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Study of the technological processes of zinc hydroxide nitrate nanocrystals production applying for foliar fertilizer for important agricultural plants

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The public defense of the dissertation will be held online on ………. at …… at an open meeting of the scientific jury, determined by order №: РД-16-1089/26.11.2.2020 of the Rector of AU-Plovdiv, consisting of:

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The materials on the defense are available to those interested in the Educational information Center of the Agricultural University of Plovdiv, department of masters and doctoral students.

Summary

The normal development of plants requires the absorption of sufficient quantities of macro- and microelements and their deficiency significantly affects the quantity and quality of the production obtained. Zinc (Zn) is among the eight trace elements (manganese, copper, boron, iron, zinc, chlorine, molybdenum and nickel), which are essential for normal, healthy growth and reproduction of plants. The average concentration of Zn in plant tissues is in the range of 15-80 mg kg^{-1}. Its physiological role in plants is associated with participation in many enzyme systems (carbonic anhydrase, superoxide dismutase, dehydrogenases), the activation of many other enzymatic systems, protein synthesis, cell membrane integration, and more. The lack of Zn in plants causes a number of structural and functional disorders such as increased membrane permeability, high concentration of reactive oxygen species, reduced photosynthesis rate, growth restriction and others.

Along with the vital need for plants, zinc deficiency in food is also an important problem for human health, affecting more than 1/3 of the earth's population. It is vital for many biological functions in the human body. It is present in all parts of the body, including organs, tissues, bones, fluids and cells. It is required for more than 300 enzymes responsible for activating growth (development of height, weight and bone), cell growth and cell division, immune system, reproduction, appetite, skin, hair, nails and vision.

In addition to the humanitarian aspect, addressing the problem of the zinc status of basic crops has a purely financial expression. There is undisputed evidence that maintaining the optimum amount of zinc leads to a significant increase in yields and product quality.

The studies included in this dissertation are in two main areas (i) controlled synthesis of zinc-containing hydroxy nitrates and their physicochemical characterization (Sections 3.1 and 3.2) and (ii) evaluation of the potential of synthesized nano-sized materials as leaf fertilizers for basic crops (Sections 3.3 - 3.8).

The conditions for the synthesis of zinc hydroxide nitrate (Section 3.1) and mixed zinc - copper hydroxy nitrates over the entire concentration range (Section 3.2) have been studied in detail. Modern instrumental methods have been used in the physicochemical characterization of the samples - Powder X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), High-Resolution Transmission Electron Microscopy (HRTEM), Thermal analysis, HPLS and Chemical Analysis (ICP-AES). Original results were obtained for the stability of the synthesized hydroxy nitrates and the conditions for the formation of solid solutions in mixed Zn-Cu hydroxy nitrates.

As test cultures for the evaluation of the potential of the synthesized nanosized materials as leaf fertilizers, basic crops for Bulgaria (maize) and Vietnam (*Curcuma Longa* and *Phyllanthus amarus*) were used.

The impact of the synthesized Zn-containing leaf nanofertilizer on the physiological status of Zn-deficient maize plants grown under controlled and field conditions was investigated (Sections 3.3 - 3.8). The subject of the study are: (1) the effect of zinc nanofertilizer nutrition on the physiological and mineral status of plants (Section 3.3); (2) the effect of the composition of the suspension on the productivity and quality of the corn kernels (moisture, protein, fat and protein content - Sections 3.4 and 3.5); (3) effect of genotype on the efficiency of zinc-containing fertilizers containing nanotubes (Section 3.6). Experiments conducted in Vietnam with Curcuma Longa (Section 3.7) show that treatment with zinc-containing leaf fertilizer increases curcumin content in the productive parts of the plant.

The experiments allow a reasoned conclusion to be drawn that suspensions of zinc-containing hydroxy nitrates can be successfully applied as long-acting foliar fertilizers.

Резюме

Нормалното развитие на растенията изисква усвояването на достатъчно количества макро- и микроелементи и техният недостиг влияе съществено върху количеството и качеството на получената продукция. Цинкът (Zn) е между осемте микроелементи (манган, мед, бор, желязо, цинк, хлор, молибден и никел), които са от съществено значение за нормален, здравословен растеж и възпроизводство на растенията.

Физиологичната му роля в растенията се свързва с участие в редица ензимни системи (карбоанхидраза, супероксидна дисмутаза, дехидрогенази), активацията на много други ензимни системи, белтъчната синтеза, интеграцията на клетъчните мембрани и др.

Наред с жизнената необходимост за растенията дефицитът на цинк в хранителните продукти е важен проблем и за човешкото здраве, който засяга повече от 1/3 от населението на земята. Той е жизнено важен за много биологични функции в човешкото тяло. Необходим е за повече от 300 ензими, отговорни за активиране на растежа (развитие на височина, тегло и кост), растежа на клетките и клетъчното делене, имунната система, репродуктивността, апетита, кожата, косата, ноктите и зрението.

Освен хуманитарния аспект решаването на проблема с цинковия статус на основни селскостопански култури има и чисто финансово изражение. Има безспорни доказателства, че поддържането на оптимално количество цинк води до съществено повишаване на добивите и качеството на продукцията.

Изследванията, които са включени в настоящата дисертация, са в две основни направления (i) контролиран синтез на цинк съдържащи хидрокси нитрати и физикохимичното им охарактеризиране (раздели 3.1 и 3.2) и (ii) оценка на потенциала на синтезираните наноразмерни материали като листни торове за основни селскостопански култури (раздели 3.3 – 3.8).

При физикохимичното охарактеризеране на образците са използвани съвременни инструментални методи – рентгеноструктурен анализ (XRD), сканираща електронна микроскопия (SEM), трансмисионна електронна микроскопия с висока резолюция (HRTEM), термичен анализ (TG, DTG, DTA) и химически анализ (ICP-AES). Получени са оригинални резултати за стабилността на синтезираните хидрокси нитрати и за условията за образуване на твърди разтвори в смесените Zn-Cu хидрокси нитрати.

Като тест-култури за оценка на потенциала на синтезираните наноразмерни материали като листни торове са използвани основни за България (царевица) и Виетнам (*Curcuma Longa* и *Phyllanthus amarus*) земеделски култури.

Изследвано е влиянието на синтезирания Zn-съдържащ листен нанотор върху физиологичния статус на Zn-дефицитни царевични растения, отглеждани при контролирани условия и при полски условия (раздели 3.3 - 3.8). Обект на проучването са: (1) влиянието на подхранването с цинков нанотор върху физиологичния и минерален статус на растенията (раздел 3.3); (2) влиянието на състава на суспензията върху продуктивността и качеството на царевичните зърна (съдържание на влага, протеини, мазнини и белтъчни вещества – раздели 3.4 и 3.5) ; (3) влияние на генотипа върху ефективността от листното торене с цинк съдържащи наноторове (раздел 3.6).

Проведените във Виетнам експерименти с *Curcuma Longa* показват, че третирането с цинк съдържащ листен тор повишава съдържанието на куркумин в продуктивните части на растението.

Проведените експерименти позволяват да се направи обоснован извод, че суспензиите на цинк съдържащите хидрокси нитрати могат да бъдат успешно прилагани като листни торове с продължително действие.

I. Introduction

Zinc deficiency can be found in every part of the world and almost all crops respond positively to application of Zn. Besides mineralogical composition of the parent material, the total amount of Zn present in the soil is also dependent on the type, intensity of weathering, climate and numerous other predominating factors during the process of soil formation. Meanwhile, high pH and high contents of $CaCO_3$, organic matter, clay and phosphate can fix zinc in the soil and give rise to the reduction of available Zn.

The normal development of plants requires the absorption of sufficient quantities of macro- and trace elements and their deficiency significantly affects the quantity and quality of the production obtained. Zinc (Zn) is among the eight trace elements (manganese, copper, boron, iron, zinc, chlorine, molybdenum and nickel), which are essential for normal, healthy growth and reproduction of plants. Its physiological role in plants is associated with participation in a number of enzyme systems (carbonic anhydrase, superoxide dismutase, dehydrogenases), the activation of many other enzymatic systems, protein synthesis, cell membrane integration, and more. The lack of Zn in plants causes a number of structural and functional disorders such as increased membrane permeability, high concentration of reactive oxygen species, reduced photosynthesis rate, growth restriction, etc.

Along with the vital need for plants, zinc deficiency in food is also an important human health problem affecting more than 1/3 of the earth's population. It is vital for many biological functions in the human body. It is present in all parts of the body, including organs, tissues, bones, fluids and cells. It is required for more than 300 enzymes responsible for activating growth (development of height, weight and bone), cell growth and cell division, immune system, reproduction, appetite, skin, hair, nails and vision.

In addition to the humanitarian aspect, solving the problem of the zinc and copper status of basic crops has a purely financial expression. There is undisputed evidence that maintaining the optimum amount of zinc leads to a significant increase in yields and product quality.

Soil fertilization is the most commonly used method of correcting nutritional deficiencies. In certain cases, such as high soil pH, high carbonate content, insufficient organic matter, water deficiency and other factors that reduce the mobility of the elements introduced into the soil, their absorption is not effective.

Zinc deficiency is often corrected by leaf fertilizers, most commonly in the form of soluble zinc salts. A *major drawback* of soluble inorganic salts, including the use of elements in their chelated form, is the risk of phytotoxicity. *The disadvantages* of very soluble inorganic salts and hard soluble oxides can be eliminated by the use of leaf *nanofertilizers* in which the nutrients are in the form of particles smaller than 100 nm in size. The most suitable of these are zinc and copper hydroxy nitrates and hydroxy sulfates, and in particular zinc hydroxide nitrate having the composition $Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$ (ZnHN).

II. Objectives and tasks

The main objective *of the study is to obtain scientific information and new knowledge that will allow controlled synthesis of zinc containing nanofertilizers and an assessment of their potential for enhancing the yields and quality of production from basic crops.*

The working hypothesis on which the study was built is based on the understanding that a *new approach* is needed to solve problems with the zinc status of plants and the deficiency of important trace elements in basic food products based on *innovative technologies*. The subject of research is nanosized zinc containing leaf fertilizers as part of nanotechnology in the agricultural sector.

The main *focus* is on:

- *Synthesis* of Zn-containing nanosized leaf fertilizers and detailed characterization of their physicochemical properties;
- *Investigation* stability of the resulting suspensions and evaluation the possibilities for their use as slow-acting leaf fertilizers by:
- *Assessment of the effects* of leaf treatment with selected Zn containing nanosized fertilizers on the physiological status of plants grown under controlled environmental conditions.
- *Conducting field experiments* with basic crops and evaluating the synthesized materials as effective zinc containing leaf fertilizers.

III. Materials and methods

Plant materials:

Maize: In this work 11 maize hybrids were used: Pr 9241, FAO 370; P2105, FAO 700; P1535, FAO 650; P1241, FAO 620; P1049, FAO 620; P1063, FAO 500; P0937, FAO 580; P0704, FAO 520; P0217, FAO 490; P20217, FAO 480 и P0023, FAO 450. The main part of the research was conducted with hybrids Pr 9241, FAO 370, given its excellent qualities (high ecological flexibility) and proper acclimatization for the area of research. The field experiment was conducted at Research Farm, Agricultural University, Bulgaria.

Phyllanthus amarus Schum**:** *Phyllanthus amarus* Schum. seeds were locally collected and cultivated at Lam Dong province, Vietnam. Before planting, surface soil samples (0 - 20 cm depth) from each harvesting plot were collected, air-dried, mixed and analyzed for the selected physicochemical properties.

Curcuma longa L: *Curcuma longa* L. tubers were locally collected and cultivated at Lam Dong province, Vietnam. All samples were milled, mixed, digested by a mixture of HNO_3 and H_2O_2 in a Microwave Digestion System MARS 6 - CEM Corporation and analysed for Zn, Cu, Fe, P, K, Ca and Mg.

Methods

The impact of synthesized Zn-containing fertilizers on plants has been evaluated on the basis of a complex of chemical, physiological and agronomic methods. In all stages of the study, up-to-date methods have been used in accordance with the internationally accepted standards:

- *Scanning Electron Microscopy (SEM)* - JSM 6390 electron microscope (Japan) in conjunction with energy dispersive X-ray spectroscopy (EDS, Oxford INCA Energy 350) equipped with ultrahigh resolution scanning system (ASID-3D) in a regime of secondary electron image (SEI) and backscattered electrons (BEC).
- *High-Resolution Transmission Electron Microscopy (HRTEM)* - JEOL JEM 2100 transmission electron microscope with an accelerating voltage of 80 to 200 kV.
- *Thermal analysis* - *c*omputerized thermal installation “Stanton Redcroft” (England) was used for thermal analysis of the samples under the following experimental conditions: heating temperature range - 20-650^oC, heating rate - 10$^oC.min^{-1}$, specimen mass - 12.00 mg, gas environment - 100% air, pot - stabilized corundum.
- *Powder X-Ray Diffraction* - Philips PW 1050 diffractometer, equipped with Cu Kα tube and a scintillation detector. Data was collected in θ-2θ, step-scan mode in the angle interval from 10 to 90^o (2θ) at counting time of 3 s/step and steps of 0.03^o (2θ).
- *Chemical analysis* - ICP-AES (Prodigy 7, Leeman) was applied to quantify the zinc, micro and macroelements content in the solid products and filtrates. All soil and plant samples were digested in a Microwave Digestion System MARS 6 - CEM Corporation.
- *Statistical analysis*

The results were analysed statistically using IBM SPSS statistic software.

IV. EXPERIMENTAL RESULTS

IV.1. Synthesis of zinc hydroxide nitrate nanoparticles

Preparation of zinc hydroxide nitrate ($Zn_5(OH)_8(NO_3)_2 \cdot 2H_2O$) was performed by pouring NaOH solution into $Zn(NO_3)_2 \cdot 6H_2O$ under vigorous stirring. The initial OH/Zn molar ratio was 1.6 (corresponding to steheometric OH/Zn molar ratio) and the time of precipitation was 10 minutes in all cases (to prevent transformation of the synthesized zinc hydroxide nitrate to ZnO). Five series of samples (Table 1) were synthesized under the following conditions: a solution containing 120 mmol of NaOH with concentration ranged from 0.4 M to 3.2 M was poured in a solution containing 75 mmol of $Zn(NO_3)_2 \cdot 6H_2O$ with concentration ranged from 0.4 M to 3.2 M under vigorous stirring.

In order to evaluate the influence of the temperature on the parameters of the resulting zinc hydroxide nitrate, more experiments were performed under different conditions, including increasing the temperature to 70°C and monitoring the precipitate in the mother liquor for one month. The white precipitate was filtered, washed with deionized water and dried at 65 °C for 24 h. The scheme of the experiments is presented in Table 1.

Table 1. *Scheme of the experiment.*

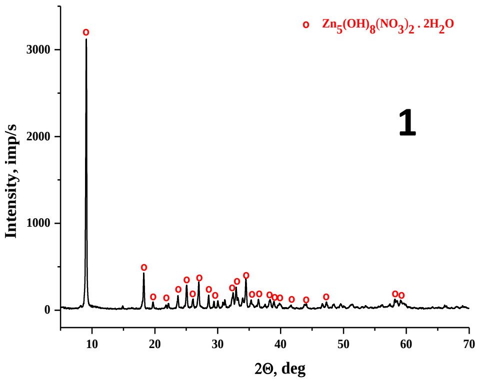
Series	S-1	S-2	S-3	S-4	S-5
$Zn(NO_3)_2.6H_2O$, mol/l	0.4M	1.2M	1.6M	2.4M	3.2M
NaOH, mol/l	0.4M	0.4M	0.4M	0.4M	0.4M
NaOH, mol/l	1.2M	1.2M	1.2M	1.2M	1.2M
NaOH, mol/l	1.6M	1.6M	1.6M	1.6M	1.6M
NaOH, mol/l	2.4M	2.4M	2.4M	2.4M	2.4M
NaOH, mol/l	3.2M	3.2M	3.2M	3.2M	3.2M

The theoretical value of zinc content in the dried at 65 °C zinc hydroxide nitrate, calculated based on the formula $Zn_5(OH)_8(NO_3)_2 \cdot 2H_2O$ is 52.47% and complete decomposition of $Zn_5(OH)_8(NO_3)_2 \cdot 2H_2O$ to ZnO ends at 300°C with the weight loss of 34.7%. The results presented in Table 2 correspond very closely to the theoretical ones and suggest the formation of $Zn_5(OH)_8(NO_3)_2 \cdot 2H_2O$ in all cases.

Table 2. *Chemical Composition (Zn, %) and weight loss at 450 °C (Δ**G,%**) of the Samples Synthesized at Room Temperature.*

Series	**S-1**	**S-2**	**S-3**	**S-4**	**S-5**
Zn,%	52.3±0.8	51.3±0.9	52.7±0.5	53.1±0.8	52.4±0.6
ΔG,%	34.48	34.57	34.63	34.3	34.8
Zn,%	53.1±0.8	53.4±0.8	51.9±0.8	52.3±0.9	52.7±0.7
ΔG,%	34.53	34.66	34.34	34.85	34.95
Zn,%	52.6±0.8	53.3±0.9	51.9±0.4	52.1±0.6	52.3±0.7
ΔG,%	34.11	34.7	34.87	34.54	34.25
Zn,%	53.3±0.9	51.8±0.8	52.4±0.5	53.1±0.7	53.6±0.8
ΔG,%	34.28	34.02	33.98	34.11	33.73
Zn,%	52.4±0.8	52.9±0.8	52.2±0.7	53.4±0.6	53.8±0.7
ΔG,%	34.46	34.00	34.25	33.60	33.62

This suggestion was verified by X-ray, SEM and TEM analysis of the fresh samples after filtration and washing with distilled water. Some of these results are shown in Figs. 1 - 3.



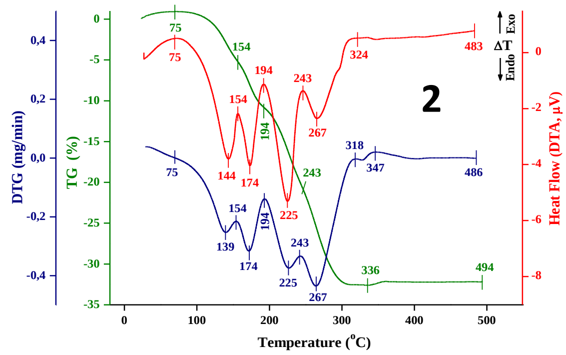
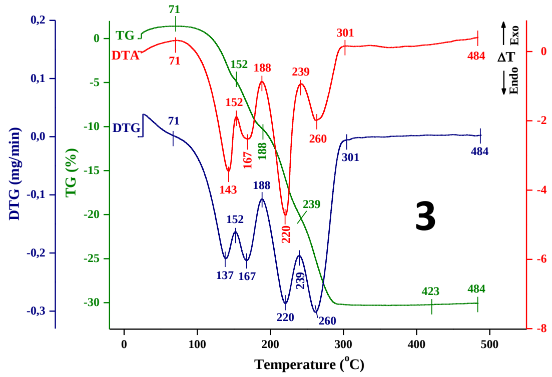


Figure 1. *X-ray pattern of the sample synthesized at 25 °C and concentration of NaOH and Zn(NO3)2 1.6 M.*

Figure 2. *TG, DTG and DTA profiles of the sample synthesized at 50°C and concentration of NaOH and Zn(NO3)2 1.6 M*

Figure 3. *TG, DTG and DTA profiles of the sample synthesized at 70°C and concentration of NaOH and Zn(NO3)2 1.6 M*



The strongest peak at 2θ = 9.2° and other characteristic peaks at 2θ = 18.4, 34.6, 35.4, 46.8, and 47.4° identified formation of pure well crystallized zinc hydroxide nitrate ($Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$, JCPDS card 24-1460).

The TG and DTG profiles of the samples, presented in Figs 4 and 5 show four steps of mass loss before reaching a constant value at around 310°C. The solid residue collected at the end of step four was identified by XRD analysis as pure ZnO. The observed total mass loss of the samples investigated is 33.4 and 32.4% respectively against an theoretical value of 34.7% for the ideal composition $Zn_5(OH)_8(NO_3)_2.2H_2O$.

Fig. 4 presents the SEM images of the sample synthesized at 25 °C and concentration of sodium hydroxide and zinc nitrate 1.6 M. As it can be seen, the sample is composed of sheet-like particles, the typical morphology of zinc hydroxide nitrate. These images are typical for all other samples, prepared according to the scheme presented in Table 1 except for the last two from series S5. The result is consistent with the observation from XRD pattern, presented in Fig. 1. and confirm the obtaining of pure $Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$.

TEM measurements and Selected area electron diffraction (SAED) patterns experimentally confirmed the preparation of pure $Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$ (Fig. 5 and Table 3).

Table 3. *Phases determined from SAED pattern and HRTEM*

Phase	PDF	Space group	Lattice parameters
$Zn_5(OH)_8(NO_3)_2.2H_2O$, monoclynic	72-0627	C2/m	a=19.48, b=6.238, ß=93.28

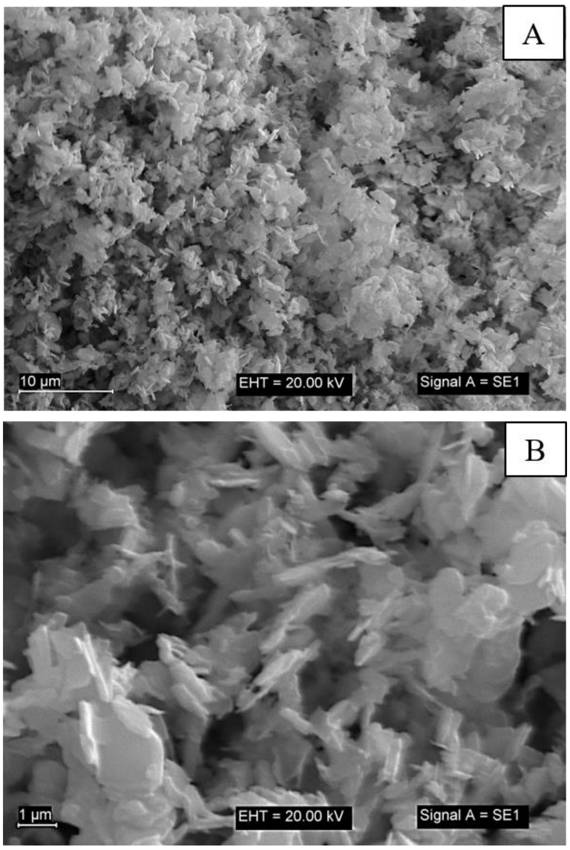


Figure 4. *SEM images of samples synthesized at 25 °C with initial OH/Zn molar ratio 1.6 and concentration of zinc nitrate and sodium hydroxide 1.6M. Scale bar: A: 10 µm; B: 1.0 µm*

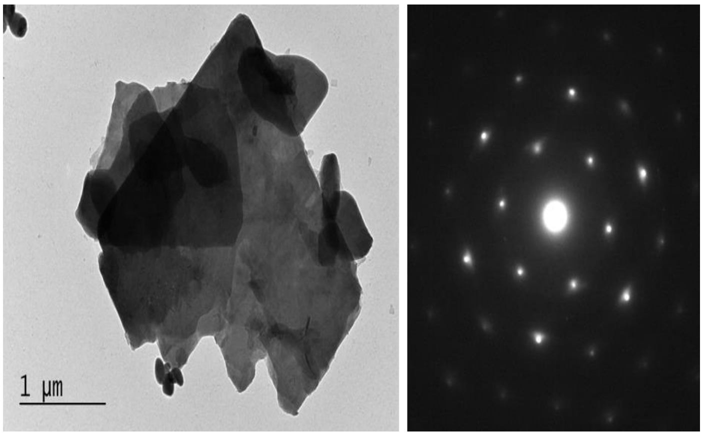


Figure 5. *The TEM micrographs of = at a magnification of 6000x and the related SAED patterns indicating the presence of Zn5(OH)8(NO3)2·2H2O.*

Fig. 6 presents the SEM images of the sample synthesized at 25 °C and concentration of NaOH and $Zn(NO_3)_2$ 3.2 M.

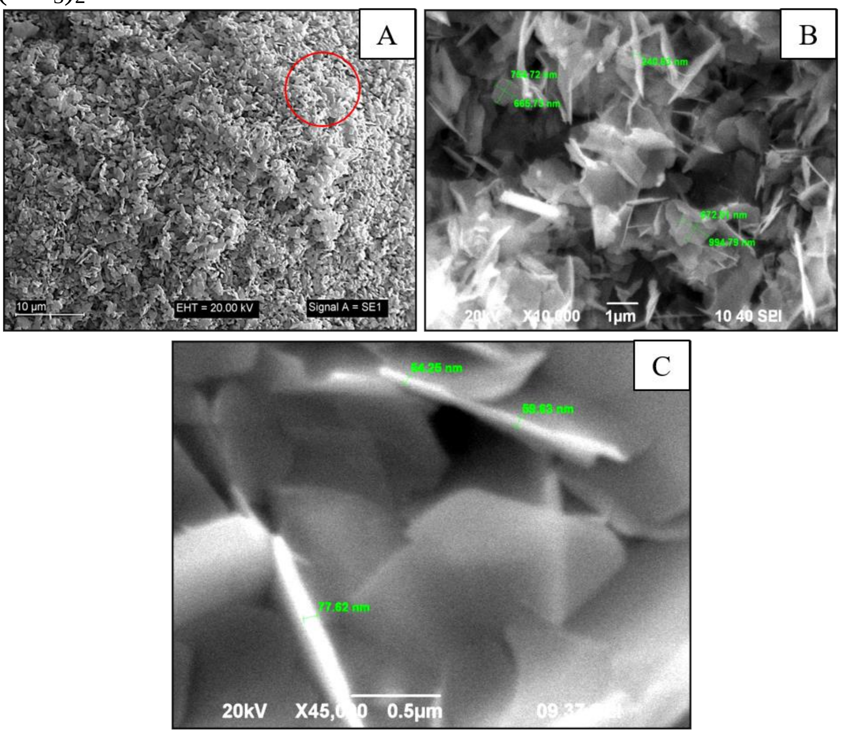


Figure 6. *SEM images of samples synthesized at 25 °C with initial OH/Zn molar ratio 1.6 and concentration of Zn(NO3)2 and NaOH 3.2 M. Scale bar: A: 10 µm; B: 1.0 µm; C: 0.5 µm.*

Fig. 6 (a) demonstrates the same morphology of the resulting precipitate but a visible decrease of the particle size, more often smaller than 1 μm. A slight morphology change can be seen after careful examination of the image, presented in Figs. 6 (b) and (c). Obvious domination of the sheet-like particles with thickness less than 100 nm, belonging to $(Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O)$ can be seen. Furthermore, although few in number, a new type of particles appears. Probably ZnO crystals or the intermediates to ZnO, as suggested by the weak characteristic peaks of ZnO in the X-ray pattern..

HRTEM measurements and Selected area electron diffraction (SAED) patterns experimentally confirmed the presence of ZnO in the samples, prepared et high concentrations of NaOH and $Zn(NO_3)_2$ (Fig. 7).

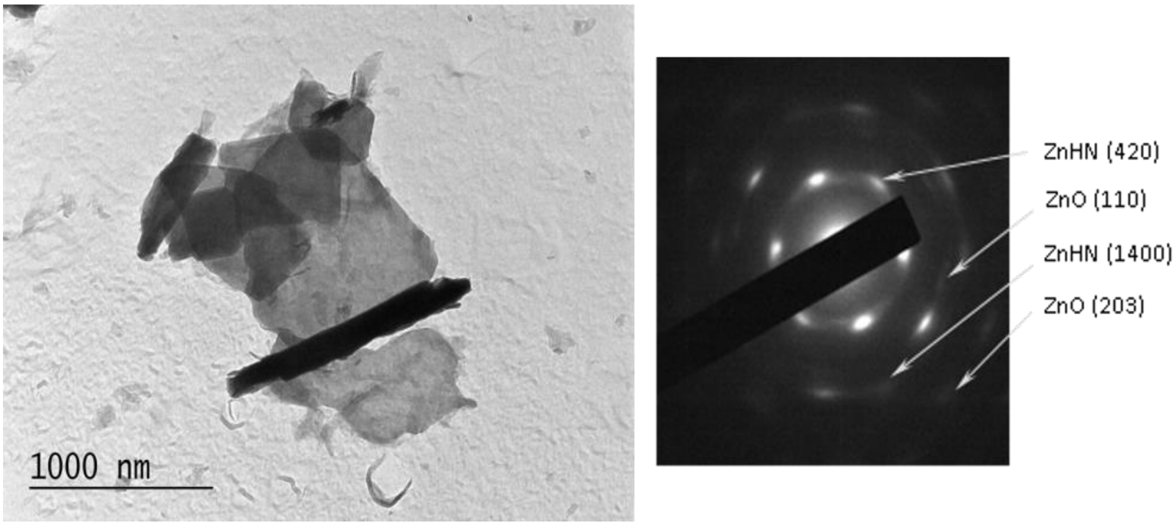


Figure 7. *TEM micrograph (at magnification 8 000 x) and SAED pattern of of sample synthesized at 25 °C with initial OH/Zn molar ratio 1.6 and concentration of zinc nitrate and sodium hydroxide 3.2 M.*

Except the concentration of the solutions used in the synthesis, the temperature and acidity of the medium are essential for the composition and stability of the resulting precipitates. Fig. 8 show the temperature influence on the samples prepared at 70 ^oC, initial OH/Zn molar ratio 1.6 and concentration of the NaOH and $Zn(NO_3)_2$ 3.2 M.

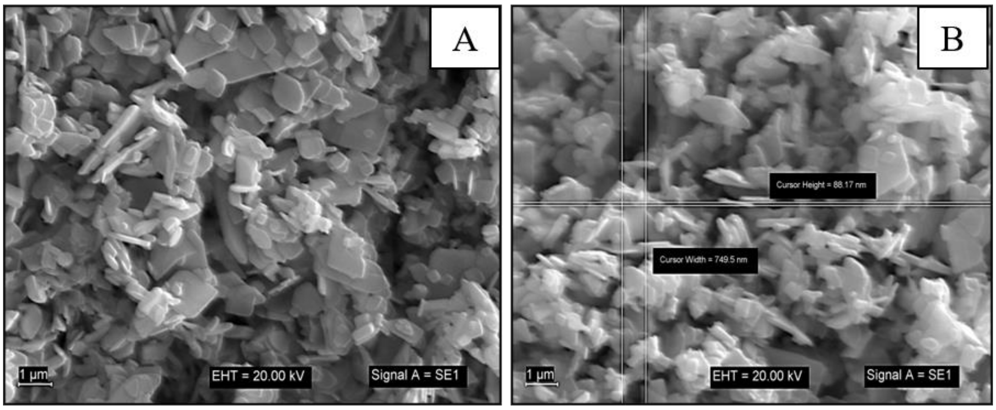


Figure 8. *SEM images of sample synthesized 70 ^oC, initial OH/Zn molar ratio 3.2 and concentration of the zinc nitrate and sodium hydroxide 3.2 M. Scale bar: A: 1.0 μm; B: 1.0 μm.*

No substantial change in the composition and morphology of the resulted sample can be observed. The sheet-like particles of $Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$ dominate and traces of ZnO and $Zn_3(OH)_4(NO_3)_2$ are also present. This result confirms the suggestion that increasing the temperature to 70 ^{o}C slightly affects the crystal phase and morphology of the precipitate.

The shelf life in the mother liquor of the precipitate obtained was monitored. The results are presented in Tables 4.

Table 4. *Chemical Composition (Zn, %) and weight loss at 450 °C (ΔG,%) of the Samples Synthesized at Room Temperature, OH/Zn Molar Ratio 1.6 and NaOH and $Zn(NO_3)_2$ Concentration 1.6 M after 60 Days Storage in Mother Liquor.*

№	Time	Zn, %	ΔG, %	pH
1	Immediately	53.4 ± 0.8	34.61	5.98
2	1 hour	52.0 ± 1.0	34.12	6.01
3	3 hours	53.1 ± 0.9	34.00	6.01
4	5 hours	52.6 ± 0.7	33.84	6.00
5	8 hours	53.2 ± 0.8	34.65	6.00
6	1 day	51.2 ± 0.9	34.13	6.00
7	10 days	52.0 ± 0.6	35.12	6.02
8	20 days	51.3 ± 1.0	34.53	6.03
9	30 days	53.2 ± 0.8	33.91	6.06
10	60 days	52.9 ± 0.8	34.50	6.10

The results presented in Tables correspond very closely to the theoretical ones and suggest no change in the composition and morphology of the precipitate. This suggestion was confirmed by the results of X-ray and SEM analysis, which are similar to the results presented above. Obviously the crystal phase does not undergo detectable change along the storage in the mother liquor (up to 60 days) which identifies the zinc hydroxide nitrate suspension as a promising feedstock for the preparation of foliar fertilizer.

IV.2. Synthesis of Layered Copper-Zinc Hydroxide Nitrate Nanoparticles

The aim of the study is to gain new knowledge about the preparation, properties and thermal decomposition of mixed Zn-Cu nanosized hydroxy nitrates. This will help to expand their use as precursors to produce important for practice nanostructured materials.

The mixed layered hydroxy salts usually are prepared by co-precipitation of dilute solutions of the corresponding nitrates in an alkaline medium. More often copper nitrate and sodium hydroxide are added simultaneously dropwise to the zinc nitrate solution. In our study, we chose a new approach using a mixture of concentrated solutions of zinc and copper nitrates (3.6 M) and initial OH/Zn molar ratio OH/(Zn+Cu) = 1.6. The time of precipitation was ten minutes in all cases. The precipitation of the mixed hydroxy nitrates was carried out at 60°C by (i) pouring 3.6 M KOH into nitrate mixture and (ii) by dropwise adding of copper nitrate and sodium hydroxide simultaneously to the zinc nitrate solution. After filtration and washing with distilled water, the resulting precipitate was dried at 65^{o}C for 12 h. The results for the mixed Cu-Zn hydroxy nitrates were very similar, so we will present only those obtained by the second method.

Zink hydroxide nitrate with the composition $Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$ was synthesized by the precipitation method. KOH solution was pouring into $Zn(NO_3)_2{\cdot}6H_2O$ solution under vigorous stirring at 60^{o}C for 10 minutes. The initial OH/Zn molar ratio was 1.6 and concentration of all compound was 1.6 M. Copper hydroxide nitrate with composition $Cu_2(OH)_3NO_3$ was synthesized by precipitation from a 3.2 M $Cu(NO_3)_2{\cdot}3H_2O$ at OH/Cu molar ratio of 1.5. The hydroxide nitrate with composition $Zn_3(OH)_4(NO_3)_2{\cdot}2H_2O$ was obtained by heating of $Zn(NO_3)_2{\cdot}6H_2O$ at 120^{f}I for 7 days.

For the study, $Zn_5(OH)_8(NO_3)_2.2H_2O$, $Zn_3(OH)_4(NO_3)_2$ and $Cu_2(OH)_3NO_3$ as reference compounds were synthesized. The resulting information on the composition, structure and properties of the pure compounds was used in the interpretation of the results for the composition, structure and properties of mixed hydroxy nitrates.

Fig. 9 presents the phase compositions of powder samples before and after calcination. No major characteristic peaks of the oxides can be found in the spectra of pure hydroxy nitrates, confirming their pure appearance.

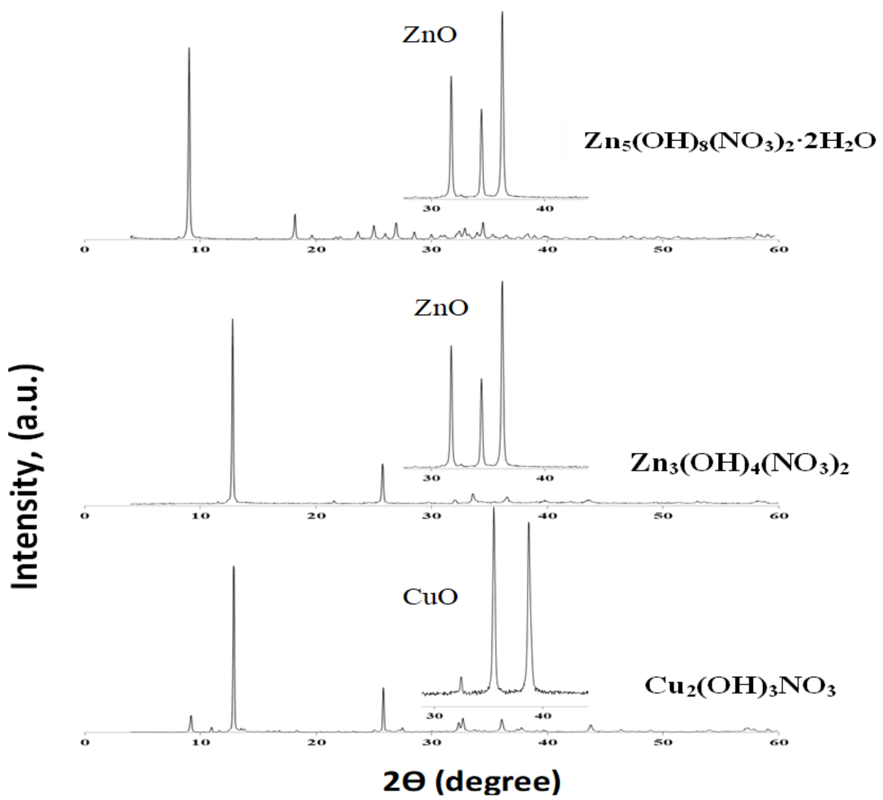


Figure 9. *X-ray pattern of $Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$, $(Zn_3(OH)_4(NO_3)_2$, $(Cu_2(OH)_3NO_3$, ZnO and CuO.*

FTIR spectra of as-prepared pure hydroxy nitrates were performed to identify the functional groups (Fig. 10).

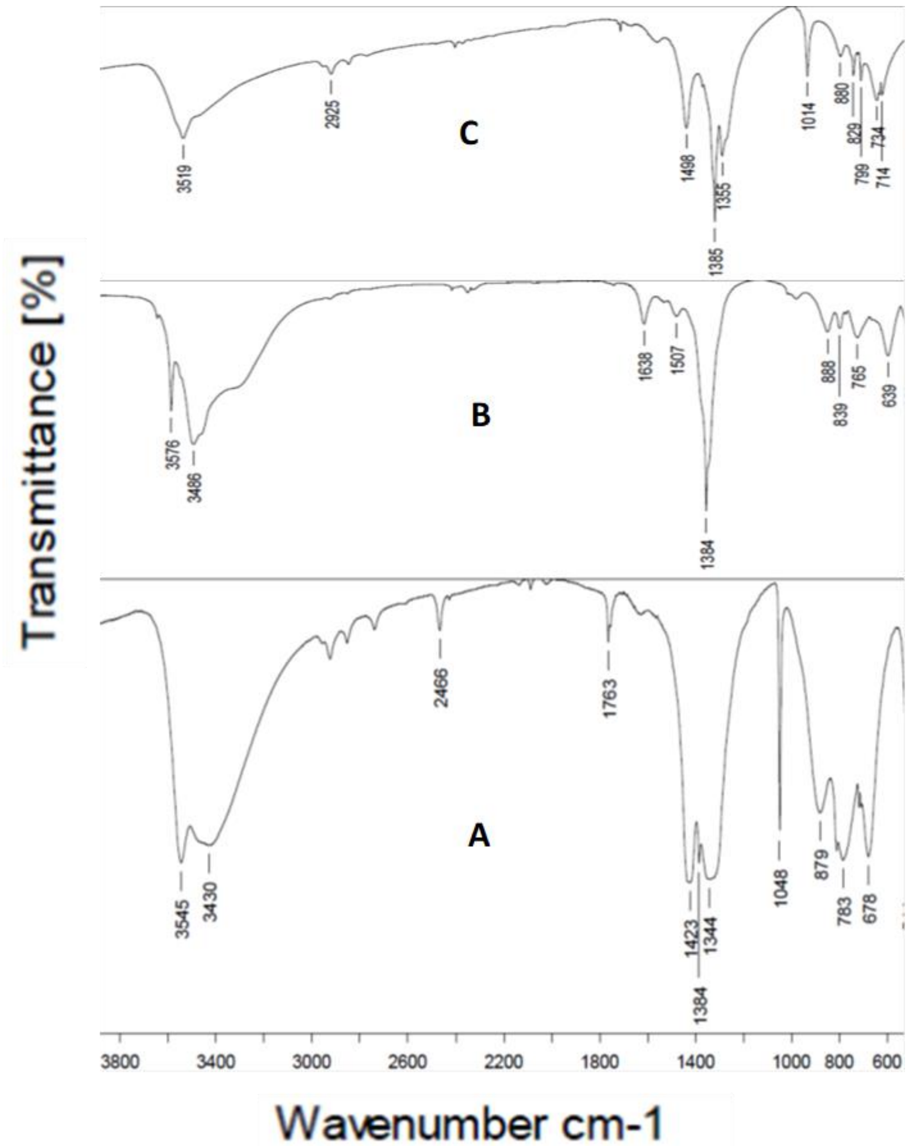
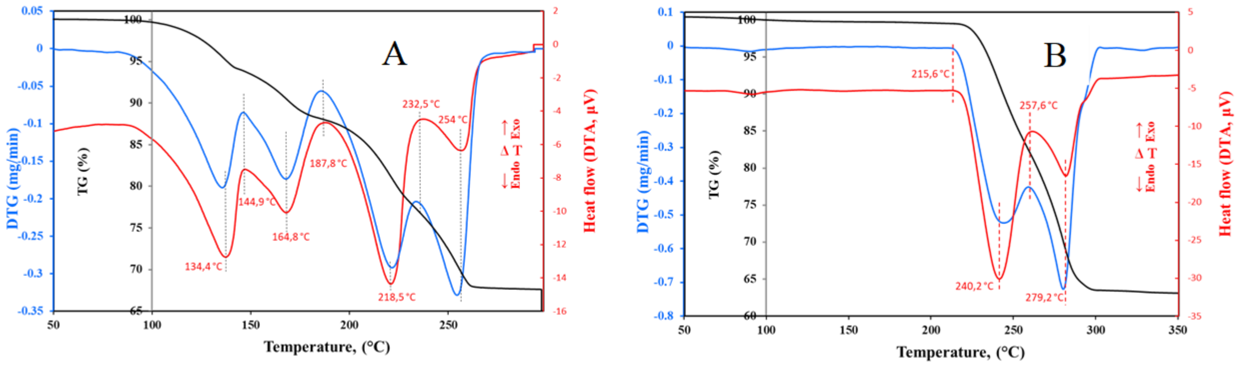


Figure 10. *FTIR spectra of ($Cu_2(OH)_3NO_3$ (A), $Zn5(OH)_8(NO_3)_2{\cdot}2H_2O$ (B), and $Zn_3(OH)_4(NO_3)_2$ (C) samples.*

Fig. 11 shows the DTA, DTG and TG curves of $Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$, ($Zn_3(OH)_4(NO_3)_2$ and ($Cu_2(OH)_3NO_3$. Analysis for H_2O, NO and NO_2 is given only for $Cu_2(OH)_3NO_3$.

From the results presented, it can be seen that the thermal decomposition of the three compounds proceeds in a fundamentally different way, in all cases the end product is the corresponding oxide. It makes the results obtained very useful in clarifying the composition and structure of mixed hydroxy nitrates.

The thermal decomposition of Zn_5HN is a four-step process occurring in the temperature range of 75 to 300^oC. There is no consensus in the scientific literature about its mechanism, but it is generally accepted that the first stage is related to the loss of 2 molecules of water (ΔG = 6.1%), the second - to the decomposition of Zn_5HN to Zn_3HN (ΔG = 5.8%) and the last two - with the decomposition of Zn_3HN to ZnO (ΔG = 21.5%). Zn_3HN undergoes decomposition in two stages and ΔG = 17.2 and 19.2% respectively, and the decomposition of CuHN occurs at a single step with ΔG = 33.4. TG/DTA curves are in full agreement with the results obtained by the XRD, FTIR and SEM analysis.



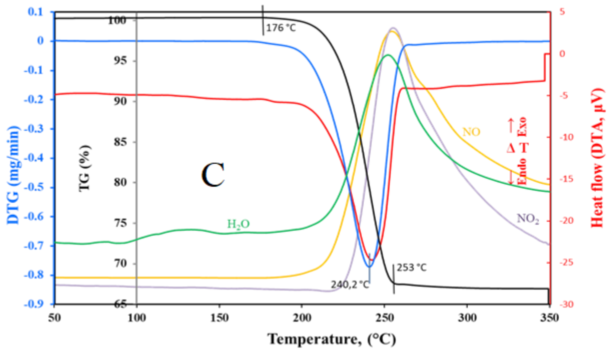
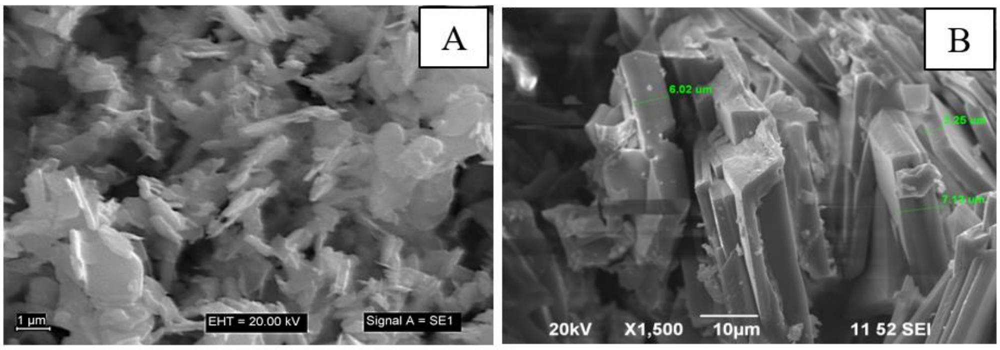


Figure 11. *Simultaneous DTA, DTG and TG cruves of $Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$ (A), $Zn_3(OH)_4(NO3)_2$ (B) and $Cu_2(OH)_3NO_3$ (C).*

Fig. 12 shows the SEM images of $Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$ (A), ($Zn_3(OH)_4(NO_3)_2$ (B), $Cu_2(OH)_3NO_3$ (C) samples.



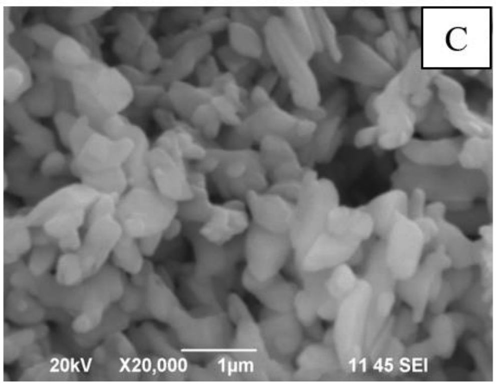


Fig. 12A. SEM image of $Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$ Scale bar: A: 10 µm; B: 1.0 µm

Fig. 12B. SEM image of $Zn_3(OH)_4(NO_3)_2$ Scale bar: A: 10 µm; B: 1.0 µm

Fig. 12C. SEM image of $Cu_2(OH)_3NO_3$ Scale bar: A: 10 µm; B: 1.0 µm

SEM images show that the synthesized hydroxy nitrates have a very different morphology. Only uniform sheet-like particles with thickness less than 100 nm, typical for zinc hydroxide nitrate with composition $Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$, can be seen in the first image. Zn_3HN (B) crystallizes in the form of well-formed square-shaped plates between 2 and

10 µm. The shape of $Cu_2(OH)_3NO_3$ (C) is characterized by belt-like particles, having a size of fewer than 1.0 µm.

All results presented are in agreement with the literature data and confirm the obtaining of pure $Zn_5(OH)_8(NO_3)_2·2H_2O$, $Zn_3(OH)_4(NO_3)_2$ and $Cu_2(OH)_3NO_3$.

Five mixed Cu-Zn hydroxy nitrates covering the entire concentration range of 20.0 to 80% Cu were synthesized: $Cu_{20}Zn_{80}$, $Cu_{40}Zn_{60}$, $Cu_{50}Zn_{50}$, $Cu_{60}Zn_{40}$ and $Cu_{80}Zn_{20}$ (Table 5). The table presents calculated and found Cu/Zn molar ratio, weight loss after calcination at 400 °C (ΔG) and Cu and Zn content in dried at 65°C samples.

Table 5. *Composition of $Cu_{20}Zn_{80}$, $Cu_{40}Zn_{60}$, $Cu_{50}Zn_{50}$, $Cu_{60}Zn_{40}$ and $Cu_{80}Zn_{20}$ samples.*

Sample	**Cu/Zn molar ratio**		**ΔG, %***	**Cu, wt. %**	**Zn, wt. %****
	Calculated	**Observed**			
$Cu_{20}Zn_{80}$	20/80	21.5/78.5	33,84 ± 1.02	11,21 ± 0.28	42,22 ± 1.22
$Cu_{40}Zn_{60}$	40/60	40.9/59.1	33,47 ± 0.96	20,02 ± 0.46	29.81 ± 1.46
$Cu_{50}Zn_{50}$	50/50	49.9/50.1	33. 48 ± 0.86	25.10 ± 0.48	25.00 ± 0.48
$Cu_{60}Zn_{40}$	60/40	61.6/38.4	33,48 ± 0.88	33,12 ± 1.15	21,23 ± 1.12
$Cu_{80}Zn_{20}$	80/20	81.0/19.0	33,67 ± 1.00	43,21 ± 1.28	10,44 ± 1.00

***ΔG –** Weight loss after calcination at 400 °C. **Data represent the mean of three independent replicates ± standard deviation.

The results presented in Table 5 confirm that the precipitation of the mixed hydroxy nitrates is complete and the content of Cu and Zn in them is very close to the theoretical calculation. The ΔG values for all samples are between the values of the pure compounds $Zn_5(OH)_8(NO_3)_2·2H_2O$ (34.67) and $Cu_2(OH)_3NO_3$ (33.4). This suggests that mixed hydroxy nitrates contain only these compounds in pure form or mixture, but not $Zn_3(OH)_4(NO_3)_2·2H_2O$, ΔG of which is 37%.

Figure 13 shows the XRD images of $Cu_{20}Zn_{80}$ (A), $Cu_{40}Zn_{60}$ (B), $Cu_{50}Zn_{50}$ (C), $Cu_{60}Zn_{40}$ (D), and $Cu_{80}Zn_{20}$ (E) samples.

The first diffractogram (A) indicates that $Cu_{20}Zn_{80}$ sample is probably a mixture of Zn_5HN and CuHN. The strongest peaks of Zn_5HN (at 2θ = 9.2) and CuHN (at 2θ = 12.8) are well expressed. No major characteristic peaks of ZnO or CuO can be found.

The spectra shown in Fig. 13 allow us to conclude that $Cu_{20}Zn_{80}$ and $Cu_{40}Zn_{60}$ samples are a mixture of Zn_5HN and mixed $(Zn_xCu_{2-x})(OH)_3NO_3$. The remaining samples do not contain Zn_5HN. They most likely represent pure $(Zn_xCu_{2-x})(OH)_3NO_3$ or a mixture of $(Zn_xCu_{2-x})(OH)_3NO_3$ and $Cu_2(OH)_3NO_3$.

In our view, the formation of mixed hydroxy nitrates based on Zn_5HN as host structure is impossible due to the large difference in the solubility of zinc and copper hydroxy nitrates. The reason for the presence of copper in the ZnO obtained after decomposition is the precipitation of mixed fine crystalline $(Zn_xCu_{2-x})(OH)_3NO_3$, whose decomposition leads to the formation of Cu doped ZnO.

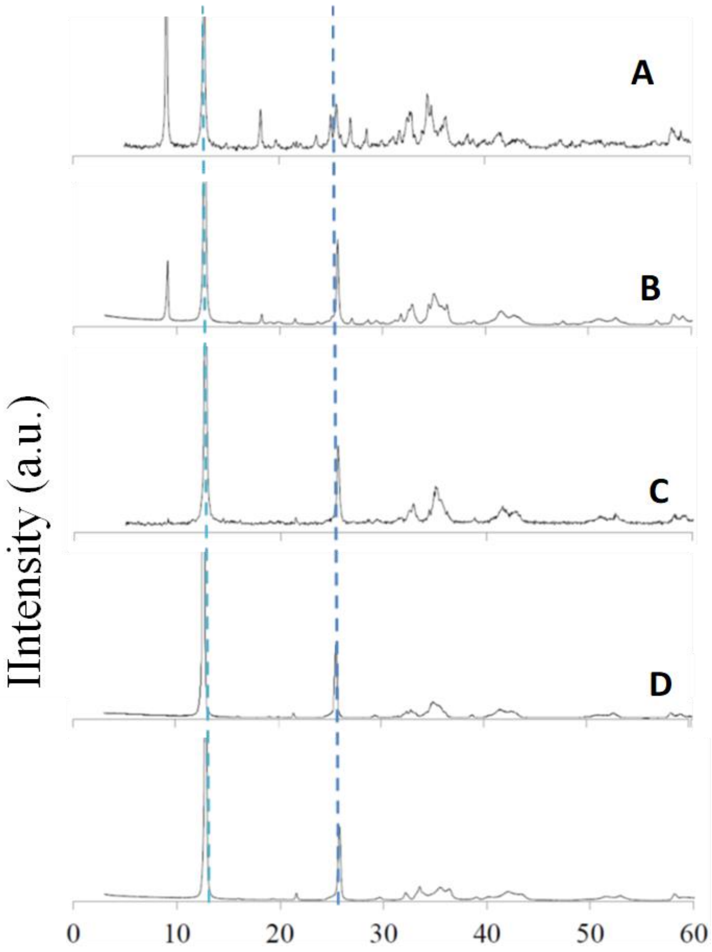


Figure 13. *XRD images of $Cu_{20}Zn_{80}$ (A), $Cu_{40}Zn_{60}$ (B), $Cu_{50}Zn_{50}$ (C), $Cu_{60}Zn_{40}$ (D), and $Cu_{80}Zn_{20}$ (E) samples.*

The fundamental difference between our results and those of other authors is probably due to the different conditions of the experiment. In our view, low concentrations of starting salts experiment lead to preferential precipitation of all copper ions as $Cu_2(OH)_3NO_3$, and then precipitation of zinc ions in the form of $Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$. This type of precipitation is described in detail in our earlier study. Taking into account our experience in the field of investigation, in this experiment, we have used concentrated solutions (3.6 M). Under these conditions, the concentration of Zn^{2+} is high, which does not allow preferential precipitation of $Cu_2(OH)_3NO_3$. This assumption is strongly supported by the additional FTIR, SEM and TEM studies presented below.

Figure 14 shows the FTIR spectra of $Cu_{20}Zn_{80}$ (A), $Cu_{40}Zn_{60}$ (B), $Cu_{50}Zn_{50}$ (C), $Cu_{60}Zn_{40}$ (D), and $Cu_{80}Zn_{20}$ (E) samples.

In all five spectra, a strong and broad absorption due to OH stretching vibration can be observed at approximately 3500 cm^{-1}. Well expressed absorption bands in the region from 880 to 1600 cm^{-1}, connected with interlayer anion can be seen. The single absorption band at 1640 cm^{-1}, characteristic for the presence of water in Zn_5HN is visible only in $Cu_{20}Zn_{80}$ (A) and $Cu_{40}Zn_{60}$ (B) samples which confirms the presence of this compound. The absorption lines around 1428 and 1341 cm-1, typical for asymmetric and symmetric NO_2 stretching bands of CuHN, confirm the assumption that these samples are a mixture of Zn_5HN and CuHN. The spectra of the other three samples are practically identical and are very close to the FTIR spectrum of CuHN. The obtained results give us reason to assume that $Cu_2(OH)_3NO_3$ is the host structure of all mixed Zn-Cu hydroxy nitrates contained in the synthesized samples.

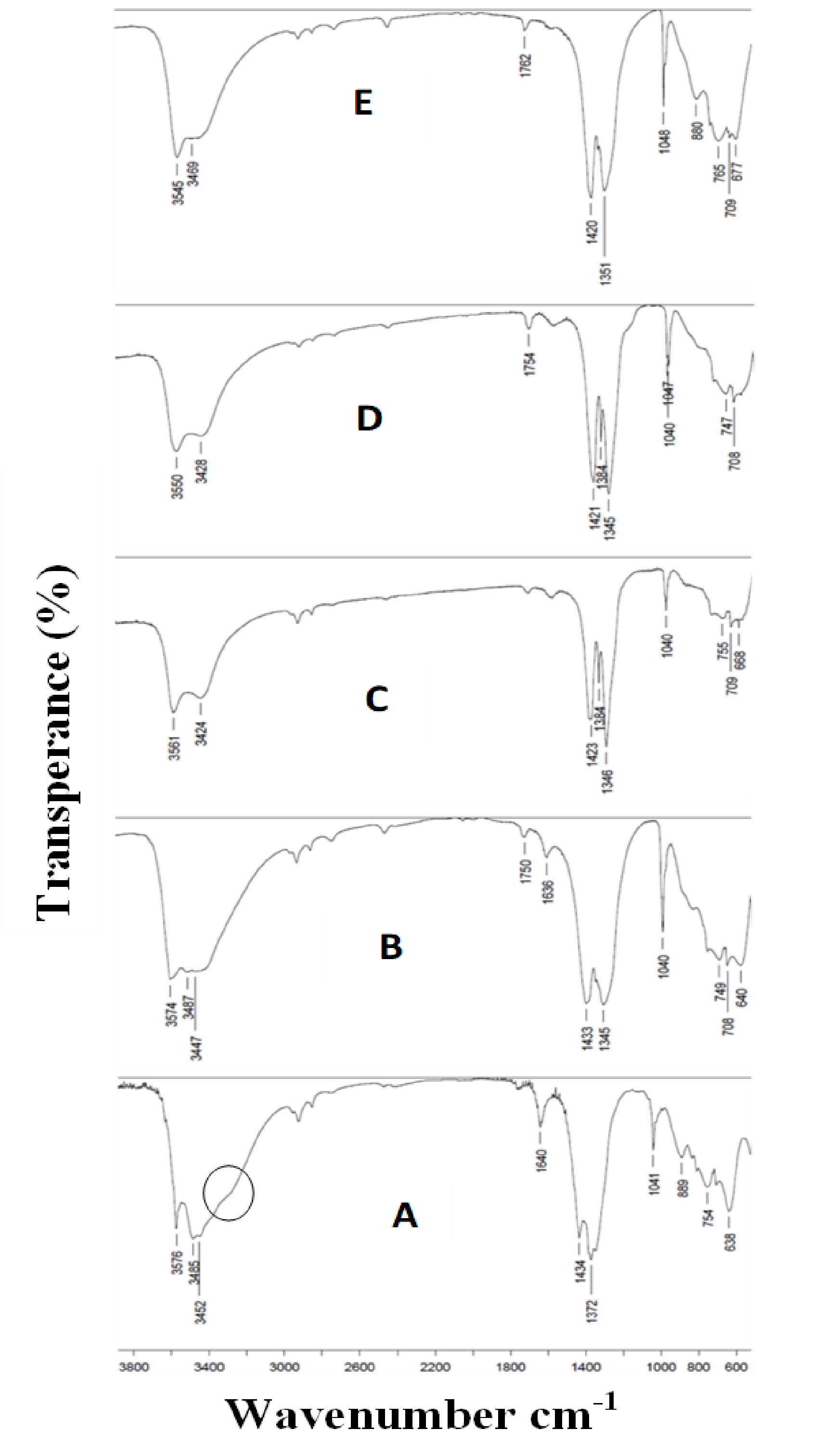


Figure 14. *FTIR spectra of $Cu_{20}Zn_{80}$ (A), Cu40Zn60 (B), Cu_{50}Zn50 (C), $Cu_{60}Zn_{40}$ (E), and $Cu_{80}Zn_{20}$ (D) samples.*

Figure 15 shows the DTA, DTG and TG curves of $Cu_{20}Zn_{80}$ (A), $Cu_{40}Zn_{60}$ (B), $Cu_{50}Zn_{50}$ (C), $Cu_{80}Zn_{20}$ (D), and $Cu_{60}Zn_{40}$ (E) samples.

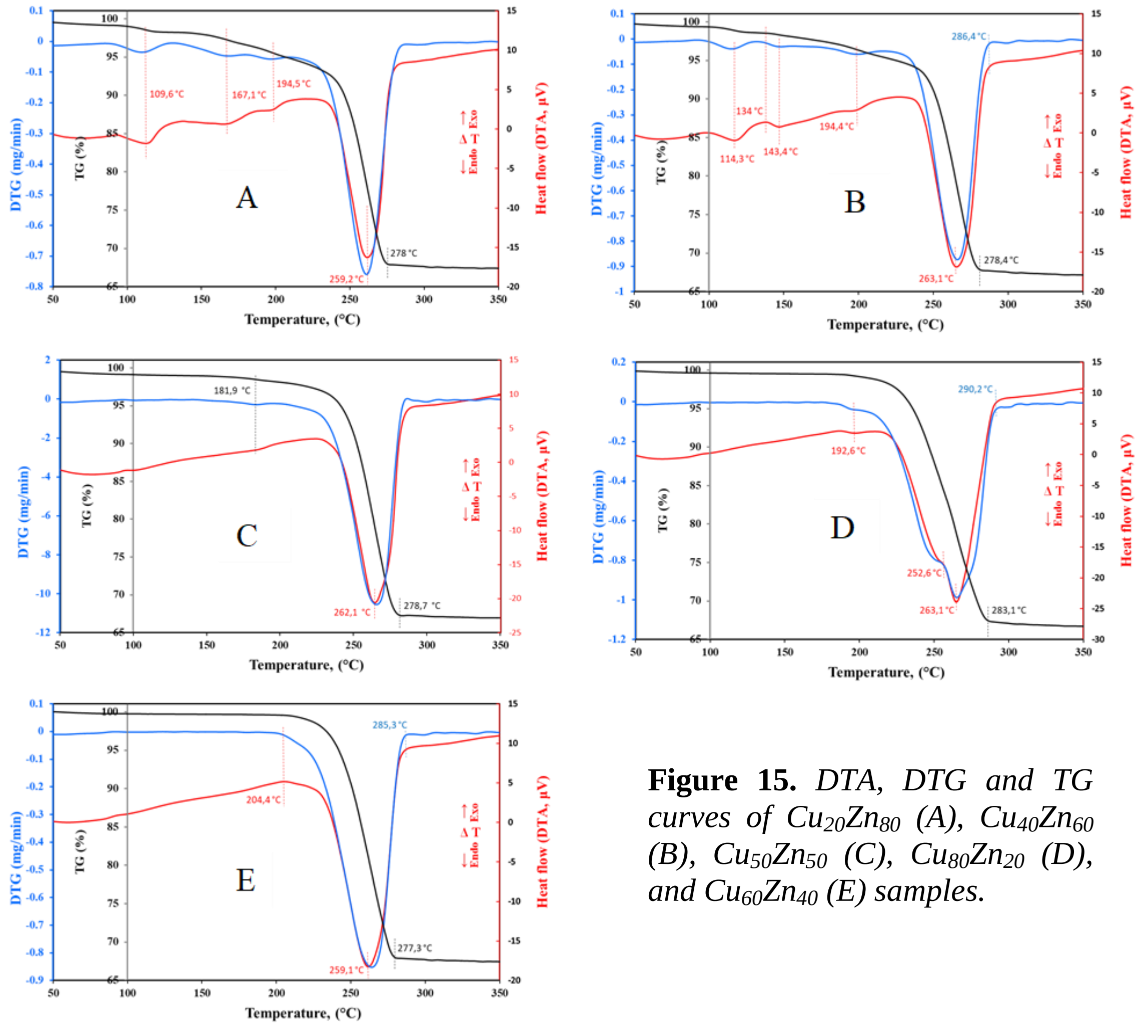


Figure 15. *DTA, DTG and TG curves of $Cu_{20}Zn_{80}$ (A), $Cu_{40}Zn_{60}$ (B), $Cu_{50}Zn_{50}$ (C), $Cu_{80}Zn_{20}$ (D), and $Cu_{60}Zn_{40}$ (E) samples.*

The starting point in our analysis of the results presented in Figs. 15 is the result of sample $Cu_{50}Zn_{50}$ (C). Chemical analysis showed that the precipitation of the two nitrates ($Cu(NO_3)_2.3H_2O$ and $Zn(NO_3)_2.6H_2O$) was complete and the molar ratio Cu/Zn in the sample dried at 65 °C was 50/50. The process of its thermal decomposition is one-step and follows that of pure CuHN. This gives us reason to believe that the compound obtained is with the composition $(Cu_xZn_{2-x})(OH)_3NO_3$, where x = 1.0. The decomposition of $Cu_{50}Zn_{50}$ is a one-step process with a weight loss of 33.48%. This value is very close to the theoretical calculated - 33.47%. Analysis of the results for the remaining samples leads to the conclusion that this compound is present in all of them together with pure Zn_5HN in the samples with a molar ratio Cu/Zn <1.0 and pure CuHN in the samples with Cu/Zn> 1.0.

The thermal decomposition of samples $Cu_{20}Zn_{80}$ and $Cu_{40}Zn_{60}$ (A and B) is a three-step process with total weight loss of 32.7 and 33.0 respectively. However, the DTG and DTA curves show that the first and the second steps of weight loss are in the temperature range up to 180°C. They are connected with the loos of water (first step) and decomposition of Zn_5XN to Zn_3HN (second step). Taking into account the fact that the weight loss of pure ZnHN in this temperature range is 11.6% (theoretically two stages of 5.8%), we can calculate with a high degree of reliability it's content in the mixed samples. For the sample with composition

$Cu_{20}Zn_{80}$ the content of Zn_5HN is 56.0%, and in the sample with composition $Cu_{40}Zn_{60}$ - 21.5%. The remaining 44.0 and 78.5% belong to the mixed $(Cu_xZn_{2-x})(OH)_3NO_3$. We suppose that the mixed samples have the composition $(Cu_1Zn_1)(OH)_3NO_3$. The results for Zn_5HN, obtained by the thermal analysis, are very close to the theoretical value of 60.8% at Cu/Zn molar ratio of 20/80 and 20.6% at Cu/Zn molar ratio 40/60 and support our suggestion.

The third weight loss is related to the simultaneous decomposition of Zn_3HN and the formed mixed hydroxy nitrate. An increase in the Cu/Zn molar ratio leads to a decrease in the weight loss in the first and second stages from 6.5 at $Cu_{20}Zn_{90}$ to 2.5% at $Cu_{40}Zn_{60}$. This is associated with the reduction of Zn_5HN content and the incorporation of Zn^{2+} in the mixed hydroxy nitrates. Based on the results presented, we can conclude that these two samples are a mixture of Zn_5HN and $(Zn_xCu_{2-x})(OH)_3NO_3$, as most likely x = 1.0.

The thermal decomposition of samples $Cu_{50}Zn_{50}$, $Cu_{60}Zn_{40}$ and $Cu_{80}Zn_{20}$ (C, E and D) is completely different. As noted above, the $Cu_{50}Zn_{50}$ sample is most likely a mixed hydroxide nitrate with a fixed composition $(Zn_{1.0}Cu_{1.}0)(OH)_3NO_3$ with one step of thermal decomposition. The decomposition of $Cu_{60}Zn_{40}$ is a one-step process with a weight loss of 32.8%. The results of the thermal analysis of both samples are very similar.

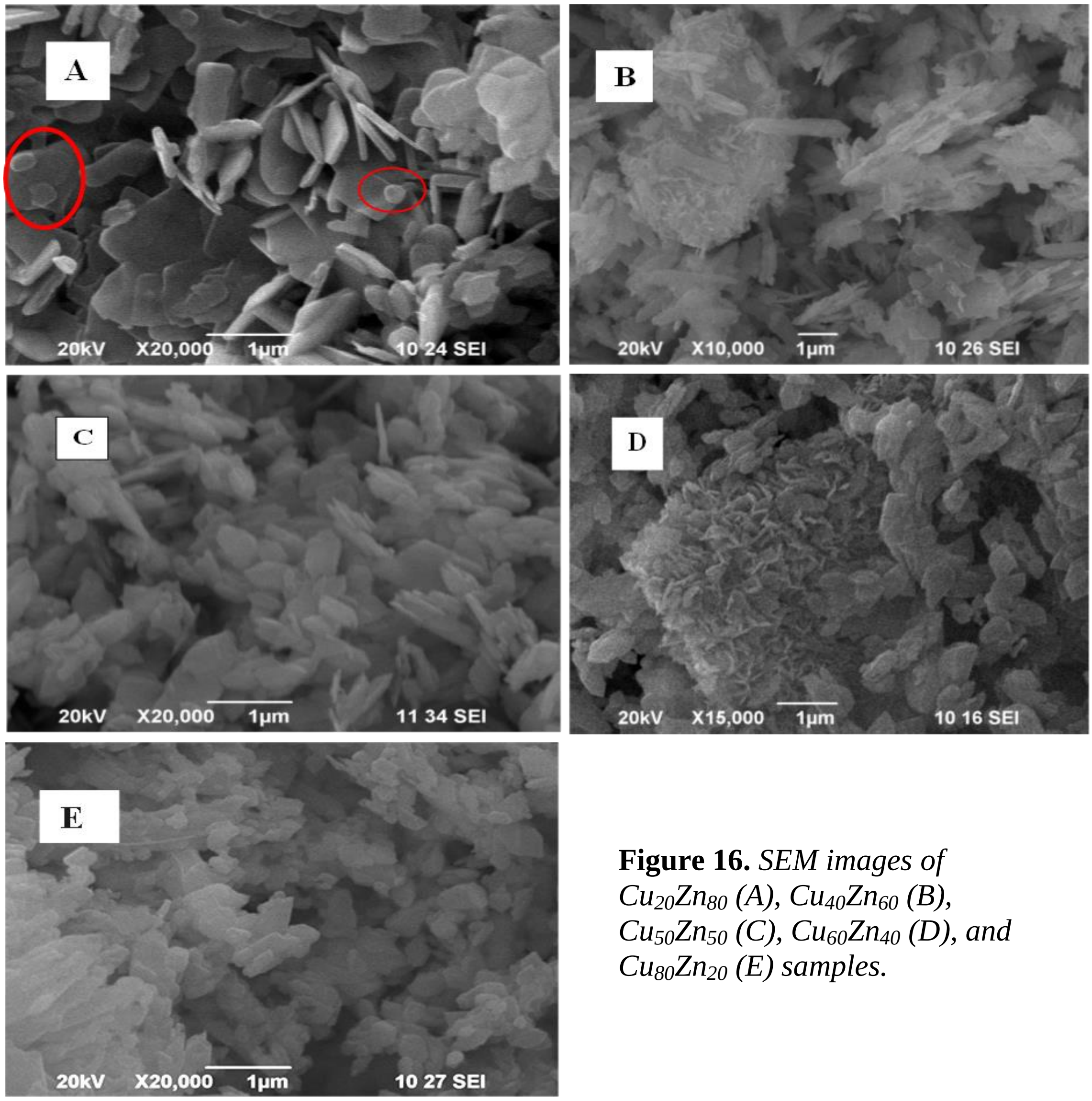


Figure 16. *SEM images of $Cu_{20}Zn_{80}$ (A), $Cu_{40}Zn_{60}$ (B), $Cu_{50}Zn_{50}$ (C), $Cu_{60}Zn_{40}$ (D), and $Cu_{80}Zn_{20}$ (E) samples.*

The results in Fig. 15D strongly indicate that the increasing copper content results in separation of $Cu_{80}Zn_{20}$ to two compounds. The onset and the edge temperatures (185 and 254^oC) of the first compound are very close to the typical temperatures of the pure $(Cu)_2(OH)_3NO_3$. No water was found in the gas products of $Cu_{50}Zn_{50}$, $Cu_{60}Zn_{40}$ and $Cu_{80}Zn_{20}$ samples, which excludes the presence of Zn_5HN. NO and NO_2 separation is a one-step process at all samples.

Fig. 16 shows the SEM images of $Cu_{20}Zn_{80}$ (A), $Cu_{40}Zn_{60}$ (B), $Cu_{50}Zn_{50}$ (C), $Cu_{60}Zn_{40}$ (D), and $Cu_{80}Zn_{20}$ (E) samples.

The image of sample $Cu_{20}Zn_{80}$ (Fig 16A) clearly shows sheet-like morphology, typical for pure Zn_5HN (nanoplates). Also noticeable are small spherical crystals with a diameter below 20 nm, which in our opinion confirm the presence of mixed ZnCuHN hydroxy nitrate. The shape of $Cu_{40}Zn_{60}$ sample (Fig. 16B) is featured with two groups of sheet-like particles with different dimensions. The larger ones are probably Zn_5HN and the smaller ones are mixed ZnCuHN hydroxy nitrates. This claim is supported by the results presented in Fig. 16D ($Cu_{60}Zn_{40}$). In our view, this sample is a mixture of Cu_3HN (large crystals) and mixed ZnCuHN (small sheet-like particles). The sample $Cu_{50}Zn_{50}$ (Fig. 20C) has uniform sheet-like morphology which we believe confirms the formation of mixed hydroxide nitrate with the composition $(Zn_1Cu_1)(OH)_3NO_3$.

IV.3. Application of Zn-containing Foliar Fertilizers for Recovery the Yielding Potential of Zn-deficient Young Maize Plants

The key objective of this paper is to evaluate the possibilities to recover the yielding potential of Zn-deficient young maize plants by application of nanosized Zn-containing foliar fertilizers. The sensitivity of maize to zinc fertilization and its effect on the physiological status of the plants are investigated also.

Bulgarian maize hybrid Kneja 300 was used in our experiment. Preliminary analysis of corn grains (4433 grains kg^{-1}) showed zinc content 14.81 mg kg^{-1}. Commercial foliar fertilizer (Zintrac 700), containing nanosized ZnO and nanosized zinc hydroxide nitrate suspension was used as foliar fertilisers.

Table 6. Nutrient solution composition.

Macroelements		Microelements	
Ca	$Ca(NO_3)_2$, 1.000 g L^{-1}	**Zn**	$ZnSO_4.7H_2O$, 0.576 mg L^{-1}
P	KH_2PO_4, 0.25 g L^{-1}	**Cl**	KCl, 3.728 mg L^{-1}
Mg	$MgSO_4.7H_2O$, 0.25 g L^{-1}	**B**	H_3BO_4, 1.546 mg L^{-1}
K	KCl, 0.125 g L^{-1}	**Mn**	$MnSO_4$, 0.338 mg L^{-1}
Fe_1	$FeCl_3$, 0.012 g L^{-1}	**Cu**	$CuSO_4$, 0.124 mg L^{-1}
Fe_2	FeEDDHA, 0.01 g L^{-1}	**Mo**	Na_2MoO_4, 0.122 mg. L^{-1}

The plants were grown as a substrate–hydroponic culture on ½ strength Hoagland nutrient solution (Table 6) which was replaced weekly to maintain nutrient concentrations at desired levels.

- *Controlled experiment*

A controlled experiment was carried out in a climatic chamber of the department of Physiology, the Agricultural University of Plovdiv in 2019 from early March to late May. The maize plants were cultivated in a controlled environment: photoperiod - 12 hours, photosynthetic photon flux density (PPFD) – 200 µmol m^{-2} s^{-1} (cool-white fluorescent lamps), temperature - 25±1°C/20±1°C (day/night) and relative air humidity - 60±5%. After the emergence, the plants (36 pieces) were divided into four groups of nine plants (three repetitions, each repetition with three plants) - control (Variant I) and Zn-deficient groups (Variants II-IV). The control group was fed with complete (all nutrients) solution, while the other by the same solution but without Zn. The nutrient solution was replaced weekly to maintain nutrient concentrations at desired levels. Single spraying in mid-April (4 - 5 fully emerged leaf) was performed as follows: (i) Variant III-spraying with commercial foliar fertilizer, containing nanosized ZnO of 3.5 g L^{-1} (3.5 g Zn L^{-1}) and (ii) Variant IV - spraying with nanosized zinc hydroxide nitrate, synthesized by us with Zn concentrations of 3.5 g L^{-1}. The effects of the applied fertilizers on Zn-deficient plants were evaluated two weeks after the foliar feeding using as criteria leaf gas exchange and chlorophyll fluorescence parameters, macro- and microelement content of plant organs as well as fresh biomass of the plants.

At the end of May, three plants of each variant were removed from the chamber (Fig. 21B) and carefully washed to remove zinc from the surface. Fresh biomass as well as macro- and microelement content in plant organs were measured.

- *Field experiment*

The remaining plants after the controlled experiment (6 plants of each variant) were moved to the Experimental Research Farm of the university. After harvesting (end of September), the cobs were collected and random samples of grain were mixed, milled and analysed for dry matter, protein, fat and starch. The roots, stems and leaves of the plants were analysed for micro- and macronutrient content.

- *Impact of foliar fertilization on plant growth and physiology*

The average amount of fresh mass of the plants varies within a relatively narrow range - 48.1, 42.0, 44.4, and 49.2 for Variants 1, 2, 3 and 4 respectively. The retarding effect of Zn deficiency (Variant 2) on the maize growth was not strong (13%), but this was not a surprise, because plant development at that stage depends, at least, partially on the seed reserves. The foliar application of ZnO containing fertilizer tended to improve to some extent (6%) the growth of maize plants. The application of ZnHN fertilizer completely compensates the lag in growth. The root system of all plants was well developed (Fig. 17B). The maize plants grown in Zn-deficiency were distinguished by visual chlorotic symptoms of the leaves.

The Zn-deficient plants showed significantly decreased the leaf gas exchange parameters (Table 7). The net photosynthetic rate (A) was reduced by 49%, the transpiration rate (E) – with 36% and the stomatal conductance (g_s) – with 40%. The relatively equal repression of these parameters indicates that stomatal limitation could be one of the leading factors for photosynthesis inhibition. Our results correspond to those of Mattiello et al. (2015), who found that zinc deficiency significantly decreased A and g_s in maize plants.

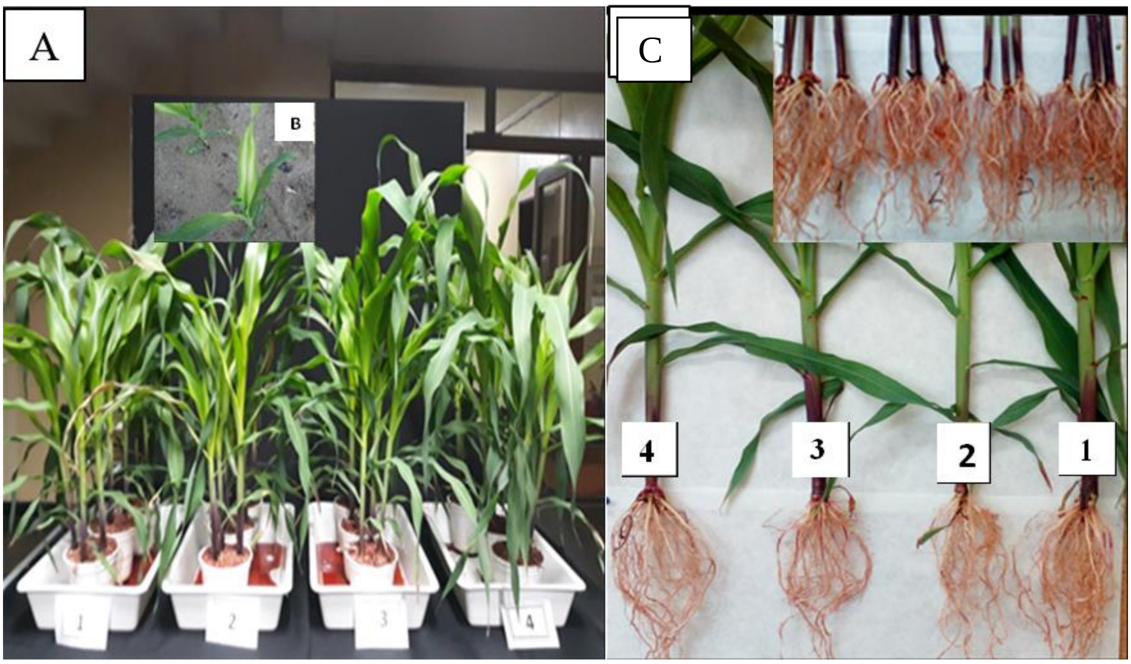


Figure 17. *Controlled experiment: A - maize plants two weeks after spraying; B - maize plants two weeks before spraying ; C - maize plants at the time of moving to Experimental farm.*

Table 1. *Influence of Zn deficiency and foliar application by Zn-containing fertilizers on leaf gas exchange parameters of young maize plants. A – net photosynthetic rate (µmol CO2 m-2 s-1), E – transpiration rate (mmol H2O m-2 s-1), gs – stomatal conductance (mol m^{-2} s^{-1})*

Variant	**A***	**E**	**gs**
Control	12.21 ± 1.00^{a}	0.76 ± 0.05^{a}	0.05 ± 0.00^{a}
2	6.26 ± 0.88^{c}	0.49 ± 0.06^{b}	0.03 ± 0.00^{b}
3	9.30 ± 0.79^{b}	0.56 ± 0.02^{b}	$0\ 04 \pm 0.01^{a}$
4	11.14 ± 0.20^{a}	0.76 ± 0.01^{a}	0.05 ± 0.00^{a}

**The data presented are sample means + SD. Different letters (a, b and c) following the SD values indicate significant differences (P<0.05).*

However, a strong improvement of leaf gas exchange was observed in Zn-deficient plants after the application of both Zn-containing products. The values of A, E and g_s of ZnO-fertilized plants were significantly higher than those of untreated Zn-deficient ones by 49, 14 and 33%, respectively, but still smaller as compared with those of the control plants. The effect of ZnHN was bigger than ZnO, leading to almost complete restoration of the leaf gas exchange of Zn-deficient maize plants. The application of this fertilizer improved A by 78% accompanied by 55 and 67% enhancement of E and g_s, respectively, as compared with Zn-deficient plants.

Zn deficiency slightly and insignificantly decreased the total chlorophyll content and maximal quantum yield of PSII (F_v/F_m) (Table 8). The decreased value was a bit lower but close to the range typical for healthy plants - 0.75-0.83.

The apparent photosynthetic electron transport rate (ETR) of Zn-deficient plants was also reduced by 31%. The quenching analysis showed that in Zn-deficient plants both photochemical quenching (qP) and non-photochemical quenching (qN) were diminished, by 25 and 27%, respectively.

Table 8. *Influence of Zn deficiency and foliar application by Zn-containing fertilizers on total chlorophyll content (mg m2) and selected chlorophyll fluorescence parameters of young maize plants: Fv/Fm – maximal quantum yield of PSII, ETR – apparent electron transport rate (µmol m^{-2} s^{-1}), qP – photochemical quenching, qN – non-photochemical quenching of chlorophyll fluorescence.*

Variant	Total chlorophyll	**Chlorophyll fluorescence parameters***			
		F_v/F_m	**ETR**	**qP**	**qN**
Control	200±15[a]	0,76[a]	40,3[a]	0,409[a]	0,341[a]
2	164±11[b]	0,72[a]	27,8[b]	0,308[b]	0,250[b]
3	175±8[b]	0,75[a]	38,9[a]	0,450[a]	0,374[a]
4	182±10[b]	0,75[a]	59,6[a]	0,665[c]	0,310[a]

**The data presented are sample means+ SD. Different letters (a, b and c) following the SD values indicate significant differences (P<0.05).*

As qP represents the relative part of open reactive centres of the PSII, its decrease could be explained as the Zn deficiency-induced lowering of these centres. The qN represents the heat dissipation losses and normally its values increased in stress situations. Here, the qN values in Zn-deficient plants surprisingly increased. In these conditions, the application of both Zn-containing fertilizers significantly improved the primary photochemistry of maize plants. The values of Y, ETR and qP increased, more significantly in the plants fertilized by ZnHN.

The adverse influence of Zn-deficiency on the light stage of photosynthesis is probably one of the possible reasons for the limitation of photosynthetic capacity in maize leaves.

- Impact of foliar zinc fertilization on yield and grain structural components

Within the mineral-related abiotic stresses, Zn deficiency is one of the most widespread limiting factors to maize production This is confirmed by the results of our experiment. Picture of corn cobs, collected after harvesting is presented in Fig. 18.

A drastic difference in grain yield between variants can be seen. Only three of the six plants from Variant 2 formed cobs, while for the control they are five and for Variants 3 and 4, six each. The same trend is observed in the number of grains - while in Variant 2 they are nearly absent, in control and Variant 3 two of the cobs are fully developed and in Variant 4 all cobs are fully developed.

The results obtained are a good indicator of the periods of maize growth during which zinc plays an important role in yield. It can confidently be concluded that zinc feeding during the initial growth stages is of great importance and plays a decisive role in the formation of the reproductive organs of maize. The explanation for this is that zinc stimulates the processes of biomass accumulation through increasing the effectiveness of other nutrients, particularly nitrogen, which is an indirect yield-forming function of zinc. The main problem is that during this period the soil temperature is low and the root system of the plants is not sufficiently developed. This does not allow them to accumulate the amount of zinc they need at this stage in their development. Soil fertilizing with zinc-containing fertilizers can help, but it cannot completely solve this problem. The application of foliar fertilizer is extremely suitable as its absorption is much faster than from the soil. The advantage of ZnHN is that its nanoscale crystals remain on the leaf surface for a long time and act as a reservoir for zinc delivery over a long period.

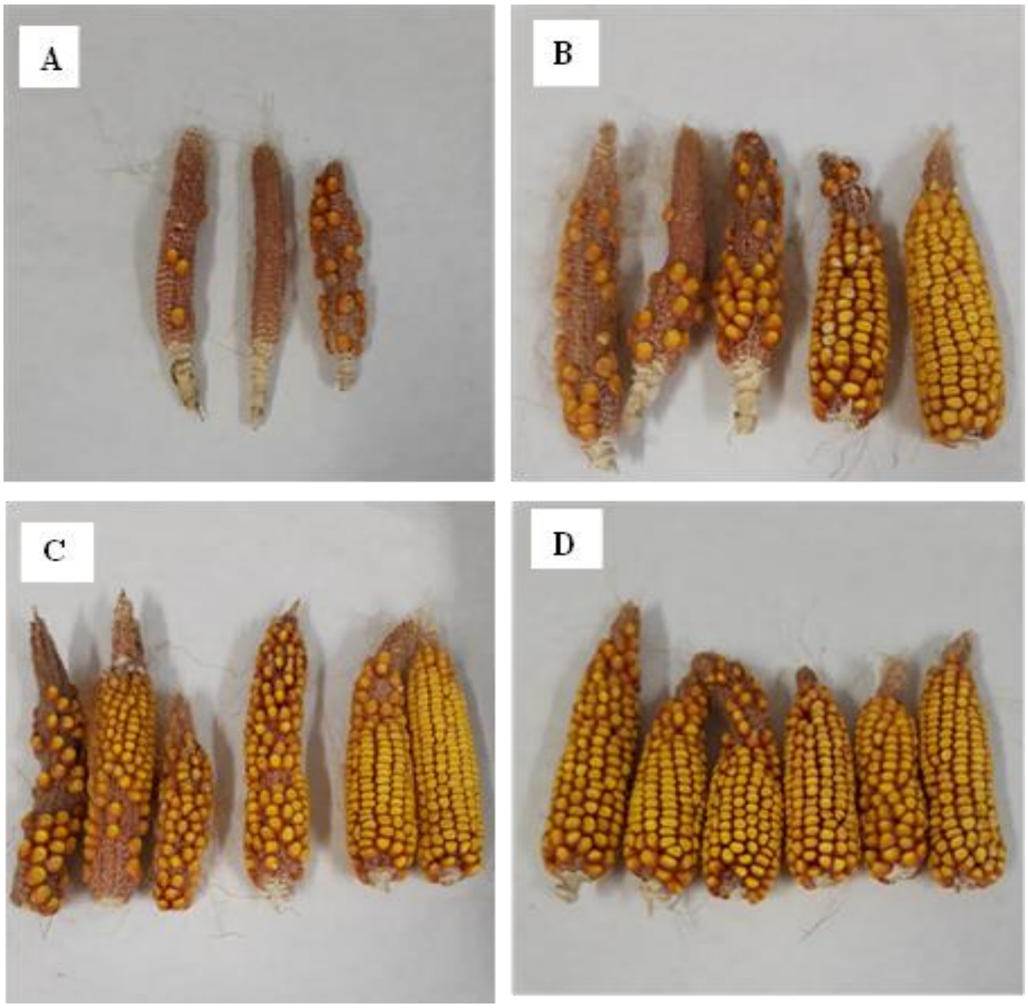


Figure 18. *Picture of corn cobs, collected after harvesting: A – Control; B – Variant II; C – Variant III; C – Variant IV.*

The impact of zinc fertilization on maize yield is not in doubt. It is important knowing to what extent this also applies to grain structural components. Tables 8 presents the results for the main structural components (dry matter, protein, fat and starch) of the grains collected in our experiment.

Table 8. *Average data for grain structural components*.*

Variant	Dry matter	Protein content, %	Fat content, %	Starch content, %
Control	88,99	11,40	4,43	61,62
2	89,05	9,04	4,47	69,07
3	88,87	9,96	4,76	65,68
4	88,81	9,02	4,89	66,36
Average	**88.93 ± 0.11**	**9.86 ± 1.12**	**4.64 ± 0.22**	**65.68 ± 3.08**

** Each value is averaged over three separate measurements*

Presented results show that the differences in the values of grain structural components are smaller than those of the yield. Data for dry matter and fat in grains varied within a narrow range, with no significant ($p < 0.05$) differences between variants. They do not differ significantly from the values obtained in our previous field experiments with maize. Protein and starch content vary in wider borders. It is noteworthy that the protein content of the corn grains of Variant 1 is greatest and the difference with the other variants is significant at $p < 0.05$ and the content of starch is lower. In the other Variants, the values for the two components are close. This result is probably due to the very low yield.

The results obtained gave evidence to conclude that both applied zinc fertilizers entirely recover the physiological performance of plants, being zinc hydroxide nitrate superior to commercial ZnO. The reasons for this are two (i) zinc supply at the time of the critical period for yield formation (ii) long-term performance of the used fertilizers after spraying. The better results with the use of zinc hydroxide nitrate are due to its better solubility and easier absorption by plants.

On the basis of the results obtained, we can conclude that Zn-fertilization of maize plants during the initial growth stages is of great importance and plays a decisive role in the formation of the reproductive organs of maize. The application of foliar fertilizers is extremely suitable as the possibility of much faster zinc absorption than from the soil.

IV.4. Impact of foliar fertilization with nanosized zinc hydroxide nitrate on maize yield and quality – field experiments 2016

The field experiment was conducted at the Research Farm, Agricultural University, Bulgaria, via a randomized block design with eight variants in 3 replications. The size of the harvesting plots was 20 m^2, and the number of plants/plot was 133 ± 2. The base fertilization (NPK 15:15:15, 5.0 kg are^{-1}) and drip irrigation were the same for all variants. The hybrid Pr 9241, group 370 according to the FAO, was chosen for the experiment, given its excellent qualities (high ecological flexibility) and acclimatization to the area of research.

Table 9. *Soil properties and total and available Ca, Mg, Cu, Fe and Zn concentrations.*

Soil test parameter	Test level	Test rating
Soil pH (1:5)	8.20 ± 0.02	Alkaline
Electrical conductivity (μS cm^{-1})	300	-
Organic matter (%)	0.952 (1.64)	low
(Nutrients mg kg^{-1})		
Nitrogen (available, 1,0% KCl)	42.65 ± 2.10	Medium
Potassium (EPA 3051)	4320.2 ± 27.4	Medium
Potassium (available, 2 N HCl) (K_2O)	205.74 ± 7.43	Medium
Phosphorous (P_2O_5) (available, laktaten)	43.86 ± 2.20	Medium
Calcium (EPA 3051)	432.20 ± 2.76	Low
Calcium (available, 1N KCl)	269.50 ± 5.66	Low
Magnesium (EPA 3051)	4710 ± 57.43	Medium
Magnesium (available, 1N KCl)	128.75± 4.54	Medium
Copper (EPA 3051A)	16,22 ± 0.38	Medium
Copper (available, DTPA)	2.24 ± 0,08	high
Manganese (EPA 3051A)	160.26 ± 3.24	Medium
Manganese (available, DTPA)	13.84 ± 0.30	high
Iron (EPA 3051A)	10970 ± 270	Medium
Iron (available, DTPA)	7.40 ± 0.06	Low
Zinc (EPA 3051A)	135 ± 1.02	Low
Zinc (available, DTPA)	2.82 ± 0.10	Low

Before planting, surface soil samples (0 - 20 cm depth) from each harvesting plot were collected, mixed, air dried and analysed for the selected physicochemical properties in the university laboratory, which is accredited under BDS EN ISO 17025/2006 for soil and plant analyses (Table 9).

The soil in this research area was alluvial with a highly alkaline pH (8.20) and low content of organic matter. It is characterized by a low content of available Ca, Cu, Mn, and Fe and a medium content of available N, P and K and Mg compared to the average content of these elements in Bulgarian soils. The content of available Zn is less than 3.0 mg kg^{-1} and can be classified as low according to the MAAF 1998 classification.

Eight suspension with different Zn concentrations were used in our experiment (Table 10), including a commercial ZnO suspension (CmZnO, 1A and 1B), containing 70% Zn, and suspensions synthesized by us with ZnHN alone (2A and 2B) or mixed with 10% $Zn(NO_3)_2$ (3A and 3B) or 2.0 g l^{-1} organic materials, containing 44% dry matter (organic compounds 82%, including 35% monoacids, 3.8% total nitrogen, 3% total phosphorus as P_2O_5 and 4% potassium as K_2O) (4A and 4B). The eight variants were divided into two groups. The first group included the variants in which the Zn content in the applied foliar fertilizer was 0.70 g plot^{-1} (350 g ha^{-1}, variants 1A – 4A), and the second group included the variants in which the Zn content in the applied foliar fertilizer was 1.40 g plot^{-1} (700 g ha^{-1}, variants 1B – 4B).

Table 102. *Scheme of the experiment.*

	VARIANTS			
Control	**1A**	**2A**	**3A**	**4A**
	CmZnO Zn 0.70 g plot^{-1}	ZnHN Zn 0.70 g plot^{-1}	ZnHN + $Zn(NO_3)_2$ Zn 0.70 g plot^{-1}	ZnHN + Organics Zn 0.70 g plot^{-1}
	1B	**2B**	**3B**	**4B**
	CmZnO Zn 1.40 g plot^{-1}	ZnHN Zn 1.40 g plot^{-1}	ZnHN + $Zn(NO_3)_2$ Zn 1.40 g plot^{-1}	ZnHN + Organics Zn 1.40 g plot^{-1}

After harvesting, the cobs were air dried and weighed. Random samples of grain were milled, mixed and analysed for zinc, dry matter, nitrogen, phosphorus, potassium, protein, fat, starch and micro - and macroelements (Cu, Fe, Mg, N, P, K and Ca).

- *Impact of foliar fertilization on the zinc distribution in the maize organs*

To more clearly understand zinc uptake and its transport to the maize organs, the results obtained three weeks after the first spraying and three weeks after the second spraying were compared (Fig. 19).

The zinc concentration of the plant roots (Figure 19A) increased in the same manner over the entire investigated growth period. The lack of a statistically significant difference between the root zinc content in the control and the variants suggests that the entire amount of zinc in the roots is absorbed by the soil solution. The results for the zinc content in the leaves were similar to those in the roots; its concentration increased substantially both in control and in all the variants (Figure 19C). These results show that zinc does not move from the leaves to the roots and are in agreement with the results of other authors.

The results for the accumulation and transport of zinc in the stems for the two sprayings are completely different (Figure 19B).

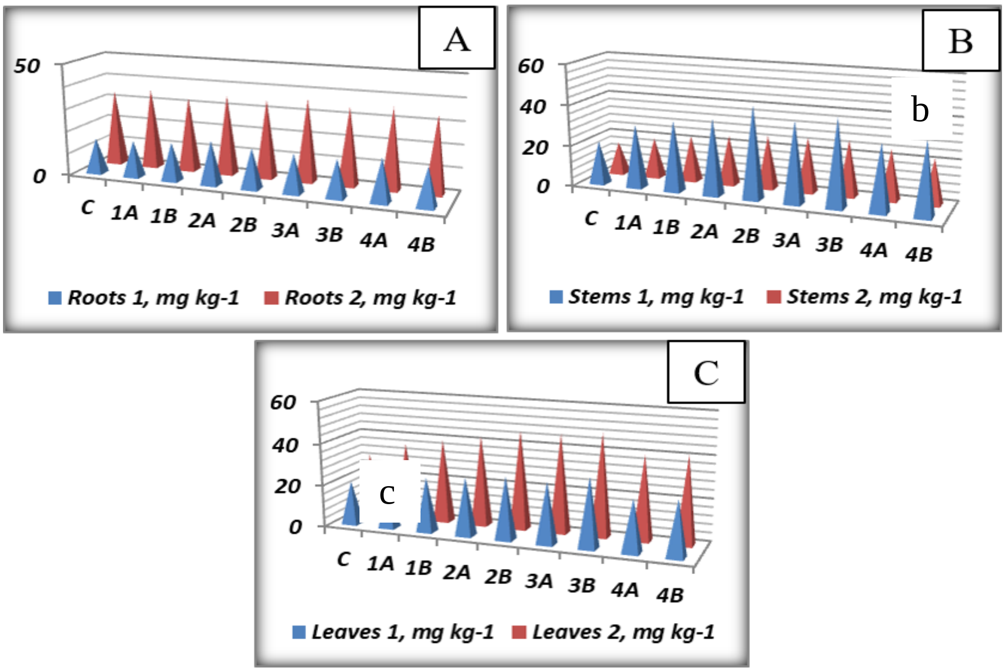


Figure 19. *Concentration of Zn in the roots (a), stems (b) and leaves (c) three weeks after the first spraying and three weeks after the second spraying.*

The first spraying resulted in a high concentration of Zn in the stems. Regardless of the additional amount of zinc deposited by the second spraying, the contents both in the control and in the variants decreased by almost 2 fold. Apparently, in the initial period of growth, the stems act as reservoirs in which the zinc that the plant needs in the next stages of its growth accumulates. This suggestion is in agreement with the results of other authors. It seems that the transport of zinc in maize organs strongly depends on the growth period. During the first growth period, zinc movement is directed from the roots and leaves to the stem; then, the trend is reversed - the zinc that accumulated in the stem moves to other organs in the plant.

- *Impact of foliar fertilization on the grain yield and quality*

Table 11 presents the impact of ZnHN application on the grain yield. The average yield from the three replicates for the control was 18.2 kg/20 м^2 (recalculated 9.10 t ha^{-1}); the variants from the first group, 20.40 kg/20 м^2 (recalculated as 10,20 t ha^{-1}); and the variants from the second group, 21.09 kg/20 м^2 (recalculated as 10.55 t ha^{-1}).

Table 11. *Impact of ZHN application on the grain yield (kernels and cob).*

Grain yield	Control	Variants, 0.70 g plot^{-1}				Variants, 1.40 g plot^{-1}			
		1 A	2 A	3 A	4 A	1 B	2 B	3 B	4 B
kg/ 20 м^2	18.20	19.30	20.51	21.90	19.90	20.42	21.30	22.41	20.22
		Average 20.40 ± 1.11				Average 21.09 ± 1.00			
t.ha^{-1}	9.10	9.65	10.26	10.95	9.95	10.21	10.65	11.21	10.11
		Average 10.20 ± 0.56				Average 10.55 ± 0.50			
*Δ, %	-	6.04	12.75	20.33	9.34	12.20	17.03	23.19	11.10
		Average 12.12 ± 6.12				Average 15.88 ± 5.51			

*Differences between the yield of the control and yield of the variants in %.

The results showed that plants fertilized with ZnHN had a significantly increased grain yield. The difference in the yield between the control and the yield of the variants ranged from 6.04 to 23.19% (mean 12.12% for the first group and 15.88% for the second group). The effect of different levels of Zn fertilization (350 and 700 g ha^{-1}) on the grain yield was significant ($p < 0.05$), and the yield of variant 3 was highest in both groups (ZnHN suspension + $Zn(NO_3)_2$). The addition of organic matter to the ZnHN suspension did not lead to higher yields compared to those of the pure ZnHN variants. More investigations are needed to estimate the effect of the simultaneous application of ZnHN and other nutrients.

Table 12 present the impact of ZnHN application on the content of grain structural components.

Table 12. *Impact of ZHN application on the grain structural components.*

Indicator,%	Control	Variants, Zn 0.70 g $plot^{-1}$				Variants, Zn 1.40 g $plot^{-1}$			
		1 A	**2 A**	**3 A**	**4 A**	**1 B**	**2 B**	**3 B**	**4 B**
Dry matter	86.58	86.16	85.63	85.29	86.12	85.88	86.68	86.19	86.22
		Average 85.8 ± 0.42				Average 86.24 ± 0.08			
Nitrogen	1.05	1.24	1.14	1.27	1,33	1,19	1.20	1.23	1.22
		Average 1.25 ± 0.08				Average 1.21 ± 0.02			
Protein	6.57	7.76	7.08	7.91	8,27	7,50	7.48	7,69	7.61
		Average 7.76 ± 0.50				Average 7.57 ± 0.10			
Fat	4.72	4.77	4.58	4.60	4,74	5,20	4.79	4,99	4.78
		Average 4.67 ± 0.10				Average 4.94 ± 0.20			
Starch	79.78	75.44	79.07	76.21	75,31	75.69	73.94	72.28	76.96
		Average 76.51 ± 1.75				Average 74.71 ± 1.75			
Phosphorus	0.24	0.26	0.20	0.17	0.17	0.22	0.20	0.17	0.18
		Average 0.20 ± 0.04				Average 0.19 ± 0.02			
Potassium	0.31	0.31	0.26	0.23	0.24	0.31	0.26	0.23	0.25
		Average 0.26 ± 0.04				Average 0.26 ± 0.03			

The dry matter and fat content varied within a narrow range of 85.63 to 86.68 and of 4.58 to 5.20, respectively, with no significant difference between the control sample and the variants ($p < 0.05$). The nitrogen and protein content increased significantly ($p < 0.05$), while the content of phosphorus and potassium decreased significantly ($p < 0.05$). The results show that the measured values for all elements are within the typical range for maize corn.

Based on the results presented, we can conclude that the synthesized zinc hydroxide nitrate has potential as a long-term foliar fertilizer. A significant ($p < 0.05$) effect on Zn accumulation in the maize stems and leaves by foliar zinc application during the first growth stage was found. The accumulation of zinc was followed by its remobilization from the stems to other plant organs during the second growth stage. Ensuring the optimal concentration of Zn at different times during the vegetative period lead to a substantial increase in the grain yield along with an improvement in the quality of the corn grain for all variants compared to those of the control. More investigations are needed to develop the potential of nanosized zinc hydroxide nitrate as a foliar fertilizer. Further attention should be directed to improving its adhesion to the leaf surface as well as to the possibilities for its simultaneous application with other nutrients.

IV.5. Impact of foliar fertilization with nanosized zinc hydroxide nitrate on maize yield and quality – field experiment 2017

The field experiment was conducted at the Research Farm, Agricultural University, Bulgaria, via a randomized block design with eight variants in 3 replications. The size of the harvesting plots was 20 m^2, and the number of plants per plot was 160 ± 2. The base fertilization and irrigation were the same for all variants. The hybrid Pr 9241, group 370, according to the FAO, was chosen for the experiment, given its excellent qualities and acclimatization to the area of research.

Seven suspension with different zinc concentrations-0.70 g $plot^{-1}$ (recalculated 350 g ha^{-1}) and 1.40 g $plot^{-1}$(recalculated 700 g ha^{-1}) were used in our experiment (Table 13), including a commercial ZnO suspension (CmZnO), containing 70% Zn, and synthesized by us suspensions with ZnHN alone (Variants 2 and 3) or mixed with 10% $Zn(NO_3)_2$ (Variant4) or 10 % copper hydroxide nitrate (CuHN) (Variant 5). In the latter two variants, the suspension of ZnHN is enriched with nitrogen and phosphorus (Variant 6) and essential micronutrients (Variant 7).

Table 33. *Scheme of the experiment.*

	VARIANTS				
Control 1	1	2	3	4	Control 2
	CmZnO Zn 1.40 g $plot^{-1}$	ZnHN Zn 0.70 g $plot^{-1}$	ZnHN Zn 1.40 g $plot^{-1}$	ZnHN + $Zn(NO_3)_2$ Zn 0.70 g $plot^{-1}$	
	5	6	7		
	ZnHN + CuHN Zn+Cu 1.40 g $plot^{-1}$	ZnHN, 1.40 g $plot^{-1}$, 5% urea 5 % NaH_2PO_4	ZnHN, 1.40 g $plot^{-1}$ 5% urea, 5 % NaH_2PO_4, Fe (20 mg/l), Mn (20 mg/l), Cu (20 mg/l,		

The Zn content and the content of selected micro (Cu, Fe) and macro (Ca, Mg, P and K) elements in maize leaves were determined two weeks after the first spraying (5-6 leaf), two weeks after the second spraying (9-10 leaf) and before harvest. After harvesting, the cobs were air-dried and weighed. Random samples of grain were mixed, milled and analysed for zinc, dry matter, nitrogen, phosphorus, potassium, protein, fat and starch.

- *Impact of foliar fertilization on the micro and macroelements distribution in the maize leaves*

In our previous investigation (2017) we tracked the dynamics of zinc movement in the roots, stems and leaves of maize throughout the growing period. In this study, we tracked the impact of foliar fertilization by zinc on other nutrients content in maize leaves. The foliar fertilizer was applied twice during the growth period. The first spraying was carried out at 5-6 fully emerged leaf, and the second spraying was carried out at 10-11 fully developed leaf. Two weeks after each spraying, random composite samples (1-5 and 6-10 leaves after firs sparing and 1–5, 6-10 and 11-15 leaves after second sparing) were collected and analysed for micro and macroelements content after drying for 12 h at 85 °C. Leaves from the whole plant were analyzed after harvesting.

Table 14 and 15 present the Zn, Cu and Fe concentration in the maize leaves two weeks after the first spraying and two weeks after the second spraying.

Table 14. *Zn, Cu and Fe concentration in the plant tissue two weeks after first spraying.*

Variant	Zn, мg/kg		Cu, мg/kg		Fe, мg/kg	
	1–5 leaf	**6–10 leaf**	**1–5 Leaf**	**6–10 leaf**	**1–5 leaf**	**6–10 leaf**
K1	26.6	28.0	10.5	7.7	137.0	78.4
K2	24.2	24.4	12.2	6.6	159.5	74.0
K_{av}	**25.2**	**26.2**	**11.4**	**7.2**	**148.3**	**76.2**
1	41.1	28.5	12.8	7.7	209.3	70.4
2	45.5	24.7	13.5	7.4	232.9	68.5
3	50.7	29.9	12.4	7.1	223.0	78.7
4	48.2	22.5	16.2	7.6	215.6	73.0
5	46.1	24.8	12.6	7.4	210.6	79.4
6	44.3	21.5	12.3	6.6	204.2	64.3
7	51.0	23.0	13.4	6.2	203.1	55.1
V_{av}	**46.7**	**25.0**	**13.3**	**7.1**	**214.1**	**69.9**

* K_{av} - average value for controls; $\mathbf{V_{av}}$ - average value

Table15. *Zn, Cu and Fe concentration in the plant tissue two weeks after second spraying.*

Variant	Zn, мg/kg			Cu, мg/kg			Fe, мg/kg		
	1–5 leaf	**6–10 leaf**	**11–15 leaf**	**1–5 leaf**	**6–10 Leaf**	**11–15 leaf**	**1–5 leaf**	**6–10 Leaf**	**11–15 leaf**
K1	28.6	29.0	31.3	4.2	5.4	5.9	124.2	110.2	94.7
K2	26.2	26.8	34.6	4.4	7.0	7.5	129.1	108.1	123.0
K_{av}	**27.4**	**27.9**	**33.0**	**4.3**	**6.2**	**6.7**	**126.7**	**104.2**	**108.9**
1	41.4	36.1	39.2	6.3	7.6	8.5	114.4	92.4	118.2
2	40.0	31.0	32.2	3.3	8.4	5.3	122.6	104.6	95.4
3	43.8	45.8	39.5	3.2	5.8	6.7	120.4	105.5	115.8
4	43.2	40.4	35.4	2.8	5.6	5.8	111.2	98.1	101.2
5	36.7	37.8	35.2	3.7	6.3	5.9	123.4	104.2	100.8
6	44.2	39.7	34.8	4.7	5.5	6.1	135.0	111.4	106.3
7	35.7	42.7	34.2	4.4	6.5	6.3	134.3	105.2	83.8
V_{av}	**40.7**	**39.1**	**35.8**	**4.1**	**6.5**	**6.3**	**123.0**	**103.1**	**103.1**

* K_{av} - average value for controls; $\mathbf{V_{av}}$ - average value for variants

The results for the accumulation and transport of zinc in the leaves for the two sprayings are entirely different. The first spraying resulted in a significantly higher concentration of Zn in the sprayed leaves and lower concentration in non-sprayed leaves. After the second spray, the concentrations of zinc in the leaves of the sprayed plants are practically equalized. The content of zinc in the young leaves of the control (11-15 leaves) also increases significantly. The results obtained support the assumption that in the initial period of growth, stems act as reservoirs in which the zinc that plants need in the next stages of growth accumulates and after that remobilizes to other parts of the plants in case of a diminished external supply. Apparently, the transport of Zn in maize organs strongly depends on the growth period. During the first growth period, zinc movement is directed from roots and leaves to stem after which the trend is reversed - the Zn that accumulated in the stem moves to other organs in the plant. Equalization of the concentration in the leaves of whole plants is also observed at the copper and iron.

The trends after the second spraying are even more pronounced in the results after harvesting (Table 16). The zinc content of the leaves of the upper part of the plants (11-15 leaf) increases significantly, reaching values above 60 mg kg^{-1} as in all variants and controls. The increase is associated with a decrease in its content in the leaves of the lower part of plants (5 - 10 leaf). Apparently, the second growth period (after second spraying) is associated with the intense movement of zinc to the top of the plants, and during which a significant part of it acquired through the root system. The same trends, but less pronounced, are also observed in copper. Iron has the opposite tendency - its content decreases in plant height almost twice.

Table 16. *Zn, Cu and Fe concentration in the plant tissue after harvesting.*

Variant	**Zn, мg/kg**			**Cu, мg/kg**			**Fe, мg/kg**		
	1–5 leaf	**5–10 leaf**	**10-15 leaf**	**1–5 leaf**	**5–10 Leaf**	**10-15 leaf**	**1–5 leaf**	**5–10 leaf**	**10–15 leaf**
K1	30.6	33.0	63.7	13.9	12.4	16.4	140.7	113.5	82.6
K2	28.2	31.8	63.9	9.5	11.0	15.5	134.5	117.2	86.8
$^*K_{av}$	**29.4**	**32.4**	**63.8**	**11.7**	**11.7**	**16.0**	**137.61**	**115.4**	**84.7**
1	35.1	45.4	54.6	12.5	16.1	20.3	140.6	81.5	78.2
2	27.3	40.0	62.5	13.8	14.6	17.7	148.4	80.5	75.6
3	35.8	51.6	67.4	14.6	14.5	18.4	147.2	78.8	75.8
4	38.6	45.6	63.9	12.1	14.0	16.7	106.8	87.6	74.4
5	26.7	29.4	62.0	8.3	12.1	21.0	116.4	78.5	77.5
6	25.0	35.9	62.8	9.5	15.8	18.2	122.3	100.0	85.6
7	30.5	51.6	63.2	9.1	14.2	17.1	115.6	110.8	82.8
V_{av}	**31.3**	**42.8**	**62.3**	**11.4**	**14.5**	**17.3**	**128.2**	**88.2**	**78.6**

* K_{av} - average value for controls; V_{av} - average value for variants

Table 17. *K, Ca and Mg and P concentration in the plant tissue two weeks after first spraying.*

Variant	K, %		Ca, %		Mg, %		P, %	
	1–5 leaf	6–10 leaf	1–5 leaf	6–10 Leaf	1–5 leaf	6–10 leaf	1–5 Leaf	6–10 leaf
K1	1.95	2.47	1.38	0.22	0.62	0.15	0.23	0.35
K2	1.97	2.85	1.50	0.20	0.77	0.15	0.23	0.40
$^*K_{av}$	**1.96**	**2.66**	**1.44**	**0.21**	**0.70**	**0.15**	**0.23**	**0.38**
1	2.16	2.37	1.62	0.22	0.78	0.17	0.31	0.32
2	2.35	2.67	1.66	0.24	0.77	0.17	0.32	0.36
3	2.55	2.54	1.73	0.23	0.83	0.18	0.32	0.36
4	2.95	3.26	1.34	0.25	0.72	0.19	0.33	0.39
5	3.06	3.12	1.46	0.24	0.73	0.20	0.31	0.40
6	2.75	2.63	1.52	0.23	0.80	0.17	0.35	0.35
7	2.15	2.51	1.70	0.20	0.91	0.16	0.34	0.34
V_{av}	**2.57**	**2.73**	**1.58**	**0.23**	**0.79**	**0.18**	**0.33**	**0.36**

* K_{av} - average value for controls; $\mathbf{V_{av}}$ - average value for variants

Table 18. *K, Ca and Mg concentration in the plant tissue two weeks after second spraying.*

Variant	K, %			Ca, %			Mg, %			P, %		
	1–5 leaf	6–10 leaf	11–15 leaf	1–5 leaf	6–10 leaf	11–15 leaf	1–5 leaf	6–10 leaf	11–15 leaf	1–5 leaf	6–10 leaf	11–15 leaf
K1	1.76	1.47	1.13	0.68	0.50	0.59	0.45	0.30	0.29	0.17	0.20	0.20
K2	1.58	1.33	1.01	0.85	0.50	0.56	0.45	0.30	0.26	0.19	0.20	0.19
$^*K_{av}$	**1.67**	**1.40**	**1.07**	**0.77**	**0.50**	**0.57**	**0.45**	**0.30**	**0.28**	**0.18**	**0.20**	**0.20**
2	1.61	1.01	0.98	0.70	0.47	0.53	0.45	0.25	0.25	0.20	0.20	0.20
3	2.10	1.04	0.89	0.68	0.51	0.49	0.40	0.28	0.26	0.20	0.20	0.20
4	2.30	1.06	1.16	0.65	0.46	0.55	0.42	0.30	0.28	0.19	0.21	0.20
5	2.14	1.43	1.09	0.67	0.47	0.47	0.42	0.30	0.27	0.18	0.19	0.19
6	1.92	1.34	1.06	0.71	0.48	0.46	0.39	0.28	0.29	0.20	0.21	0.19
7	1.89	1.35	0.95	0.78	0.47	0.46	0.40	0.26	0.28	0.19	0.20	0.21
8	1.75	1.15	1.31	0.88	0.44	0.45	0.44	0.26	0.27	0.17	0.19	0.20
V_{av}	**1.96**	**1.20**	**1.06**	**0.72**	**0.47**	**0.49**	**0.42**	**0.28**	**0.27**	**0.19**	**0.20**	**0.20**

* K_{av} - average value for controls; $\mathbf{V_{av}}$ **-** average value for variants

The results for the content of the basic nutrients K and P are quite different. The concentration of both elements in the young leaves increases slightly compared with the sprayed leaves (Table 17). In the controls, the difference was statistically significant ($p < 0.05$), while in the variants, it was insignificant. The probable cause is the better life status of the sprayed plants and the accumulation of more nutrients in the period between treatment and sampling. With the growth of plants between the two sprays, the concentration of K and P decreases both in absolute value and in the plant height (Tables 18 and 19).

Table 4. *K, Ca, Mg and P concentration in the plant tissue two weeks after harvesting.*

Variant	**K, %**			**Ca, %**			**Mg, %**			**P, %**		
	1– 5 leaf	**5–10 leaf**	**10–15 leaf**	**1–5 leaf**	**5–10 leaf**	**10–15 leaf**	**1–5 leaf**	**5–10 leaf**	**10–15 leaf**	**1–5 leaf**	**5–10 leaf**	**10–15 leaf**
K 1	1.00	0.79	0.67	1.10	0.93	1.33	0.56	0.55	0.62	0.10	0.05	0.05
K 2	0.94	0.62	0.65	1.06	0.96	1.25	0.62	0.55	0.61	0.08	0.06	0.06
$^*K_{av}$	**0.97**	**0.71**	**0.66**	**1.08**	**0.94**	**1.29**	**0.59**	**0.55**	**0.61**	**0.09**	**0.06**	**0.06**
1	1.07	0.69	0.51	0.95	0.92	1.26	0.55	0.48	0.52	0.09	0.06	0.06
2	1.11	0.61	0.50	0.94	0.92	1.22	0.56	0.52	0.56	0.09	0.07	0.05
3	1.22	0.82	0.52	0.87	0.74	1.08	0.50	0.49	0.57	0.08	0.06	0.04
4	1.15	0.69	0.66	0.80	0.84	1.00	0.51	0.56	0.57	0.07	0.06	0.07
5	0.84	0.61	0.60	0.91	0.80	1.14	0.50	0.45	0.51	0.07	0.07	0.04
6	0.82	0.65	0.53	0.88	0.87	1.15	0.49	0.46	0.55	0.08	0.05	0.05
7	1.05	0.62	0.61	0.84	0.84	1.12	0.45	0.46	0.51	0.08	0.07	0.05
V_{av}	**1.04**	**0.67**	**0.56**	**0.88**	**0.85**	**1.14**	**0.51**	**0.49**	**0.54**	**0.08**	**0.06**	**0.05**

* K_{av} - average value for controls; $\mathbf{V_{av}}$ **-** average value for variants

Tables 17-19 show the concentrations of the essential macronutrients K, Ca, Mg and P concentration in the maize leaves two weeks after the first and second spraying and after harvesting.

The content of Ca and Mg in the leaves sprayed with ZnHN suspensions (1–5 leaf) ranges in narrow limits from 1.46 to 1.70 for Ca and from 0.72 to 0.91 for Mg (Table 17). The difference in their concentration in the variants and controls is insignificant. Quite different are the results for the young leaves (6–10 leaf). The content of Ca and Mg decreases drastically, the values for controls and variants being indistinguishable. This tendency persists after the second spraying, but is much less pronounced (Table 18) and completely changes in the last period of plant development (Table 19).

The results obtained by us do not indicate a significant impact of the foliar-applied zinc on the concentration of macronutrients in maize leaves.

- *Impact of foliar fertilization on the grain yield and quality*

Our previous study (2016) showed that foliar spraying with ZnHN has positive effects on the corn yield and quality. The results of this study confirm this conclusion (Table 20).

Table 20. *Impact of ZHN application on the grain yield (kernels and cob).*

Variant	Total weight, kg	ΔG_1, %*	Grain weight, kg	ΔG_2, %*	t.ha^{-1}
K1	27.77	Average	24.25	Average	Average
K2	27.70	**27.74**	24.15	**24.20**	**12.10**
1	29.83	+ 7.62	26.01	+ 7.48	13.00
2	31.34	+ 13.0	27.24	+ 12.53	13.62
3	34.54	+ 24.54	30.12	+ 24.45	15.06
4	31.25	+ 12.65	27.20	+ 12.40	13.60
5	31.04	+ 11.92	27.07	+ 11.85	13.51
6	30.43	+ 9.70	26.53	+ 9.63	13.26
7	29.78	+ 7.43	25.96	+ 7.27	12.98
V_{av}	**31.17**	**12.41**	**27.16**	**12.23**	**13.58**

*ΔG_1 **and** ΔG_2 **-** Differences between the yield of the control and yield of the variants in %.

The average yield for the controls (mean of all replicates) was 27.74 kg total weight and 24.20 - grain weight from 20 m^2 (recalculated 9.10 t ha^{-1}). The average yield of the variants ranged from 29.83 to 34.54 kg total weight (mean of all replicates 31.17) and from 26.1 to 30.12 kg grain weight from 20 m^2 (recalculated respectively 13.00 to 15.06 t ha^{-1}). These results show that foliar feeding of zinc at appropriate stages of plant growth (5 - 6 and 9-10 leaves) has a substantial impact on yield. Increasing the yield of variants compared to that of controls varies from 7.62 to 24.54% (mean 12.41% for the all variants). Adding other nutrients to the suspension does not improve the results. The difference of the levels of zinc fertilization (350 and 700 g ha^{-1}) on the grain yield was significant ($p < 0.05$), and the yield from variant 4 was highest. These results confirm the conclusion that maize required additional Zn to achieve its full yield potential.

The analysis of maize grains for dry matter, nitrogen, protein, fats and starch shows increasing the protein content from an average of 8.12 for controls to 8.42 for variants and fat from 3.98 for controls to 4.45 for variants. No difference in dry matter was detected, and the starch in variants decreased by 1.2% compared to controls.

IV.6. Impact of foliar fertilization with nanosized zinc hydroxide nitrate on maize yield and quality – field experiments 2019

The objective of the study was to investigate the agronomic responses of maize hybrids from different maturity groups to foliar fertilization by zinc in the form of zinc hydroxide nitrate suspension. The evaluation was performed on the base of grain yield and its components.

The investigation was carried out in 2019 on ten of the most common maize hybrids, belonging to three maturity groups: early maturity group - P0023 (FAO 450), P20217 (FAO

480), P0217 (FAO 490); medium maturity group - P0704 (FAO 520) and P0937 (FAO 580) and medium-late maturity group – P1241 (FAO 620), P1049 (FAO 620), P1535 (FAO 650) and P2105 (FAO 700). Nanosized zinc hydroxide nitrate (ZnHN) suspension with zinc content of 12.2% was used as a foliar fertilizer.

- *Field experiment*

The field experiment was conducted at the Research Farm, Agricultural University, Bulgaria (Fig. 20) in four variants, three repetitions for each hybrid: Variant I - single spraying at the end of May (4 - 5 fully emerged leaf); Variant II - double spraying at the end of May (4 - 5 fully emerged leaf) and at the end of Jun (8 -9 fully developed leaf); Variant III - single spraying at the end of Jun (8 - 9 fully developed leaf) and Variant IV - control.

Figure 20. *Pictures of the corn plants at the beginning and end of the experiment.*

The size of the harvesting plots was 60 m^2. A suspension with zinc concentrations of 4.2 g $plot^{-1}$ (recalculated 700 g ha^{-1}) was used in all variants. The base fertilization (NPK 15:15:15 - 50 kg dka^{-1} pre-sowing and 50 kg dka^{-1} ammonium nitrate at 6 - 7 fully emerged leaf) and drip irrigation were the same for all variants. Weed control was carried out by using Click combi 250 ml dka^{-1} applied after sowing before the emergence of the crop and weeds and a mixture of Equip SK 250 ml/dka and Laudis OD 200 ml dka^{-1} at 6 - 7 fully emerged leaf.

After harvesting, the cobs were air-dried and weighed. Random samples of grain were mixed, milled and analysed for protein, fat and starch.

- *Impact of foliar fertilization by ZnHN suspension on the grain yield components*

Tables 21-23 present the impact of zinc hydroxide nitrate application on the grain yield, grain moisture content and the part of the corn in the cob for the hybrids from the three maturity groups.

Significant differences ($p < 0.05$) in grain yield between different maturity grope hybrids were observed. The average yield and standard deviation for all variants, including control, for early maturity group, was 1343 ± 84,62 kg dka^{-1}. For the medium and medium-late maturity groups, the results were 1549 ± 69,17 and 1725 ± 64,70 kg dka^{-1} respectively. The results obtained confirm that short-season hybrids (group FAO 400) have significantly less genetic potential for yield compared to medium (group FAO 500) and long-season (group FAO 600/700) hybrids. This shortcoming of short-season hybrids is largely offset by more favourable values of stability parameters. The yields of the different hybrids within the same group also differ, although to a much lesser extent.

Table 21. *Average yield and yield component data for maize hybrids in maturity group FAO 600/700.*

Hybrid	Variant*	Corn in cob, %	Moisture, %	$Kg.dka^{-1}$	% versus control
P2105 FAO 700	1	88,25	17,2	1780	101,4
	2	88,15	17,1	1828	104,1
	3	87,94	17,4	1800	102,5
	4	86,98	17,3	1756	-
P1535 FAO 650	1	89,32	16,9	1772	102,9
	2	88,38	16,7	1800	104,5
	3	88,77	16,6	1756	102.0
	4	88,73	16,8	1722	-
P1241 FAO 620	1	88,85	16,1	1712	104,8
	2	89,92	16,1	1782	109,7
	3	88,81	15,9	1668	102,1
	4	88,65	15,8	1634	-
P1049 FAO 620	1	89,53	16,1	1732	105.0
	2	88,77	15,7	1788	108,4
	3	88,72	15,9	1716	104.0
	4	88,34	15,8	1650	-
P1063 FAO 600	1	88,32	15,3	1666	103,3
	2	88,58	15,2	1696	105,2
	3	88,58	15,5	1632	101,2
	4	88,32	15,6	1612	-
Average for the group		**88,60 ± 0,61**	**16,25 ± 0,69**	**1725 ± 64,70**	**103,59 ± 2,63**

Table 22. *Average yield and yield component data for maize hybrids in maturity group FAO 500.*

Hybrid	Variant*	Corn in cob, %	Moisture, %	Yield, $Kg.dka^{-1}$	% versus control
P0937 FAO 580	1	89,34	14,9	1596	102,1
	2	89,31	14,7	1640	105,0
	3	89,06	14,7	1582	101,3
	4	89,38	14,8	1562	-
P0704 FAO 520	1	86,32	14,1	1512	106,0
	2	86,47	13,7	1588	111,3
	3	86,82	13,9	1488	104,3
	4	86,67	13,8	1426	-
Average for the group		**87,92 ± 1,45**	**14,33 ± 0,50**	**1549 ± 69,17**	**105,0 ± 3,56**

Table 23. *Average yield and yield component data for maize hybrids in maturity group FAO 400.*

Hybrid	Variant*	Corn in cob, %	Moisture, %	Kg.dka^{-1}	% versus control
P0217 FAO 490	1	89,92	12,8	1444	106,2
	2	89,11	12,6	1488	109,4
	3	89,26	12,4	1426	104,8
	4	89,62	12,7	1360	-
P0216 FAO 480	1	89,08	12,5	1342	107,9
	2	88,26	12,6	1396	112,2
	3	88,54	12,8	1322	106,3
	4	88,44	12,5	1244	-
P0023 FAO 450	1	89,01	12,2	1288	106,6
	2	88,11	12,4	1332	110,3
	3	88,38	12,1	1266	104,8
	4	88,78	12,0	1208	-
Average for the group		**88,88 ± 0,56**	**12,47 ± 0,26**	**1343 ± 84,62**	**107,61 ± 2,55**

Presented in Table. 21-23 results allow drawing definite conclusions about the effect of foliar fertilization with ZnHN on the yield of hybrids from different maturity groups. The average yield increase for the hybrids from the FAO 400 maturity group compared to the control was 7.61 ± 2.55%. For the FAO 500 maturity group and the FAO 600/700 maturity group, the increase was 5.0 ± 3.56% and 3.59 ± 2.63%, respectively. This is due to the different periods of maize phenophases in which the reproductive organs of the plant are formed. In the early hybrids of the FAO 400 maturity group, this period was drawn forward when the conditions for plant development were less favourable. During this period the temperature is relatively low and the root system is underdeveloped. At this time the supply of nutrients, including zinc, is insufficient to meet the needs of the plant. Zinc feeding during this period is of great importance and plays an important role in the formation of the reproductive organs of maize. The application of foliar fertilizer is extremely suitable as its absorption is much faster than from the soil. Apart between the different maturity groups, there is a difference between the variants within each group. In all three maturity groups, the highest yields were recorded after double treatment of the plants at 4 - 5 fully emerged leaf and 8 - 9 fully developed leaf. Most significant is the increase in yield after double treatment of P0216 hybrid by FAO 480 maturity group (12.2%). A single treatment at 8-9 fully developed leaf also has a positive effect, but in all hybrids, it is less pronounced.

The results for grain moisture content at maturity are quite different too. The average value and standard deviation for all variants, including control, for early maturity group, was 12,47 ± 0,26%. For the medium and medium-late maturity groups the results were 14,33 ± 0,50% and 16,25 ± 0,69% respectively. The differences between the hybrids within the groups as well as between the treated plants and the controls were negligible. There is no noticeable relationship between the number and time of treatment with zinc hydroxide nitrate and the values of this indicator.

The data for the part of the corn in the cob at maturity is quite different from that for yield and grain moisture. The results for all variants varied within a narrow range of 86.98 for hybrid P2105 FAO 700 (control) to 89.92 for the same hybrid (double treated). The reason for this result, despite the significant differences in yields, is the larger grains and larger cobs of the hybrids from later maturity groups.

- *Impact of foliar fertilization by ZnHN suspension on the grain structural components*

Tables 24 - 26 present the impact of ZnHN application on the content of the main grain structural components (protein, fat and starch). The results of Variant 2 (double treatment at 4 - 5 fully emerged leaf and 8 -9 fully developed leaf) and the control for each hybrid were compared.

Table 24. *Average data for grain structural components of maize hybrids in maturity group FAO 600/700.*

Hybrid	Variant	Protein content, %	Fat content, %	Starch content, %
P1063 FAO 600	2	6,45	1,60	71,78
	4	6,39	2,33	71,78
P1041 FAO 620	2	6,57	1,68	70,42
	4	6,81	2,21	70,42
P1249 FAO 620	2	6,76	1,60	73,13
	4	6,47	1,53	71,78
P1535 FAO 650	2	7,22	2,21	69,07
	4	6,20	1,76	70,42
P2105 FAO 700	2	6,59	1,75	70,42
	4	6,61	1,52	67,71
Average for the group		**6,61 ± 0,28**	**1,82 ± 0,31**	**70,69 ± 1,54**

Table 25. *Average data for grain structural components of maize hybrids in maturity group FAO 500.*

Hybrid	Variant	Protein content, %	Fat content, %	Starch content, %
P0704 FAO 520	2	6,51	2,53	69,07
	4	6,45	2,11	67,71
P0937 FAO 580	2	6,38	2,32	67,71
	4	6,31	2,14	70,13
Average for the group		**6,41 ± 0,09**	**2,28 ± 0,19**	**68,66 ± 1,17**

Presented in Tables 24-26 results show that the differences in the values of grain structural components of the hybrids from the different maturity groups are smaller than those of the yield and its components. Protein, fat and starch content varied within a narrow range, with differences between maturity groups not significant at $p < 0.05$. It can be summarized, that the impact of foliar zinc fertilization on protein, fat and starch content in the grains of the hybrids investigated is negligible. Probably critical factors for the grain structural components content, in this case, are climate and soil properties.

Table 26. *Average data for grain structural components of maize hybrids in maturity group FAO 400.*

Hybrid	Variant	Protein content, %	Fat content, %	Starch content, %
P0023 FAO 450	2	7,76	3,45	66,16
	4	8,46	3,60	65,34
P0216 FAO 480	2	6,57	2,31	71,49
	4	6,63	2,19	70,42
P0217 FAO 490	2	6,40	2,22	69,07
	4	6,49	1,81	70,42
Average for the group		**7,05 ± 0,85**	**2,60 ± 0,74**	**68,82 ± 2,51**

On the base of the results obtained, we can conclude that the synthesized zinc hydroxide nitrate has indubitable potential as a successful long-term foliar fertilizer. A significant positive effect on grain yield up to 12.2% for variants compared to those of the controls was found. The best effect is obtained by double spraying at 4 - 5 fully emerged leaf and 8 - 9 fully developed leaf. The most sensitive to foliar zinc fertilization are the hybrids from the early maturity group FAO 400.

IV.7. Impact of foliar application of zinc on micro- and macro elements distribution in *Phyllanthus amarus*

The field experiment was conducted in Lam Dong province, Vietnam. The soil in this research area was with an acidic pH (5.87) and low content of organic matter. It is characterized by a low content of Ca, Cu, Mn, K and Zn and medium content of N, P and Fe. Since the soil is low in organic matter and nutrients in our experiment we used a mixture of 4 parts of soil, 1 part of coco fiber and 0.5 parts of organic fertilizer.

Eight suspension solutions with different Zn concentrations were used. The scheme of the experiment is presented in Table 27. The foliar fertilizer was applied triple during the growth period – first, fourth and seventh week after germination. The size of the plots was 4.4 m^2 for all variants and the working solution for each spraying – 0.2 l/plot. After harvesting, random samples were collected and air dried at 85 ^{o}C for 24 hours

Table 275. *Scheme of the experiment*

Variant	$Zn_5(OH)_8(NO_3)_2$ suspension, g/l	$Zn_5(OH)_8(NO_3)_2$ suspension, g/plot	Zn, g/plot
Control	-	-	-
2	6.6	1.32	0.10
3	10.7	2.14	0.17
4	14.8	2.96	0.23
5	18.9	3.78	0.30
6	23.0	4.60	0.36
7	27.0	5.40	0.43
8	32.8	6.56	0.52

All samples were carefully separated into its roots, stems and leaves, after which all parts were milled, mixed and analysed for Zn, Cu, Mn, Fe, P, K, Ca and Mg.

- *Impact of Foliar Fertilization on the Zinc Distribution in the Phyllanthus Amarus Organs.*

The results in Table 28 present the impact of the foliar fertilization on the zinc distribution in the *Phyllanthus amarus* organs.

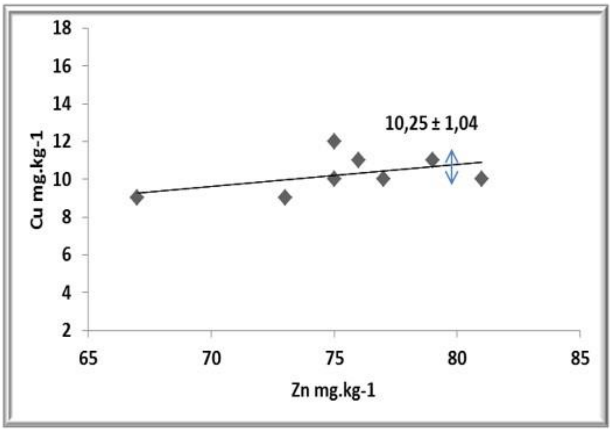
Table 286. *Zinc concentration in Phyllanthus amarus organs, mg kg-1 d.wt*

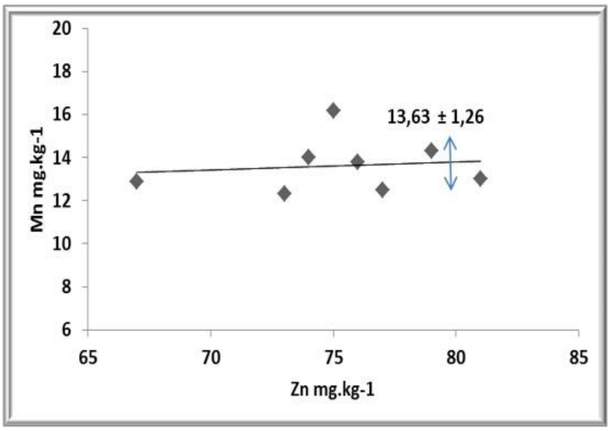
Variant	Roots	Stem	Leaves
Control	75.22 ± 2.02	54.22 ± 2.12	90.24 ± 1.56
2	73.30 ± 1.82	93.12 ± 2.64	171.12 ± 2.02
3	75.42 ± 1.90	104.42± 1.82	191.32 ± 2.58
4	76.12 ± 1.80	110.63 ± 2.22	247.16 ± 2.92
5	67.24 ± 1.72	115.00 ± 2.42	333.04 ± 3.84
6	81.31 ± 1.96	119.02 ± 1.02	333.46 ± 5.36
7	77.02 ± 1.68	158.42 ± 3.12	338.23 ± 5.48
8	79.25 ± 1.94	211.04 ± 3.22	314.12 ± 4.98

The presented results show that the concentration of the zinc fertilizer used significantly influences the zinc content in the stems and leaves of the plants, but not in the roots.

- *Impact of Foliar Fertilization on Micro and Macro Elements Distribution in the Phyllanthus Amarus Organs.*

Fig. 21 present the impact of Zn fertilization on micronutrients (Cu, Mn and Fe) content in roots of *P. amarus*.





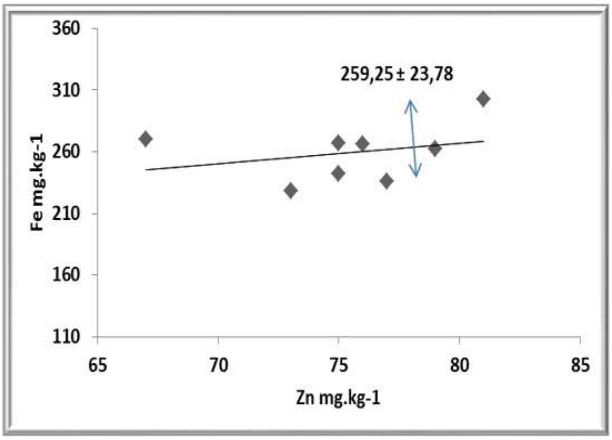


Figure 21. *Impact of Zn fertilization on Cu, Mn and Fe content in roots of P. amarus.*

The copper and zinc content of the soil used in our experiment is low and a noticeable competitive interaction between the two elements cannot be expected. The content of Mn and Fe varies narrowly (13.63 ± 1.26 mg kg^{-1} for Mn and 259.25 ± 23.78 mg kg^{-1} for Fe). Obviously, the limiting factors in determining the content of micronutrient at the roots of *P. amarus* are pH and soil composition.

Fig. 22 present the impact of Zn fertilization on macronutrients (P, K, Ca and Mg) content in roots of *P. amarus*.

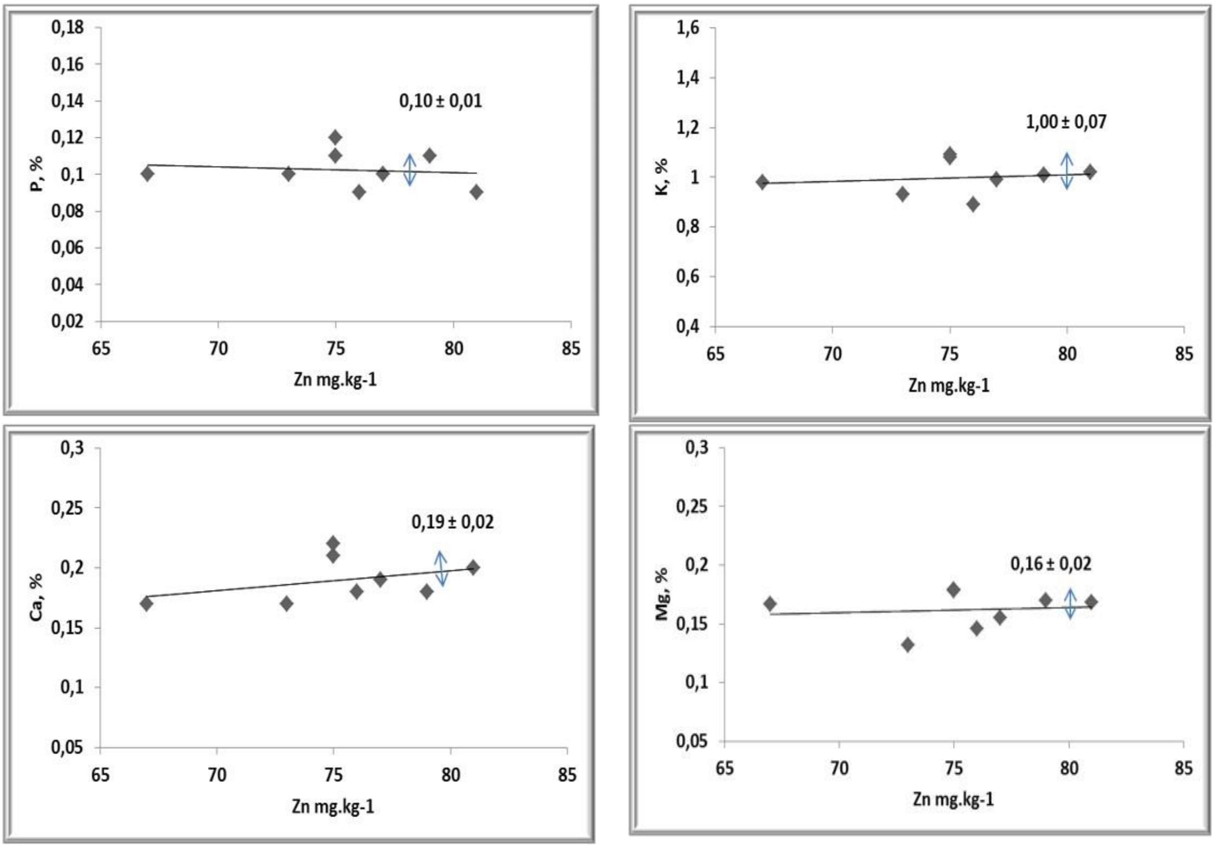
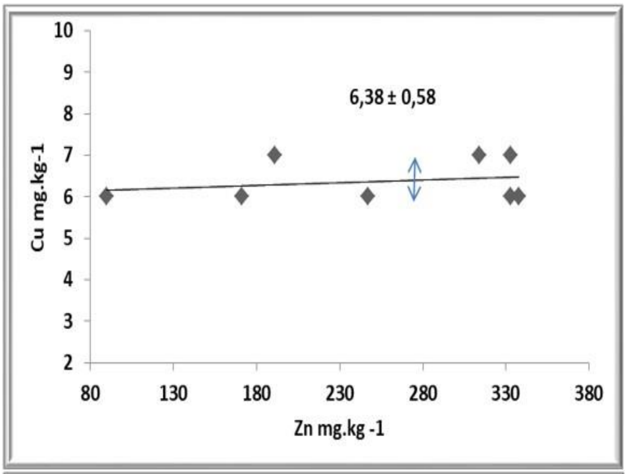


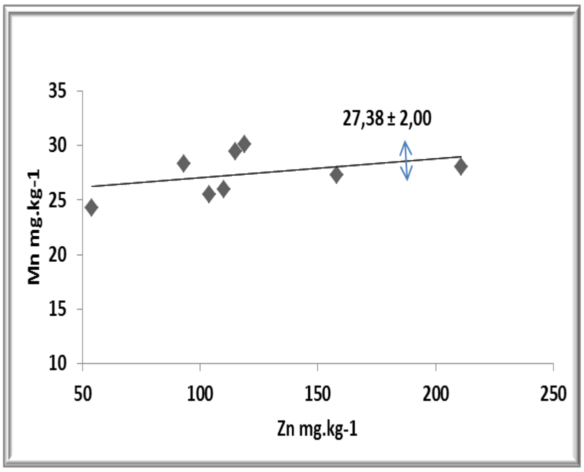
Figure 22. *Impact of Zn fertilization on P, K, Ca and Mg content in roots of P. amarus.*

The results presented in Fig. 28 show that foliar application of zinc in no way affects the content of macronutrients at the roots of *P. amarus*. The content of P, K, Ca and Mg varies in a narrow range (0.10 ± 0.01% for P, 1.00 ± 0.07% for K, 0.19 ± 0.02% for Ca and 0.16 ± 0.01% for Mg).

The content of micro and macronutrients in the above-ground parts of plants strongly depends on their concentration and the interaction between them in the soil solution. However, unambiguous conclusions cannot be extracted and the experimental results obtained are contradictory.

Fig. 23 present the impact of Zn fertilization on micronutrients (Cu, Mn and Fe) content in stems of *P. amarus*.





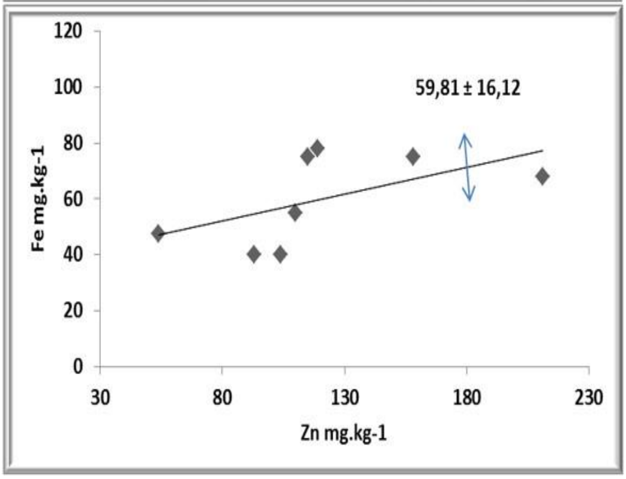


Figure 23. *Effect of Zn fertilization on Cu, Mn and Fe content in stems of P. amarus.*

The results presented in Fig. 23 show that the concentration of the applied zinc fertilizer has no significant influence on the Cu and Mn content in the stems of the plants. The content of both elements varies in a narrow range of 6.38 ± 0.58 mg kg^{-1} for Cu and 27.38 ± 2.00 mg kg^{-1} for Mn. A little different is the result of iron. Its content is ranging from 40.0 to 78.2 with the mean value for all variants 59.81 ± 16.12 mg kg^{-1}**.** This difference, however, does not allow for the conclusion of synergy between the two elements.

These results show that the interaction of zinc with Cu, Mn and Fe depends not only on the type of plant but also on the mode of application of zinc. Obviously, the interaction of zinc with micronutrients is much more pronounced in the soil solution and the roots of the plants than in their above-ground parts after using zinc-containing leaf fertilizers.

Figs. 24 present the impact of Zn fertilization on macronutrients (P, K, Ca and Mg) content in stems of *P. amarus*.

The content of all four elements in the stems of *P. amarus* varies within a narrow range, which confirms the above conclusion that the interaction of soil-zinc and foliar applied Zn with nutrients differ substantially.

Fig. 25 present the impact of Zn fertilization on micronutrients (Cu, Mn and Fe) content in leaves of *P. amarus*.

Many publications report negative interaction between zinc and copper due to the effect of antagonism and the same membrane transport protein. In our study, such an effect is not noticeable. The content of Cu in the leaf of *P. amarus* varies within a relatively narrow range from 4.02 to 7.88 with the mean value for all variants 6,67 ± 0.78 mg kg^{-1}**.** Manganese ranged from 38.10 to 43.20 with a mean value of 36.88 ± 4.32.

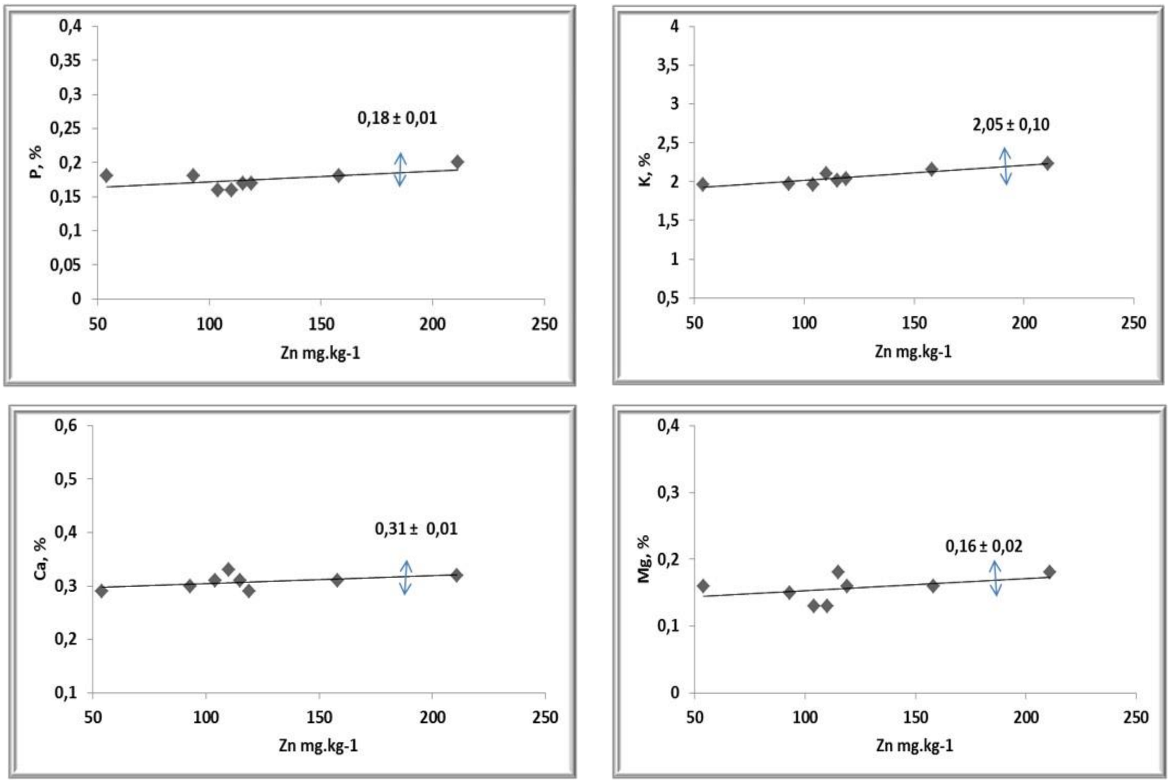
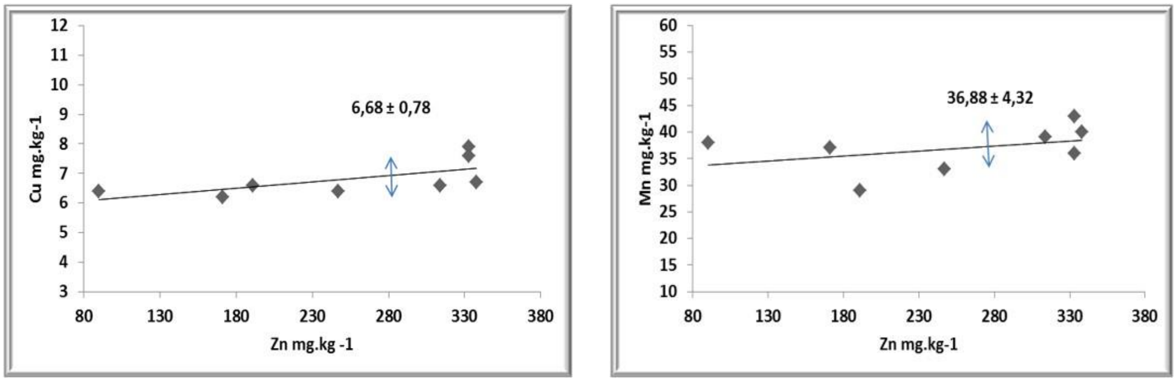


Figure 24. *Impact of Zn fertilization on P, K, Ca and Mg content in stems of P. amarus.*



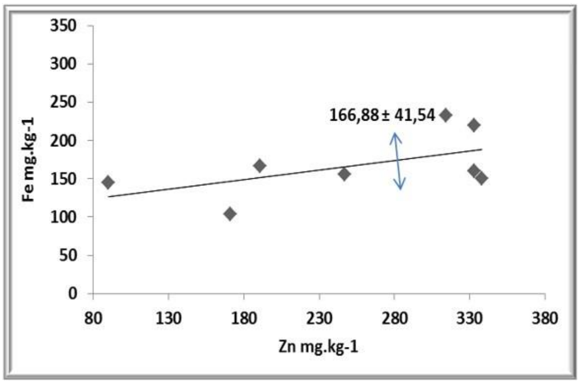


Figure 25. *Impact of Zn fertilization on Cu, Mn and Fe content in leaves of P.amarus.*

The summarized results for micronutrient content in *P. amarus* show that the content of copper increases in order: roots > stems ≅ leaves. The manganese content in the different organs of the plant differs significantly, as in this case, the order is: leaves > stems > roots. The main part of the iron is localized in the roots > leaves > stems.

Fig. 26 present the impact of Zn fertilization on macronutrients (P, K, Ca and Mg) content in leaves of *P. amarus*.

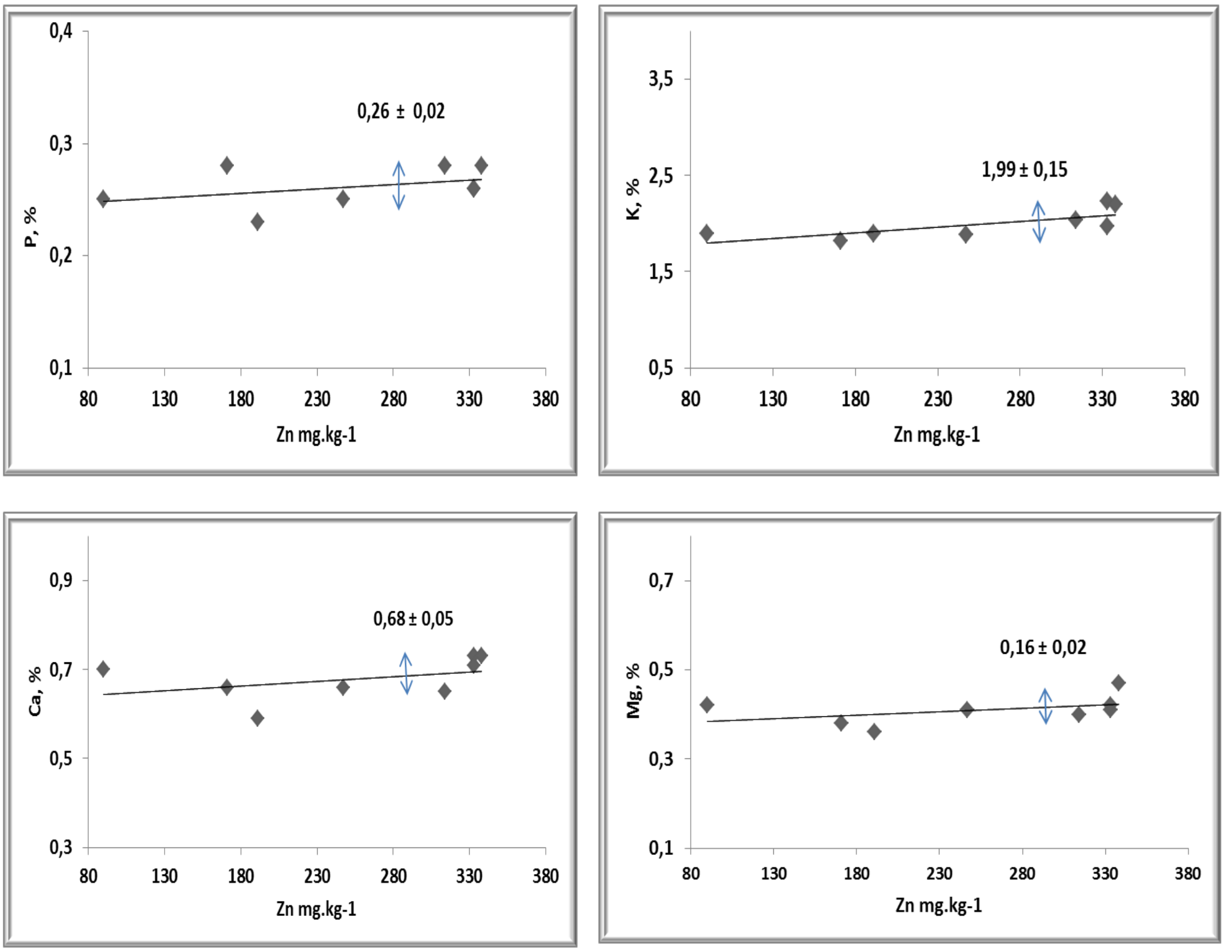


Figure 26. *Effect of Zn fertilization on P, K, Ca and Mg content in leaves of P. amarus.*

It's generally accepted, that there are antagonistic effects of P application on absorption and uptake of Zn. Formation of Zn phosphate or/and phytate is considered responsible for Zn immobilization on root surfaces and in leaves. Antagonistic effects of Ca and Zn have been reported by many authors. The results, presented in Fig. 26 don't show any effect of foliar Zn fertilization on macronutrients in the leaves of *P. amarus* and the content of P, K, Ca and Mg at all variants is comparable with those of control plants. The main part of P and Ca is localized in the leaves, followed by stems and roots. The content of K increases in order: stems ≅ leaves > roots. The magnesium content in the different organs of the plant differs significantly, as in this case, the order is: leaves > stems > roots.

The summary of the results presented in Figs 21-26 shows that the mineral composition in each of the three parts investigated differs from each other. The minerals in the root were in order of P ≅ K > Ca > Mg for macronutrients and Fe > Zn > Mn> Cu for micronutrients. In the steams the content for macronutrients was in order of Ca > K > P > Mg and Zn > Fe > Mn > Cu for micronutrients and for the leaves, the order was Ca > Mg > P > K and Zn > Fe > Mn > Cu for micronutrients.

IV.8. Impact of foliar application of zinc on micro and macro elements distribution in *Curcuma Longa*

The field experiment was conducted in Lam Dong province, Vietnam. The soil in this research area was with an acidic pH (5.87) and low content of organic matter. It is characterized by a low content of Ca, Cu, Mn, K and Zn and medium content of N, P and Fe. Since the soil is low in organic matter and nutrients in our experiment we used a mixture of 4 parts of soil, 1 part of coco fibre and 0.5 parts of organic fertilizer.

Seven suspension solutions with different Zn concentrations were used. The scheme of the experiment is presented in Table 27. The foliar fertilizer was applied triple during the growth period – first, fourth and seventh week after germination. The size of the plots was 4.4 m^2 for all variants and the working solution for each spraying – 0.2 L $plot^{-1}$. After harvesting, random samples were collected. The rhizomes were washed, cleaned and air-dried at 40 ^oC for 24 hours. All samples were milled, mixed, digested by a mixture of HNO_3 and H_2O_2 in a Microwave Digestion System MARS 6 - CEM Corporation and analysed for Zn, Cu, Fe, P, K, Ca and Mg.

Table 27. *Scheme of the experiment*

Variant	$Zn_5(OH)_8(NO_3)_2$ suspension, g l^{-1}	$Zn_5(OH)_8(NO_3)_2$ suspension, g $plot^{-1}$
Control	-	-
1	6.6	1.32
2	10.7	2.14
3	14.8	2.96
4	18.9	3.78
5	23.0	4.60
6	27.0	5.40
7	32.8	6.56

- *Impact of foliar fertilization on micro and macro elements content in the Curcuma Longa.*

Figs 27 present the impact of Zn fertilization on micronutrients (Cu and Fe) content in the rhizome of Curcuma Longa. The copper and zinc content of the soil used in our experiment is low and a noticeable competitive interaction between the two elements cannot be expected.

The content of iron also varied within a wide range from 75.5 to 169.0 mg kg^{-1}, with the mean value for all variants 108.2 ± 30.0 mg kg^{-1} (Fig. 28). No clear tendency can be seen and in this case. According to some authors Zn inhibited Fe translocation in some cases, which can lead to its accumulation in the roots. Probably in our case, the limiting factors in determining the content of micronutrient at the rhizome of Curcuma Longa are the soil properties.

Figs. 28 present the impact of Zn fertilization on macronutrients (K, P, Ca and Mg) content in the rhizome of *Curcuma Longa*.

The results, presented in Fig. 28 don't show any effect of foliar Zn fertilization on P, K and Ca content in variants comparing with those of control plants. A little different is the result of magnesium. Its content varies within a wide range from 0.25 to 0.33% with the mean value for all variants 0.30 ± 0.02%. This substantial variation, however, does not allow for the conclusion of synergy or antagonism between the two elements.

These results show that the interaction of zinc with P, K, Ca and Mg depends not only on the type of plant but also on the mode of application of zinc. The interaction of zinc with macronutrients is much more pronounced in the soil solution and the roots of the plants than in their above-ground parts after using zinc-containing leaf fertilizers.

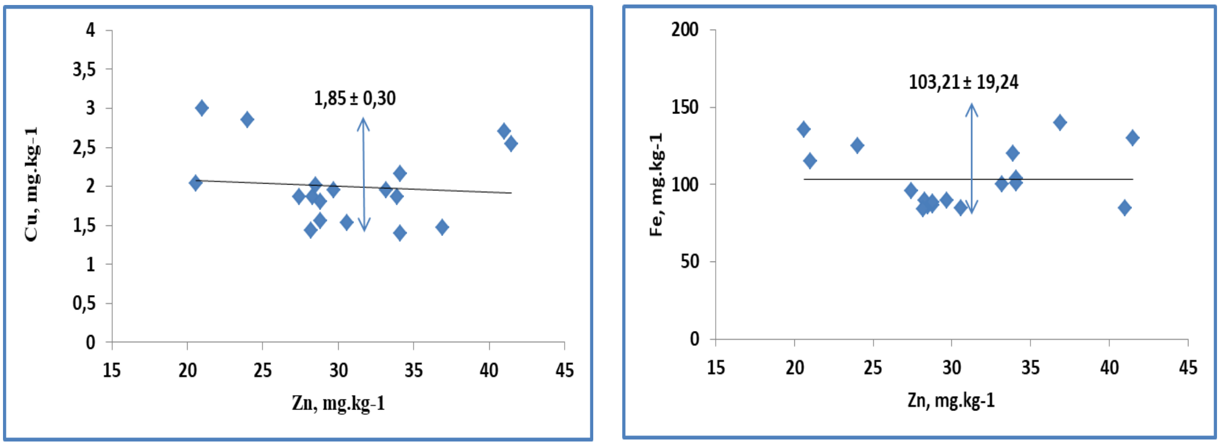


Figure 271. *Impact of Zn fertilization on Cu content in the rhizome of Curcuma Longa.*

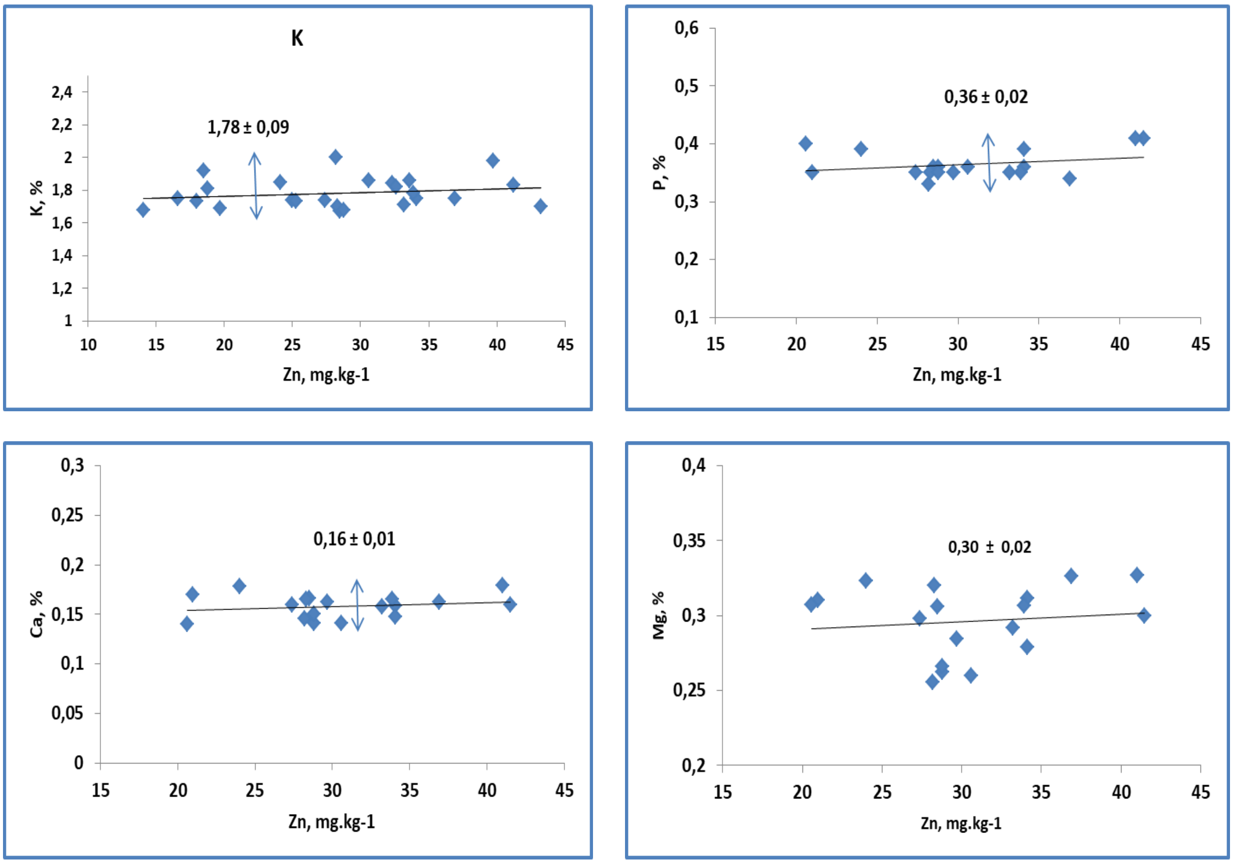


Figure 28. *Impact of Zn fertilization on K, P, Ca and Mg content in the rhizome of P. amarus.*

The summarized results for investigated micro and macroelement show that the foliar fertilization by Zn does not affect their content in the rhizome of *Curcuma Longa*. The positive effect can be sought in improving the plant physiological status and as a result a change in the quality of turmeric.

- *Impact of foliar fertilization on curcuminoids content in the rhizome of Curcuma Longa.*

Fig. 29 show our results for the impact of foliar fertilization by ZnHN suspension on the content of curcuminoids in the rhizomes of *Curcuma Longa.*

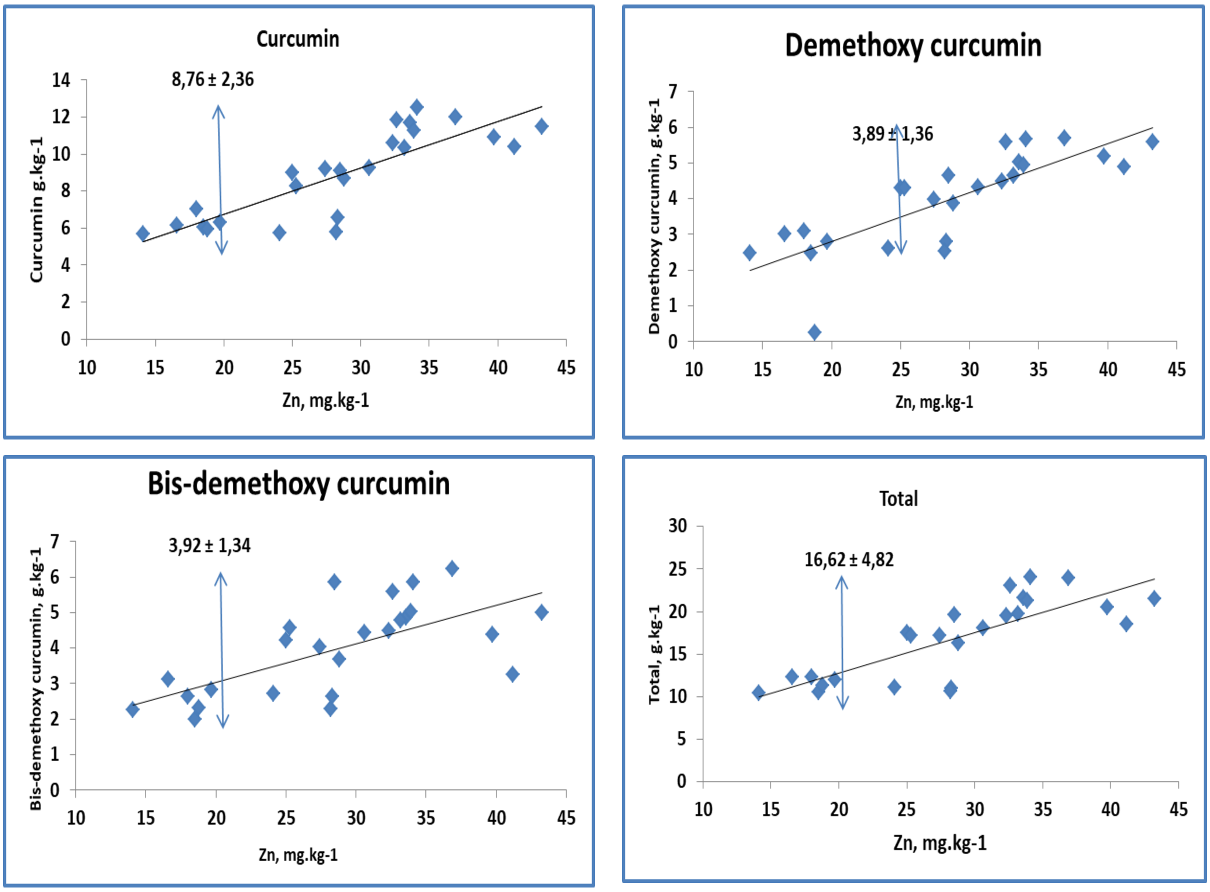


Figure 2. *Impact of Zn fertilization on* curcuminoids *content in the rhizome of Curcuma Longa.*

The content of curcumin varied within a wide range from 5.66 to 12.53 g kg^{-1}, with the mean value for all variants 8.76 ± 2.36 g kg^{-1} with a clear tendency of increasing with the increase of Zn content. The same trend is observed for desmethoxycurcumin and bisdemethoxycurcumin, but their amount is significantly less than that of curcumin – respectively 3.98 ± 1.36 and 3.92 ± 1.34 g kg^{-1}. The total content of curcuminoids varies from 10.38 to 24.05 with a clear upward trend with the increasing zinc content.

- *Impact of foliar fertilization on other main components of turmeric*

The results obtained show that the content of all components varied within a narrow range with no significant difference between the control sample and the variants ($p < 0.05$). It can be concluded, that Zn foliar fertilization has no impact on the investigated ingredients.

A significant ($p < 0.05$) effect of foliar fertilization by nanosized zinc hydroxide nitrate on curcuminoids in the rhizome of *Curcuma Longa* was found. The other ingredients forming the quality of turmeric remain unaffected.

Table 28. *Impact of foliar fertilization on dry matter, protein, sugar, fats, vitamin C and ashes in turmeric.*

Variant, №	Dry mather,%	Protein,%	Sugar,%	Fats,%	Vitamin C, mg kg^{-1}	Ashes,%
Control	*86,63*±0.28	*8,70*±0.14	*10,07*±0.12	*11,84*±0,26	*83.5*±1.64	*7.53*±0.25
1	87,28	8,82	9,99	11,85	77,3	7,12
2	86,13	8,13	10,12	12,71	83,6	7,30
3	87,41	7,49	12,65	12,30	82,4	6,94
4	86,40	7,74	11,61	12,46	72,9	6,86
5	86,63	7,62	8,84	12,45	83,1	7,78
6	86,42	8,24	12,80	13,15	72,9	7,49
7	87,25	7,93	8,34	12,01	77,4	6,53
8	86,89	7,97	11,54	12,47	82,9	7,00
Average	86.80±0,48	7.99±0,42	10.74±1,67	12.43±0,40	79.1±4,54	7.13±0,39

MAIN RESULTS AND CONCLUSIONS

The main idea *of the study was to obtain new scientific information and new knowledge that will allow controlled synthesis of zinc-containing nanofertilizers and an assessment of their potential for enhancing the yields and quality of production from basic crops.*

Synthesis of zinc hydroxy nitrates

- *Main results*

Zinc hydroxide nitrate has been synthesized by precipitation of zinc nitrate in sodium hydroxide solution under various conditions. The phase transformation from zinc hydroxide nitrate to ZnO or other intermediate compounds has been examined. As a result, the conditions for the synthesis of nanosized crystals of zinc hydroxide nitrate were optimized and new information on its stability in suspension was obtained. New information has been obtained on the equilibrium of $Zn_5(OH)_8(NO_3)_2{\cdot}2H_2O$ - ZnO and the conditions for controlled synthesis of a product with predetermined properties.

- *Main conclusion*

Long-term stability of the synthesized nanosized zinc hydroxide nitrate suspension identifies it as a promising feedstock for the preparation of a long-term foliar fertilizer.

Synthesis of mixed zinc-copper hydroxide nitrate nanoparticles

- *Main results*

New knowledge about the preparation, properties and thermal decomposition of mixed Zn-Cu nanosized hydroxy nitrates was obtained. A method for controlled synthesis of nanosized zinc and copper-containing materials based on zinc and copper hydroxide nitrate in the entire concentration range has been developed, which has enriched the knowledge in the field of inorganic synthesis. The limits of formation of solid solutions in mixed hydroxy nitrates based on $(Cu)_2(OH)_3NO_3$ were determined.

- *Main conclusions*

- A method for control preparation of mixed Cu-Zn hydroxy nitrates by using concentrated solutions of $Cu(NO_3).3H_2O$ and $Zn(NO_3).6H_2O$ is developed.
- In all cases of copper nitrate and zinc nitrate coprecipitation, the host material in the mixed samples is $Cu_2(OH)_3(NO_3)_2$.

- The composition of the mixed Co-Zn hydroxy nitrates strongly depends on the molar ratio Cu/Zn in the stock solution. At a molar ratio Cu/Zn <1.0 the synthesized samples contain $Zn_5(OH)_8(NO_3)_2 \cdot 2H_2O$ and $(Zn_1Cu_1)(OH)_3NO_3$, and at a molar ratio Cu / Zn >1.0 – $Cu_2(OH)_3NO_3$ and $(Zn_1Cu_1)(OH)_3NO_3$.
- The result will help to expand the use of nanostructured materials as precursors to produce important for practice mixed nanostructured materials, allowing controlled release of elements and their assimilation by plants.

Application of Zn-containing foliar fertilizers for recovery the yielding potential of Zn-deficient young maize plants

- *Main results*

The agronomic response of Zn-deficient maize plants to foliar fertilization with nanoscale zinc-containing foliar fertilizers is investigated. The study is conducted in two stages: (i) planting and growing the plants under controlled conditions in a zinc-deficient environment for three months and (ii) moving the plants and continuing the experiment in field conditions. The physiological status of the plants and the dynamic of zinc and micro- and macroelements concentration in plant organs are monitored. The influence of foliar zinc fertilization on yield and grain structural components is determined.

- *Main conclusions*

- Zinc fertilization throughout the initial growth stages plays a decisive role in the formation of the reproductive organs of maize plants.
- Foliar zinc fertilizers can entirely recover the physiological performance of plants grown under conditions of zinc deficiency, being zinc hydroxide nitrate superior to commercial ZnO.

Impact of foliar fertilization with nanosized zinc hydroxide nitrate on maize yield and quality – field experiments.

- *Main results*

The impact of foliar fertilization by zinc in the form of nanosized zinc hydroxide nitrate suspension alone and mixed with other nutrients on maize grain yield and quality is investigated in the frame of four years experiment. The impact of foliar applied zinc on other micro and macroelements is also estimated. The agronomic responses of 10 maize hybrids to foliar fertilization by zinc in the form of zinc hydroxide nitrate suspension is investigated too.

- *Main conclusions*

- The synthesized zinc hydroxide nitrate has indubitable potential as a successful long-term foliar fertilizer. A significant positive effect on grain yield up to 25.0% for variants compared to those of the controls was found. The best effect can be obtained by double spraying at 4 - 5 fully emerged leaf and 8 - 9 fully developed leaf.
- The most sensitive to foliar zinc fertilization are the hybrids from the early maturity group FAO 400. No direct correlation was found between foliar zinc fertilization and protein, fat and starch content in the maize grains. The critical factor of determining the yield and yield components is the maturity season of the hybrids.
- The foliar zinc application during the first growth stage has a significant ($p < 0.05$) effect on Zn accumulation in the maize stems and leaves. The accumulation of zinc is followed by its remobilization from the stems to other plant organs during the second growth stage.

Impact of foliar application of zinc on micro- and macro elements distribution in Phyllanthus amarus

- *Main results*

The interaction of foliar applied zinc with other elements in *Phyllanthus amarus* plants is investigated. The Zn content and the content of selected micro (Cu, Fe, Mn) and macro (Ca, Mg, P and K) nutrients in plant roots, stems and leaves are determined.

- *Main conclusions*

- The Zn, Cu, Mn and macronutrients content of plant roots varies narrowly, with no significant impact of ZnHN fertilization.
- The zinc content of plant stems and leaves varies within wide limits, with the significant impact of ZnHN fertilization. The trends in the content of Cu, Mn and macronutrients are kept the same as in the root, whereas the iron trends to increase its content at increasing the zinc content.

Impact of foliar application of zinc on micro and macro elements distribution in Curcuma Longa.

- *Main results*

The interaction of foliar applied zinc in the form of zinc hydroxide nitrate suspension with other elements in *Curcuma Longa* plants is investigated. The impact of the zinc application on the quality of turmeric was also estimated. The Zn content and the content of selected micro (Cu and Fe) and macro (K, Ca, Mg, and P) elements in the plant's rhizome are determined. Separation and quantification of curcuminoids were accomplished too.

- *Main conclusions*

- The fertilization by zinc in the form of nanosized zinc hydroxide nitrate suspension has a significant ($p < 0.05$) effect on curcuminoids in the rhizome of *Curcuma Longa*. The other ingredients forming the quality of turmeric remain unaffected.
- The content of Cu, Fe and macronutrients varies narrowly with no significant impact of zinc hydroxide nitrate fertilization. The interaction of zinc with micro and macronutrients is much more pronounced in the soil solution and the roots of the plants than in their above-ground parts after using zinc-containing leaf fertilizers.

List of publications:

A. Published

1. Ivanov, K., E. Kolentsova, **N. Nguyen**, A. Peltekov and V. Angelova, Synthesis and stability of zinc hydroxide nitrate nanoparticles. *Bulg. Chem. Commun*. 49: 225-230, (2017). **IF=0.242**
2. Krasimir Ivanov, Tonyo Tonev, **Nguyen Nguyen**, Alexander Peltekov, Anyo Mitkov, Impact of foliar fertilization with nanosized zinc hydroxide nitrate on maize yield and quality, *Emirates Journal of Food and Agriculture*, **31**(8): 597-604, (2019). **IF=0.921.**
3. **Nguyen Cao Nguyen**, Krasimir I. Ivanov, Penka S. Zapryanova, Impact of Foliar Application of Zinc on Micro and Macro Elements Distribution in Phyllanthus Amarus, *International Journal of Agricultural and Biosystems Engineering*, *13*(7), 193-198, (2019), **Google Scholar.**

Б. Given for printing:

1. T. Tonev, E. Kolentsova, A. Mitkov, **N. Nguyen**, K. Ivanov, Nanofertilizers in Sustainable Agriculture: Foliar Application of Zinc to Enhance Productivity of Maize, *J. of Environmental Protection and Ecology*, (2000), **IF= 0.634.**
2. **Nguyen Nguyen**, Penka Zapryanova, Magdalena Stoyanova, Krasimir Ivanov, Impact of foliar application of zinc on curcumin and macro and micro elements distribution in curcuma longa, *Agricultural Sciences*, (2019), **Google Scholar.**

Participation in scientific conferences:

A. International:

1. Krasimir Ivanov, Elitsa Kolentsova, **Nguyen Nguyen**, Alexander Peltekov, Violina Angelov, Some Observations on the Preparation of Zinc Hydroxide Nitrate Nanoparticles, *ICNT 2016 : 18th International Conference on Nanotechnology and Therapeutics*, Singapore, SG, September 08-09, 2016.
2. Krasimir Ivanov, Tonyo Tonev, **Nguyen Nguyen**, Alexander Peltekov, Anyo Mitkov, *ICAIE 2018 : International Conference on Agricultural Infrastructure and Environment*, Paris, France, July 19-20, 2018.
3. **Nguyen Cao Nguyen**, Krasimir I. Ivanov, Penka S. Zapryanova, Impact of Foliar Application of Zinc on Micro and Macro Elements Distribution in Phyllanthus Amarus, *ICSAFS 2019 : International Conference on Sustainable Agricultural and Food Systems*, Amsterdam, The Netherlands, May 14-15, 2019.

Б. National:

1. Krasimir Ivanov, Alexander Peltekov, Nguyen Nguyen, Elitsa Kolentsova, Thermal decomposition of nano-sized zinc hydroxy nitrate, *11th Chemistry Conference (11cc*), 11 – 13 October 2018, Plovdiv, Bulgaria.

Citations:

I. Ivanov. K I, Kolentsova. E N, **Nguyen. Cao N**, Peltekov. A B, Angelova. V R (2017). Synthesis and Stability of Zinc Hydroxide Nitrate Nanoparticles. Bulgarian Chemical Communications, Volume 49 Special Issue G (pp.225 –230) 2017

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1. Shadreck Mtambo**, "**Two dimensional layered material, a potential slow release fertilizer". PhD Thesis, Faculty of science, Chemistry department, Bindura university of science education, (2018). Link.
2. WL de Almeida, W. L., Rodembusch, F. S., Ferreira, N. S., & de Sousa, V. C., Eco-friendly and cost-effective synthesis of ZnO nanopowders by Tapioca-assisted sol-gel route. *Ceramics Internationa*, 46 (8), Part A, (2020), 10835-10842. Link.
3. Westrup, Kátia CM, et al. "Light-assisted cyclohexane oxidation catalysis by a manganese (III) porphyrin immobilized onto zinc hydroxide salt and zinc oxide obtained by zinc hydroxide salt hydrothermal decomposition." *Applied Catalysis A: General* (2020): 117708. Link.
4. Batista, Elisson Andrade, et al. "Effect of the location of Mn+ 2 ions in the optical and magnetic properties of ZnO nanocrystals." *Journal of Alloys and Compounds* (2020): 156611. Link.
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2. Brankov, M., Simić, M., Dolijanović, Ž., Rajković, M., Mandić, V., & Dragičević, V. (2020). The Response of Maize Lines to Foliar Fertilizing. *Agriculture*, *10*(9), 365. Link