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# Use of Siliceous Minerals as Natural Nitrification Inhibitors †

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**Abstract:** The comparative study on the effects of nitrapyrin and the mixture of natural siliceous minerals on physiological processes in economically important C3 and C4 crops (viz. wheat and corn) grown under high nitrogen fertilization conditions, as well as on soil microbiota and nitrogen balance was conducted in the laboratory and long-term field experiments. The siliceous minerals were shown to have significantly higher inhibitory effects on nitrification process in different types of soils, amended with urea, the number of nitrifiers and micromicetes producing phytotoxic allelochemicals compared with nitrapyrin. Crops treated with siliceous minerals had higher photosynthetic pigment content and higher glutamic acid concentration, which indicated the intensification of photosynthetic processes. Nitrapyrin reduced the concentration of chlorophyll b and carotenoids, but slightly increased the content of chlorophyll a in the leaves of wheat and corn. The content of aspartate and some aromatic amino acids decreased, while arginine and lysine increased. Such metabolic changes suggested disintegration of nitrogen and phosphate metabolism in the studied crops. Thus, the use of siliceous minerals is more advantageous than nitrapyrin in terms of their effectiveness, persistence in various types of soils and beneficial effect on soil microbiota and crop’s functional state and productivity.

**Keywords:** nitrification inhibitors; siliceous minerals; nitrogen balance; corn; winter wheat; growth; photosynthesis; soil microbiota

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## 1. Introduction

Nitrogen (N) is an essential nutrient element and also a key limiting factor for the growth and development of plants in agricultural systems [1]. About 25% of nitrogen available to plants in the soil is presented in the form of ammonia and nitrate ions produced by microbiological transformation of residues and humus [1]. In the majority of agricultural soils, $NH_4^+$ is rapidly converted to $NO_3^-$ as a result of the biological oxidation of ammonia ($NH_3$) or ammonium ($NH_4^+$) to oxidized nitrogen in the form of nitrite ($NO_2^-$) and further to nitrate ($NO_3^-$). This process is referred to as nitrification and takes a relatively short period of time [2]. The nitrate formed is susceptible to losses via leaching and conversion to gaseous forms via denitrification [2]. Often less than 30% of the applied N fertilizer is recovered in intensive agricultural systems, largely due to losses associated with the following nitrification [3]. $NO_3^-$ leaching from intensive agricultural systems typically represents the major N loss. Unlike ammonium $(NH_4)^+$, which is strongly held on soil particles, $NO_3^-$ is a negative ion and weakly held by soil making it susceptible to leaching under higher rainfall or irrigation, particularly if it is present in much greater quantities than plants can uptake [4]. Besides, nitrates are readily denitrificated by soil microorganisms to gaseous forms of nitrogen, mainly $N_2O$, which is a long-lasting greenhouse gas (lifetime—150 years), and is also the major source of ozone depleting nitric oxide NO, and nitrogen $N_2$ [5].

In the next 20–30 years, a significant increase in the world's population is expected, and, accordingly, it is necessary to produce food in sufficient quantities. To achieve this, the use of nitrogen fertilizers will have to double by 2050 [1]. For environmental reasons, this is not possible as nitrate levels in drinking water, eutrophication of surface waters and greenhouse gas emissions have already reached critical levels in many countries of the world. The use of nitrification inhibitors can reduce the use of fertilizers and significantly increase their efficiency. Therefore, there is a need for environmentally friendly nitrification inhibitors, as well as methods for their study.

Approaches to the management of nitrification include those that control ammonium substrate availability and those that inhibit nitrifiers directly [2]. Strategies for controlling ammonium substrate availability include timing of fertilization, formulation of fertilizers for slow release, intensification of nitrogen cycling (immobilization). Another effective strategy is to inhibit nitrification directly with either synthetic or biological nitrification inhibitors [2,3]. The latter are chemical compounds temporarily reducing populations of *Nitrosomonas* and *Nitrobacter* bacteria in soil. Nitrification inhibitors protect against both denitrification and leaching by retaining fertilizer N in the ammonium form.

There are at least eight compounds commercially recognized as nitrification inhibitors, although the most commonly used and most studied are 2-chloro-6-(trichloromethyl)-pyridine (nitrapyrine), dicycanediamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP). These compounds inhibit microbial activity for several days to weeks depending on soil moisture and soil type, although there are differences in the way they are used. In general, nitrification inhibitors are more effective on sandy soils or soils with low organic matter content and low temperature effects.

Another group of chemicals used for regulation of the soil nitrogen include urease inhibitors. These chemicals block the activity of the enzyme urease. Urease is found in soil as well as in plant residues. Urease, along with water, will hydrolyze, or break down, urea into ammonium. The loss process that urease inhibitors protect against is ammonia volatilization. With high pH soil, or soil/residue environments that are poorly buffered against an increase in pH, the rapid hydrolysis of urea will result in an accumulation of ammonia ($NH_3$). The latter can be lost to the atmosphere as a gas. By keeping urea from hydrolyzing, urease inhibitors protect against ammonia volatilization, keeping nitrogen fertilizer in the urea form. Among commercially available urease inhibitors (N-(n-butyl) thiophosphorictriamide (NBPT) и N-(n-propyl) thiophosphorictriamide (NPPT) are the most studied [6].

The use of the mentioned above both groups of chemicals allows reducing the use of fertilizers and significantly increasing their efficiency. However, the use of nitrification and urease inhibitors increases cost and the risk of environmental contamination [7]. Recently, nitrapyrin has been detected in streams, suggesting off-site transport of this nitrogen stabilizing compound [8]. DCD residues were detected in milk in New Zealand resulting in the suspension of DCD use in pastures [9]. Commercially available urease inhibitors NBPT and NPPT were shown to be phytotoxic to some sensitive crops [6]. Besides, chemical nitrification and urease inhibitors are not permitted in certified organic management systems. Therefore it is necessary to use nitrification inhibitors which are environmentally safe, non-toxic, non-volatile and have a prolonged effect. In this regard the natural siliceous minerals have been suggested as organic alternatives for management of nitrification.

Natural siliceous minerals present promise as environmentally friendly regulators of the biogeochemical nitrogen cycle. In contrast to synthetic nitrification inhibitors natural siliceous minerals do not impose any harm to soil microbiota and were shown to have beneficial effect on crop productivity [10,11]. They are stable in the soil environment, and, after having been applied once, express their effect for many years. Silicon was shown to increase nitrates utilization by crops by stimulating photosynthetic rate, root activities and nitrate reductase activity in plants [12,13]. Rice grains accumulated 17%–37% more N with an exogenous application of Si between 100 $kg \cdot ha^{-1}$ $SiO_2$ and 400 $kg \cdot ha^{-1}$ $SiO_2$ compared to the control. Similar increase in N accumulation for rice straw and total biomass with the same Si doses was in the ranges of 19%–29% and 18%–33%, respectively [13].

Fertilization with sodium metasilicate (50–800 mg Si kg$^{-1}$) stimulated uptake of N and Ca by cowpea and wheat and improved nodulation and $N_2$ fixation in cowpea [14].

In the greenhouse study $NH_4$-charged zeolite was shown to minimize $NO_3$-leaching from soil and to optimize water-saving soil capacity as well as corn growth and yield under different fertilizing conditions (i.e., standard, high or 70%, medium or 50% and low or 30% of conventional fertilization rate). The results suggested that plants may have a better response if $NH_4$-charged zeolite is used with a limited amount of conventional fertilizer, allowing a reduction of nitrate concentration in drainage [15]. The field studies on the effect of soil amendments (i.e., lime or zeolite (clinoptilonite)) on nitrous oxide ($N_2O$) and dinitrogen ($N_2$) emissions from pastoral soil conducted on Topehaehae silt loam soil (Aeric Haplaquent, a dairy catchment area at Toenepi, Hamilton, New Zealand) demonstrated that zeolite (clinoptilonite) significantly reduced total $N_2O$ emissions by 11% from cow urine treated soils probably because of $NH_4^+$ sorption by zeolite; while it had no such effect on $N_2O$ emission in $KNO_3$ treated soils (the both nitrogen fertilizers were applied at a rate of 200 kg N ha ha$^{-1}$). While lime did not have any effect on $N_2O$ emission in either urine or $KNO_3$ treated soils [16].

The objectives of the given study was to compare the effectiveness of nitrapyrin and the mixture of natural siliceous minerals (analcite and tripoli) on the balance soil nitrogen, dynamics of the different functional groups of microorganisms in the soil, metabolism and productivity in economically important C3 and C4 crops (viz. wheat and corn) grown under high nitrogen fertilization conditions.

The mentioned siliceous minerals are readily available in Ukraine (Vinnytsia and Rivne regions) and present inexpensive and environmentally safe source of fertilizers for agricultural needs. In addition, unlike nitrapyrin, these minerals neither are hazardous for human health nor explosive [17].

## 2. Experiments

### 2.1. Experimental Procedures

#### 2.1.1. Pot Experiments

The effectiveness of nitrapyrin and a mixture of siliceous minerals (analcite and tripoli) as nitification inhibitors was compared using different types of substrates (sand and soil mix) and crop species in the laboratory pot experiments. The latter were conducted in the plant growth chamber at the department of Allelopathy of the M.M.Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv, Ukraine). Wheat (*Triticum aestivum* L. **cv.** «Smuglianka»**)** and corn (*Zea mays* L., Hybrid Pereyaslovsky 230 SV) seeds were obtained from the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine.

The test-plants were grown in 1 L plastic pots for 45 days under controlled conditions: air temperature of 20–22 °C, illumination of 1700 lux. The rate of application of nitrapyrin was 1 mL (1,58 g) per 1 L of soil mixture or sand, and a mixture of minerals of analcite and tripoli in the amount of 1 g per 1 L of sand or soil mixture, which included sand, peat, meadow soil, humus in proportion 1: 1: 1: 0.2. As a source of nitrogen 0.5% urea solution was used.

Qualitative and quantitative analysis of soil microbiota and assessment of the content of nitrates in the sand and soil substrates were conducted on 15-th, 30-th and 45-th day of the test-plant cultivation.

#### 2.1.2. Field Experiments

5-years field studies were conducted on the experimental plots (plot area—2 ha) situated at the Agricultural Research Station of the Institute of Bioenergy Crops and Sugar Beet of the National Agrarian Academy of Sciences of Ukraine (Kyiv region, Ksaverovka Village, Kagarlytsky District during 2015–2019). The nitrification inhibitors were applied to winter wheat(*Triticum aestivum* L.) cv. «Samurai» and corn *Zea mays* L. hybrid «Adevei 4014»). The rate of the applied nitrapyrin was 1.7 L per 1 ha (2,69 kg per 1 ha), and the amount of siliceous minerals was 10 kg per 1 ha for winter wheat and 6 kg per 1 ha for corn. For winter wheat, the rate of urea application was 100 kg/ha, and for corn—60 kg/ha. Nitrification inhibitors were applied simultaneously with fertilizers ($N_{20}P_{60}K_{60}$): for winter wheat—at the stage of spring tillering (on frozen thawed ground), for corn—in the phase of 3–5 leaves under harrowing. The soil type was dark-gray podzol, sandy slightly loamy with the following content of the basic macroelements: nitrogen 92 mg/kg, phosphorus—150 mg/kg, water-soluble potassium—1.8 mg/kg. The pH of soil solution was 6.7.

The measurements of the content of nitrate nitrogen in soil were timed to crop's phenological phases (tillering, first node, flowering, physiological maturity). Allelopathic activity of soil and the number of microorganisms producing phytotoxic allelochemicals were evaluated two months after the application of nitrification inhibitors. Changes in the amino acid composition and photosynthetic pigments content in the leaves of winter wheat and corn were evaluated 30 days after the application of nitrification inhibitors and urea.

### *2.2. Measurements*

The macro- and microelements in soil samples were determined using inductively coupled plasma spectrometer iCAP 6300 DUO from Thermo Fisher Scientific, USA (2006). Their extraction was conducted with 1N HCl [18].

The photosynthetic pigments (chlorophylls a, b and carotenoids) were extracted from freshly collected leaves of the test-plants with dimethylsulfoxide (DMSO) [19]. Their content was determined spectrophotometrically with SPECORD 200 (Analytik Jena). Qualitative and quantitative content of free amino acids was determined by amino acid analyzer Hitachi [20].

Allelopathic activity of the rhizosphere soil was assessed by direct bioassay (Grodzinski et al., 1990) on germination of radish (Raphanus sativus var. radiculata, cv. «Krasny s belym konchikon») seeds. The seeds (20 seeds per a Petri dish) were germinated at a temperature of + 23–26 °C in darkness. The number of germinated seeds was counted on the 2nd day, when in the control germinated 50% of the seeds [21]. Phenolic allelochemicals were extracted from the soil samples with ethanol and 70% acetone in distill water [22]. Their quantity was assessed spectrophotometrically using color reaction with Folin Ciocalteu reagent with SPECORD 200 (Analytik Jena) [22].

Analysis of the number of microorganisms from freshly collected soil samples was carried out by sowing soil suspensions in appropriate dilutions on selective agar nutrient media: ammonifying bacteria (peptone ammoniation medium); nitrifying bacteria (Winogradsky medium); denitrifying bacteria (Trypticase soy agar supplemented with nitrate), micromycetes (Chapek's medium); bacteria (meat peptone agar + wort agar); actinomycetes (starch-ammonia agar). The count of the colonies was conducted visually using light illuminated microscope Zeiss on 3-th—7th days after sowing [23].

### *2.3. Statistical Analysis*

The data were subjected to the analysis of variance (ANOVA) appropriate to the randomized complete block design applied after testing the homogeneity of error variances using Levene's mean-based F-test procedure with modifications outlined by D. Sharma and B. M. Golam Kibria [24]. The significant differences between treatments were compared with the critical difference at 5% probability level by the LSD (the least significant difference) test. The statistical operations were conducted using Statistica 10.0 software.

## **3. Results**

### *3.1. Pot Experiments*

The data presented in the Table 1 clearly show that application of 0.5% urea solution caused gradual increase of nitrate concentration in both sand and soil mix, reaching maximum values to the end of the experiment (45-th day). The mixture of analcite and tripoli was the most effective in inhibiting the nitrification process in the both types of substrates (sand and soil mix) irrespective of the test-plant species. For nitrapyrin treatments minimum nitrates level in the substrates was observed on the 30-rd day after application. On the 45-th day the nitrates concentration slightly increased. Application of the mixture of siliceous minerals (analcite and tripoli) had been reducing the nitrates concentration from the beginning till the end of the experiments reaching the minimum on the last (45-th day) of the experimentation, indicating higher persistence comparing to nitrapyrin.

The decrease in pH of the soil solution and % of humus in the substrates stimulated nitrification processes in treatments with nitrapyrin more significantly compared with the similar treatments exposed to siliceous minerals.

**Table 1.** The effect of exogenous nitrapyrin and a mixture of siliceous minerals (analcite and tripoli) on the content of nitrate nitrogen in the soil.

| Treatment | Type of Substrate | pH of Soil Solution | Humus % | The Content of Nitrate Nitrogen, mg/kg Duration of Plant Exposure, Days | | |
|---|---|---|---|---|---|---|
| | | | | **15** | **30** | **45** |
| | | | **Wheat** | | | |
| Control | Sand | 7,2 | 0,03 | 115,7 | 193,6 | 298,1 |
| | Soil mix | 6,2 | 7,5 | 146,9 | 235,8 | 334,2 |
| Nitrapyrin | Sand | 7,2 | 0,03 | 97,5 | 83,9 | 92,4 |
| | Soil mix | 6,2 | 7,5 | 101,7 | 88,3 | 95,6 |
| Analcite + Tripoli | Sand | 7,2 | 0,03 | 71,3 | 64,9 | 61,7 |
| | Soil mix | 6,2 | 7,5 | 73,8 | 66,1 | 64,3 |
| *LSD (P = 0,05)* | | *0,01* | *0,01* | *1,23* | *1,88* | *1,74* |
| | | | **Corn** | | | |
| Control | Sand | 7,2 | 0,03 | 99,3 | 168,4 | 255,8 |
| | Soil mix | 6,2 | 7,5 | 125,7 | 191,0 | 287,1 |
| Nitrapyrin | Sand | 7,2 | 0,03 | 81,2 | 73,7 | 77,6 |
| | Soil mix | 6,2 | 7,5 | 87,9 | 76,4 | 82,3 |
| Analcite + Tripoli | Sand | 7,2 | 0,03 | 52,4 | 43,9 | 42,5 |
| | Soil mix | 6,2 | 7,5 | 54,3 | 45,1 | 43,2 |
| *LSD (P = 0,05)* | | *0,01* | *0,01* | *1,74* | *1,45* | *1,58* |

* Once a week, a 0.5% solution of urea was added in an amount of 50 mL per 1 L of soil substrate.

Microbiological analysis of the soil samples of the pot experiment showed that application of urea caused an increase in the number of nitrifying and denitrifying microorganisms from 15 to 30 days of the test-plants cultivation, followed by some decrease. When nitrapyrin was applied with urea, a sharp decrease in the number of nitrifiers and denitrifiers was observed on the 30th day of the experiment with a gradual increase in their number on the 45th day (Table 2). Application of analcite-tripoli mixture with urea demonstrated more pronounced effect on the quantity of these two groups of microorganisms for the whole period of experimentation with the minimum values registered on the 45-th day, which indicated a more prolonged effect of silicon compounds on the inhibition of nitrification processes in the soil compared to nitrapyrin. The both nitrification inhibitors studied had a positive effect on the content of ammonifiers in the soil. However, the siliceous mixture again had a more prolonged effect on this group of microorganisms compared to nitrapyrin.

**Table 2.** The dynamics of the different functional groups of microorganisms in the soil from pots where winter wheat was cultivated with different nitrification inhibitors.

| Treatment | Microorganisms | Period of Winter Wheat Cultivation, Days | | |
|---|---|---|---|---|
| | | **15** | **30** | **45** |
| Control | Total number, million/g | 20,6 | 16,2 | 11,9 |

| | | | | |
|---|---|---|---|---|
| Nitrapyrin | | 21,4 | 24,7 | 14,3 |
| Analcite + Tripoli | | 23,8 | 24,3 | 25,1 |
| *LSD (P = 0,05)* | | *0,62* | *0,89* | *097* |
| Control | Ammonifying, million/g | 4,6 | 10,4 | 3,2 |
| Nitrapyrin | | 9,7 | 17,5 | 12,0 |
| Analcite + Tripoli | | 11,5 | 17,4 | 16,9 |
| *LSD (P = 0,05)* | | *0,71* | *0,35* | *037* |
| Control | Denitrifying, million/g | 3,1 | 4,3 | 3,8 |
| Nitrapyrin | | 1,8 | 1,3 | 1,5 |
| Tripoli + Analcite | | 0,7 | 0,6 | 0,4 |
| *LSD (P = 0,05)* | | *0,48* | *069* | *077* |
| Control | Nitrifying, million/g | 3,9 | 4,1 | 3,3 |
| Nitrapyrin | | 3,0 | 2,1 | 2,3 |
| Analcite + Tripoli | | 2,4 | 1,8 | 1,5 |
| *LSD (P = 0,05)* | | *0,43* | *0,31* | *0,44* |

### *3.2. Field Experiments*

The results of agrochemical analysis of the rhizosphere soil of field experiments were in good agreement with the tendencies observed in the laboratory tests (Table 3). In the absence of nitrification inhibitors, the nitrate nitrogen content in the soil gradually decreased during the growing season, reaching during the phase of physiological maturity of crops the level of 20% of the initial (tillering phase). Combined application of urea (100 kg/ha) and analcite + tripoli mixture (10 kg/ha) to winter wheat had a positive effect on the nitrogen supply to plants compared with nitrapyrin treatment (2.69 kg per 1 ha).

**Table 3.** The effect of nitrification inhibitors on the content of nitrate nitrogen in the soil under winter wheat, mg/L.

| **Treatment** | **Phenological phase** | | | |
|---|---|---|---|---|
| | **Tillering** | **First Node** | **Flowering** | **Physiological Maturity** |
| Control | 78,3 | 51,8 | 28,3 | 15,9 |
| Analcite + Tripoli | 63,7 | 58,4 | 50,9 | 42,1 |
| Nitrapyrin | 71,3 | 50,9 | 37,4 | 28,2 |
| *LSD (P = 0,05)* | *2,12* | *1,87* | *124* | *1,77* |

Microbiological analysis of the root layer of the soil (depth −0–20 cm) showed a significant long-term positive effect of the analcite + tripoli mixture on the microorganisms producing phytotoxic allelochemicals (Table 4). The positive effect of nitrapyrin was significantly lower. Data from microbiological analysis were in good agreement with the results of the assessment of the soil allelopathic activity using radish seeds as a bioassay.

**Table 4.** Allelopathic activity of soil and the number of microorganisms that produce phytotoxic allelochemicals 60 days after application of nitrification inhibitors.

| **Treatment** | **Wheat** | | | | **Corn** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Germination of Radish Seeds, %** | **The Number of Phytotoxic Microorganisms, million/g** | | | **Germination of radish seeds, %** | **The Number of Phytotoxic Microorganisms, million/g** | | |
| | | **Bacteria** | **Micro-Mycetes** | **Actino mycetes** | | **Bacteria** | **Micro-Mycetes** | **ACTINOMYCETES** |
| Control | 72,1 | 13,6 | 10,1 | 2,8 | 70,7 | 14,5 | 10,8 | 3,1 |
| Analcite + Tripoli | 92,2 | 3,3 | 4,5 | 1,7 | 91,0 | 3,8 | 5,1 | 1,9 |
| Nitrapyrin | 75,8 | 11,9 | 9,4 | 3,0 | 77,5 | 12,4 | 9,8 | 3,4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *LSD (P = 0,05)* | *2,33* | *0,67* | *1,11* | *0,25* | *1,76* | *0,78* | *0,99* | *0,76* |

The decrease in soil phytotoxicity is evidenced by a 1.2–1.9-fold decrease in the concentration of phenolic compounds (Table 5).

**Table 5.** The effect of nitrification inhibitors on the content of phenolic allelochemicals in the soil.

| **Treatment** | **Phenolic Allelochemicals, mg/kg of Dry Soil** | | | |
|---|---|---|---|---|
| | **70% Acetone Extract** | | **Ethanol Extract** | |
| | **Corn** | **Wheat** | **Corn** | **Wheat** |
| Control | 39,3 | 33,8 | 151,3 | 147,2 |
| Analcite + Tripoli | 22,7 | 19,4 | 112,7 | 104,9 |
| Nitrapyrin | 31,5 | 28,7 | 144,3 | 138,7 |
| *LSD (P = 0,05)* | *1,13* | *1,65* | *2,05* | *1,25* |

Application of the tested nitrification inhibitors caused significant biochemical changes, in particular, in the composition of free amino acids (Table 6) in the leaves of wheat and corn plants. Nitrapyrin treatment caused a decrease in aspartic acid as well as aromatic amino acids, while the content of arginine and lysine increased in the both wheat and corn plants. In the test-plants treated with analcite + tripoli mixture, an increase in the content of glutamic acid was recorded, which indicated the intensification of photosynthetic processes.

**Table 6.** Changes in amino acid composition in the leaves of winter wheat and corn 30 days after the application of nitrification inhibitors, µg/100 mg of fresh weight.

| **Amino Acids** | **Wheat** | | | **Corn** | | |
|---|---|---|---|---|---|---|
| | **Co ntr ol** | **Ni tra py** ıa | lci te + Tri | **Co ntr ol** | **Ni tra py** ıa | lci te + Tri |
| Aspartic acid | 8,5 | 8,1 | 17,3 | 6,2 | 5,5 | 14,2 |
| Threonine | 15,0 | 9,8 | 7,4 | 26,9 | 22,1 | 19,2 |
| Serine | 2,3 | 1,8 | 0,9 | 2,1 | 1,3 | 0,5 |
| Glutamic acid | 6,4 | 6,7 | 9,1 | 11,2 | 11,8 | 15,1 |
| Histidine | 4,5 | 4,8 | 6,0 | 4,7 | 4,9 | 7,4 |
| Tyrosine | 2,3 | 2,4 | 3,9 | 1,4 | 1,6 | 2,5 |
| Arginine | 9,7 | 8,2 | 4,7 | 10,9 | 10,3 | 5,1 |
| Lysine | 2,5 | 2,2 | 1,5 | 2,3 | 2,0 | 0,8 |
| Phenylalanine | 1,3 | 2,1 | 2,6 | 1,8 | 1,9 | 3,3 |
| *LSD (P = 0,05)* | *0,04* | *0,01* | *0,01* | *0,01* | *0,02* | *0,01* |

The content of photosynthetic pigments in the leaves of winter wheat and corn plants 30 days after application of urea with nitrification inhibitors differed significantly between treatmnets. Application of siliceous mixture contributed to a marked increase in the content of chlorophyll a and b, as well as carotenoids in the leaves of the studied crops. While nitrapyrin caused a decrease in the biosynthesis of chlorophyll b and carotenoids and a slight increase in the content of chlorophyll a in the leaves of the test-plants (Table 7). A significant increase in the concentration of chlorophyll b in the leaves of wheat and corn plants treated with analcite + tripoli mixture should be noted.

**Table 7.** The content of photosynthetic pigments in the leaves of winter wheat and corn treated with the different nitrification inhibitors, mg/100 g of fresh weight.

| **Treatment** | **Wheat** | | | **Corn** | | |
|---|---|---|---|---|---|---|
| | **Chlorophyll** | | **Carotenoids** | **Chlorophyll** | | **Carotenoids** |
| | **a** | **b** | | **a** | **b** | |
| Control | 74,2 | 22,7 | 92,5 | 80,9 | 37,6 | 105,7 |
| Nitrapyrin | 76,9 | 21,8 | 91,7 | 96,3 | 34,2 | 88,2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Analcite + Tripoli | 82,5 | 40,3 | 112,6 | 95,1 | 58,9 | 121,3 |
| *LSD (P = 0,05)* | *1,12* | *0,84* | *0,95* | *0,98* | *0,86* | *0,92* |

The data in Table 8 clearly show that the highest yield of winter wheat was observed on plots where mixture of tripoli and analcite was applied. Grain was characterized by the highest content of protein and fiber. Nitrapyrin showed significantly less efficacy compared to the siliceous mixture.

**Table 8.** The effect of nitrification inhibitors on the yield of winter wheat.

| Treatment | Yield, quintal/ha | Protein content, % | Fiber, % |
|---|---|---|---|
| Control | 68,4 | 12,6 | 24,3 |
| Analcite + Tripoli | 82,5 | 13,5 | 25,1 |
| Nitrapyrin | 75,3 | 12,9 | 24,7 |
| *LSD (P = 0,05)* | *0,97* | *0,54* | *0,48* |

## 4. Discussion

Despite a great interest in nitrification inhibitors to date, only a few compounds have been adopted for agricultural use. The main problems are the high cost and contamination of the environment [6–9]. There is need to continue efforts to develop nitrification inhibitors that are inexpensive, readily available locally, and effective at reasonable rates of application.

The effectiveness of zeolite in minimizing NO3-leaching from soil and optimizing crop's growth and yield under different fertilizing conditions were shown in a number of studies [15,16].

The results of our laboratory and field experiments confirmed good potential of the mixture of the natural siliceous minerals (analcite and tripoli) to reduce nitrification process and $NO_3$-leaching from different types of agricultural soils under sowings of winter wheat and corn. In our study application of mixture of the natural siliceous minerals (analcite and tripoli) was noticeably more effective in altering soil inorganic N content, composition of microbial community and nitrogen metabolism in crops as compared to nitrapyrin. The duration of the observed effects for siliceous mixture was more lasting as compared to nitrapiryn.

In particular, the mixture of tripoli and analcite more efficiently preserved nitrogen in the soil in comparison with the synthetic nitrification inhibitor (nitrapyrin). In addition, the use of the mixture of tripoli and analcite provided a longer preservation of nitrogen in the soil. At the same time it is found out that decrease in pH and quantitative parameters of humus of soil substrates stimulated processes of nitrification in treatments with nitrapyrin more essentially in comparison with treatments with siliceous minerals.

The results of microbiological analysis are in good agreement with the data of agrochemical evaluation of soil. The application of a siliceous mixture caused a more prolonged decrease in the number of nitrifiers and denitrifiers, as well as an increase in the number of ammonifiers in the soil compared with nitrapyrin.

In addition, the siliceous mixture more effectively inhibited the development of microorganisms that produce phytotoxic allelochemicals. In particular, this applies to bacteria, micromycetes and actinomycetes. It is known that long-term cultivation of wheat and corn causes changes in the microbiota composition of the rhizosphere soil, which significantly affects the properties of the soil. In particular, the development of beneficial microorganisms that produce vitamins, enzymes and organic acids is inhibited, while microbiota producing phytotoxic allelochemicals prevail. All this affects the yield of these crops [25]. Application of a siliceous mixture contributed to improving the composition of the microbiocenosis and allelopathic regime of the rhizosphere soil.

Similar results were obtained by Ellanska et al. [26]. The authors studied the effect of analcite applied at a rate of 50 $kg \cdot ha^{-1}$ on the soil microbial community under sugar beet plantations and showed that growth of soil streptomycetes was almost 2-fold as much as in the rhizosphere soil without analcite amendment. This led to the increased activity of enzymes involved in transformation of nitrogen and carbon compounds. Besides increased titer of microorganisms assimilating inorganic nitrogen. The increase of mineralization index up to 2.53 (compared to 0.81 in control) indicated the enhancement in soil mobilization processes and associated mineralization of organic compounds. Abundance of *Azotobacter chroococcum* was 100% in both cases. However application of analcite stimulated their faster development as compared to control [26]. In other study, conducted by Mali and Aery [14] exogenous silicates promoted nodule formation in legume plants and hence promotion of $N_2$ fixation. The authors reported an increase in absorption of N and Ca for cowpea and wheat fertilized with increasing doses of sodium metasilicate as well as an improvement in nodulation and $N_2$ fixation in cowpea.

In our study, the tested nitrification inhibitors demonstrated different effects on nitrogen metabolism in winter wheat and corn leaves. In particular, nitrapyrin application decreased aspartic acid content in the test-plant's leaves, indicating impaired nitrogen metabolism caused by suppression of amination and reamination reactions. In turn, with unsatisfactory nitrogen supply, a significant part of inorganic phosphorus accumulates in the vacuoles, resulting in a decrease in its entry into the cytoplasm and chloroplasts. Disorder of phosphate metabolism is also indicated by an increase in the content of arginine and lysine and a decrease in the content of amino acids of the aromatic series [27]. In particular, the synthesis of phenylalanine, histidine and tyrosine, which is associated with the formation of the benzene ring and the starting material for which are phosphorylated sugar compounds, decreases not only due to inhibition of photosynthetic carbon sequestration, but also as a result of disorder of phosphate metabolism and respiration processes. On the other hand stimulation of biosynthesis of glutamic acid by analcite-tripoli mixture indicates the activation of photosynthesis in the test-plants.

The analysis of the pigment complex of the test-plants allowed assessing the degree of variability and stability of their photosynthetic apparatus under the condition of application of nitrification inhibitors, as well as factors promoting the intensity of photosynthetic process. In particular, it was shown that the content of photosynthetic pigments in the leaves of winter wheat and corn plants significantly depended on the type of nitrification inhibitor used. Application of the siliceous mixture contributed to a significant increase in the content of chlorophyll a and b, as well as carotenoids in the leaves of the tested crops. This testifies to a better supply of nitrogen to plants and activation of photosynthetic processes. The increase in the content of chlorophyll b and carotenoids indicates an increase in plant resistance to environmental stressors. At the same time application of nitrapyrin reduced the biosynthesis of chlorophyll b and carotenoids and a slight increase in the content of chlorophyll a in the leaves. These biochemical changes were associated with higher grain yield and quality.

Thus, the study of general patterns of plant metabolism in the laboratory and field experiments allowed revealing the mechanisms of adaptation of plants to nitrogen fertilization, to develop optimal techniques for application of nitrification inhibitors and to implement into agricultural production environmentally friendly natural siliceous minerals for regulated nitrogen up-take by plants.

The use of nitrogen-containing fertilizers with nitrification inhibitors in agriculture provides a number of advantages in agronomic and economic terms. Given this, of particular importance for the environment are siliceous minerals, which are characterized by high biological activity and persistence of action, harmless to both soil microbiota and higher plants. Their production is simple and economical as they are readily available in many regions of the worlds and have beneficial effects on components of agroecosystem (i.e., soil, microbiota, water balance etc.).

## 5. Conclusions

The comparative study on the effects of nitrapyrin and the mixture of natural siliceous minerals (analcite + tripoli) on nitrification processes in different types of agricultural soils under wheat and corn sowings under high nitrogen fertilization conditions clearly indicated higher effectiveness of the siliceous mixture as compared to nitrapyrin. In particular, analcite + tripoli mix had more profound effect in slowing down nitrification processes for prolong period of time, strongly inhibited the number of nitrifiers, denitrifiers and micromicetes producing phytotoxic allelochemicals compared with nitrapyrin. Crops treated with siliceous minerals had higher photosynthetic pigment content and higher glutamic acid concentration, which indicated the intensification of photosynthetic processes. Nitrapyrin reduced the concentration of chlorophyll b and carotenoids, but slightly increased the content of chlorophyll a in the leaves of wheat and corn. The content of aspartate and some aromatic amino acids decreased, while arginine and lysine increased. Such metabolic changes suggested disintegration of nitrogen and phosphate metabolism in the studied crops. Thus, the use of siliceous minerals is more advantageous than nitrapyrin in terms of their effectiveness, persistence in various types of soils, beneficial effect on soil microbiota, crop's functional state and productivity.

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