(4): 304-314.
21. Shekarchi M., Hajimedipoor H., Khanavi M., Rustaie A. The effects of plant age and harvesting

time on chicoric and caftaric acids content of *E. purpurea* (L.) Moench. *Iranian Journal of Pharmaceutical Sciences,* 2012, 8(3): 203-208.
22. Khasina E.I. *Izvestiya Samarskogo nauchnogo tsentra Rossiiskoi akademii nauk*, 2014, 16(5-2): 1030-1032 (in Russ.).
23. Babaeva E.Yu. *Osobennosti mineral'nogo pitaniya ekhinatsei purpurnoi v usloviyakh Nechernozemnoi zony RF. Avtoreferat kandidatskoi dissertatsii* [Features of the *Echinacea purpurea* mineral nutrition in the Non-chernozem zone of the Russian Federation. PhD Thesis]. Moscow, 2000 (in Russ.).
24. Hajagha R., Kirici S., Tabrizi L., Asgharzadeh A., Hamidi A. Evaluation of growth and yield of purple coneflower (Echinacea purpurea l.) in response to biological and chemical fertilizers. *Journal of Agricultural Science*, 2017, 9(3): 160-171 (doi: 10.5539/jas.v9n3p160).
25. *Agrokhimicheskie metody issledovaniya pochv* /Pod redaktsiei A.V. Sokolova [Agrochemical methods in soil research. A.V. Sokolov (ed.)]. Moscow, 1975 (in Russ.).
26. *GOST 24027.2-80 Syr'e lekarstvennoe rastitel'noe. Metody opredeleniya vlazhnosti, soderzhaniya zoly, ekstraktivnykh i dubil'nykh veshchestv, efirnogo masla* [GOST 24027.2-80 Raw medicinal vegetable. Methods for determination of moisture, ash content, extractive compounds and tannins, essential oils]. Moscow, 1999 (in Russ.).
27. *GOST 13496.17-95 Korma. Metody opredeleniya karotina* [GOST 13496.17-95 Feed. Methods for determining carotene]. Moscow, 2011 (in Russ.).
28. Mineev V.G., Sychev V.G., Amel'yanchik O.A., Bolysheva T.N., Gomonova N.F., Durynina E.P., Egorov V.S., Edemskaya N.L., Karpova E.A., Prizhukova V.G. *Praktikum po agrokhimii* /Pod redaktsiei V.G. Mineeva [Workshop on agrochemistry. V.G. Mineev (ed.)]. Moscow, 2001 (in Russ.).
29. *GOST 30178-96 Syr'e i produkty pishchevye. Atomno-absorbtsionnyi metod opredeleniya toksichnykh elementov* [GOST 30178-96 Raw materials and food products. Atomic absorption method for determination of toxic elements]. Moscow, 1997 (in Russ.).
30. Collins J.F., Klevay L.M. Copper. *Advances in Nutrition*, 2011, 2(6): 520-522 (doi: 10.3945/an.111.001222).
31. Kaiser C., Wernery U., Kinne J., Marker L., Liesegang A. The role of copper and vitamin A deficiencies leading to neurological signs in captive cheetahs (*Acinonyx jubatus*) and lions (*Panthera leo*) in the United Arab Emirates. *Food and Nutrition Sciences*, 2014, 5(20): 1978-1990 (doi: 10.4236/fns.2014.520209).
32. Hassanpour S., Maheri-Sis N., Eshratkhah B., Mehmandar F.B. Plants and secondary metabolites (Tannins): a review. *International Journal of Forest, Soil and Erosion*, 2011, 1(1): 47-53.
33. Zaprometov M.N. *Osnovy biokhimii fenol'nykh soedinenii* [Fundamentals of biochemistry of phenolic compounds]. Moscow, 1974 (in Russ.).
34. Andrade R.G. Jr., Dalvi L.T., Silva J.M. Jr., Lopes G.K., Alonso A., Hermes-Lima M. The antioxidant effect of tannic acid on the in vitro copper-mediated formation of free radicals. *Archives of Biochemistry and Biophysics*, 2005, 437(1): 1-9 (doi: 10.1016/j.abb.2005.02.016).
35. Khathutshelo M.V., Mpumelelo N., Wonder N., Fhatuwani M.N. Effects of foliar spray application of selected micronutrients on the quality of bush tea. *Horticultural Science*, 2016, 51(7): 873-879 (doi: 10.21273/hortsci.51.7.873).
36. Santos R.M., Fortes G.A.C., Ferri P.H., Santos S.C. Influence of foliar nutrients on phenol levels in leaves of *Eugenia uniflora*. *Revista Brasileira de Farmacognosia*, 2011, 21(4): 581-586 (doi: 10.1590/S0102-695X2011005000112).
37. Araya M.M., Pizarro F., Olivares M., Arredondo M., González M., Méndez M. Understanding copper homeostasis in humans and copper effects on health. *Biological Research*, 2006, 39(1): 183-187 (doi: 10.4067/S0716-97602006000100020).
38. Napoli J.L. Vitamin A. Biochemistry and physiological role. In: *Encyclopedia of human nutrition (second edition)*. Elsevier, Berkeley, CA, 2005: 339-347 (doi: 10.1016/B0-12-226694-3/02280-8).
39. Mistríková I., Vaverková S. *Echinacea* — chemical composition, immunostimulatory activities and uses. *Thaiszia - Journal of Botany*, 2006, 16: 11-26.
40. Zagumennikov V.B., Molchanova A.V., Babaeva E.Yu., Petrova A.L. Accumulation of ascorbic acid in fresh *Echinacea purpurea* plants and their processing products. *Pharmaceutical Chemistry Journal*, 2015, 48(10): 671-674 (doi: 10.1007/s11094-015-1168-1).
41. Osredkar J., Sustar N. Copper and zinc, biological role and significance of copper/zinc imbalance. *Journal of Clinical Toxicology*, 2011, 3: 1-18 (doi: 10.4172/2161-0495.S3-001).
42. Wang Y., Ou Y.L., Liu Y.Q., Xie Q., Liu Q.F., Wu Q., Fan T.Q., Yan L.L., Wang J.Y. Correlations of trace element levels in the diet, blood, urine, and feces in the chinese male. *Biological Trace Element Research*, 2012, 145(2): 127-135 (doi: 10.1007/s12011-011-9177-8).
43. Kumar R., Mehrotra N.K., Nautiyal B.D., Kumar P., Singh P.K. Effect of copper on growth, yield and concentration of Fe, Mn, Zn and Cu in wheat plants (*Triticum aestivum* L.). *Journal of Environmental Biology*, 2009, 30(4): 485-488.
44. Kaewchangwat N., Dueansawang S., Tumcharern G., Suttisintong K. Synthesis of copper-chelates derived from amino acids and evaluation of their efficacy as copper source and growth stimulator for *Lactuca sativa* in nutrient solution culture. *Journal of Agricultural and Food Chemistry*, 2017, 65(45): 9828-9837 (doi: 10.1021/acs.jafc.7b03809).
45. Stepien A., Wojtkowiak K. Effect of foliar application of Cu, Zn, and Mn on yield and quality indicators of winter wheat grain. *Chilean Journal of Agricultural Research*, 2016, 76(2): 220-227 (doi: 10.4067/s0718-58392016000200012).