

## Article

# Effect of Urease and Nitrification Inhibitors on Heavy Metal Mobility in an Intensively Cultivated Soil

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**Abstract:** Urease and nitrification inhibitors represent ways to reduce nitrogen losses; their presence modifies microbial and enzymatic activity in the soil, affecting pH and organic matter (OM), which in turn affects the mobility of heavy metals. To evaluate the effect of urea with inhibitors, pH, OM content, and pseudo-total and mobile metal content (Cu, Cd, Ni, Pb, Cr, Zn, and Mn) were determined in soil samples fertilized with six different urea variants with inhibitors. The modification in the pseudo-total content of heavy metals following fertilization (%) was as follows: Cu ( $-39.26 \div -8.82$ ), Cd ( $10.74 \div 15.40$ ), Ni ( $5.76 \div 18.84$ ), Pb ( $-13.30 \div 12.46$ ), Cr ( $-15.55 \div 11.60$ ), Zn ( $35.10 \div 162.76$ ), and Mn ( $-1.32 \div 12.17$ ). The pH was situated in the range of 7.05 to 7.17, while OM content showed an average increase of 16%. The determined pollution indicators revealed an accumulation of Zn in the soil. The results showed a trend of accumulation of bioavailable heavy metals, with the greatest increase for Mn (43%). Only in the case of Zn, there was a decrease in mobile content with the lowest value when applying two urease inhibitors and one nitrification inhibitor. Inhibitors modify the OM content and soil pH, influencing the mobility and bioavailability of heavy metals.

**Keywords:** urease inhibitors; nitrification inhibitors; pseudo-total heavy metal; mobile heavy metal



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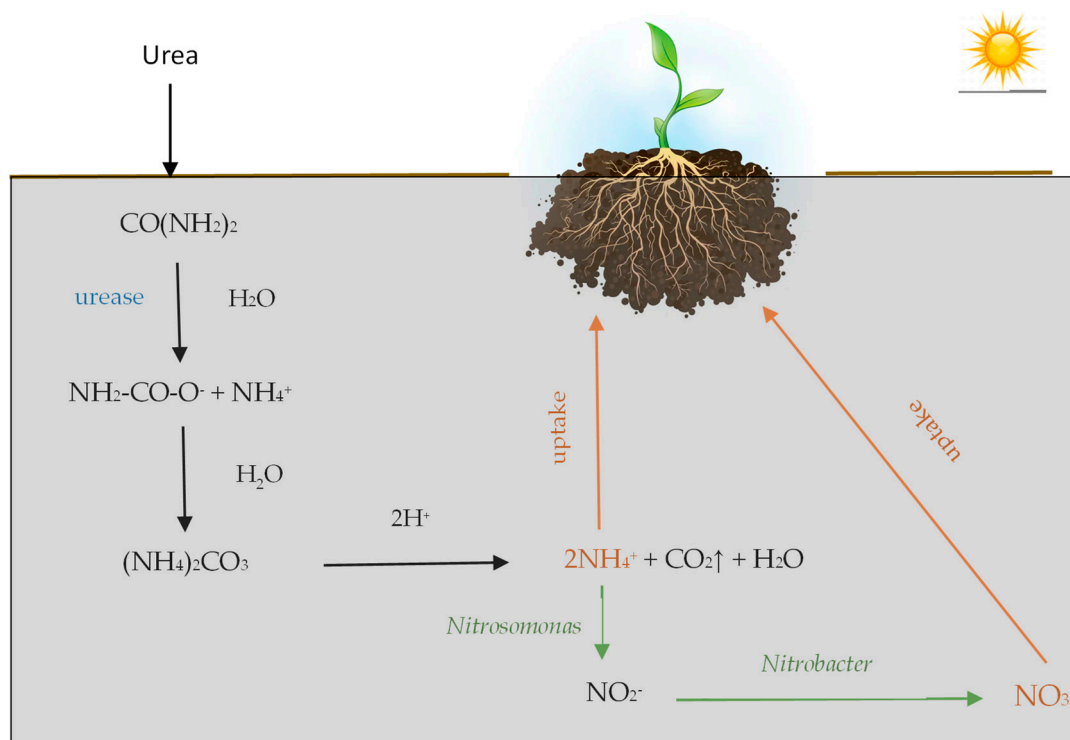
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## 1. Introduction

Fertilizers are one of several factors that contribute to soil contamination, which can ultimately affect the safety of food. Mineral fertilizers are estimated to contribute globally to approximately 50% of the inputs of Cd and Cr [1,2]. Increased Ni and Zn content in agricultural soils is primarily associated with the use of NPK fertilizers [3], while increased doses of nitrogen (N) fertilizers lead to increased accumulation of Cd and Pb [4,5].

Urea is a commonly used nitrogen fertilizer, valued for its high nitrogen content and cost-effectiveness. However, it can experience significant nitrogen loss (20–60%) and low nitrogen use efficiency (30–40%) [6,7]. When urea is applied to soil, it is quickly converted to ammonium by the enzyme urease (Figure 1) [8,9]. Ammonium nitrogen is available to plants and remains stable until nitrification begins, which is a natural two-step process where ammonium ( $\text{NH}_4^+$ ) is converted to nitrate ( $\text{NO}_3^-$ ) in the soil [10–13]. Increasing the application rate of urea can elevate the volatilization of ammonia ( $\text{NH}_3$ ), emissions of nitrous oxide ( $\text{N}_2\text{O}$ ), and nitrogen leaching. To minimize nitrogen losses and enhance the efficiency of urea, using urease and nitrification inhibitors is advisable. Urease inhibitors (UI), such as N-(n-butyl) thiophosphoric triamide (NBPT) and N-(n-propyl) thiophosphoric triamide (NPPT), prevent urea from hydrolyzing, thus reducing ammonia volatiliza-

tion and retaining nitrogen in its urea form, NBPT having a stronger inhibitory effect than NPPT [2,14,15]. Nitrification inhibitors (NIs) temporarily decrease the populations of *Nitrosomonas* and *Nitrobacter* bacteria, thereby slowing the conversion of ammonium to nitrite and nitrate [16,17]. Numerous synthetic and naturally occurring organic compounds can function as nitrification inhibitors, with dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) being two of the most widely used [18,19]. They help mitigate losses due to denitrification and leaching by keeping nitrogen in the ammonium form [20].



**Figure 1.** Urea hydrolysis in soil.

UIs and NIs slow the ammonia volatilization and nitrification process but can affect the dynamics of heavy metals in soil in various ways, both directly and indirectly. Adding NIs to urea fertilizer can change the pH value and cation exchange capacity (CEC) of the soil, which in turn affects the solubility and mobility of certain heavy metals, such as lead, cadmium, zinc, and copper, making them more available to plants and potentially more toxic [21]. Additionally, UIs and NIs can affect soil microbial activity, which can alter the chemical forms of heavy metals, potentially increasing their bioavailability (e.g., cadmium and nickel), or conversely, immobilizing them by forming organic–metal complexes [4]. Cheng et al. (2017) [22] showed that the addition of DCD can influence soil pH to increase Cd bioavailability; meanwhile, Yang et al. (2021) [23] showed that the addition of NIs (DCD and DMPP) could affect soil pH to inhibit Cd activity and reduce Cd accumulation in soil [22,23]. Research conducted by Xiao et al. (2022) [24] on rice and rapeseed crops fertilized with urea +NIs had an opposite trend; in paddy soil, the pH increased and Cd bioaccessibility decreased, while in the soil cultivated with rapeseed, the pH decreased and Cd bioaccessibility increased [25].

The available information regarding the response of heavy metal bioavailability to the presence of urease and nitrification inhibitors in soil is limited and sometimes contradictory, and only a few studies to date have considered field experiments. Taking this into account, the aim of our study was to evaluate the effect of urea with urease and nitrification inhibitors on the mobility and bioaccessibility of HM in soil; we applied six types of urea with inhibitors on a cambic chernozem cultivated with wheat. Soil samples taken from the

experimental variants were analyzed to determine the soil contamination and enrichment coefficients, as well as the changes occurring in the pseudo-total HM content and in the mobile HM content. Also, the soil OM and soil pH were monitored, and their influence on the mobility of HM was discussed.

## 2. Materials and Methods

### 2.1. Field Experiences' Organization

The field experience was conducted during 2023–2024 in the plain region of western Romania—an area characterized by intensive agriculture (Figure 2). The experimental plots, measuring 30 m × 30 m, are characterized by cambic chernozem soil. Cambic chernozem is a specific soil of steppes and forest steppes, being a dark-colored soil, having a strong structure, and presents accumulations of organic matter bases saturated. In the first horizon, cambic chernozem is characterized by medium texture: clayey–loamy, low porosity, medium field capacity, increased wilting coefficient, and increased bulk density. Soil reaction varies from weakly acid to neutral, increased humus reserve, medium nitrogen, and phosphorus content, and increased potassium. The general characteristics of the cambic chernozem are presented in Table 1.

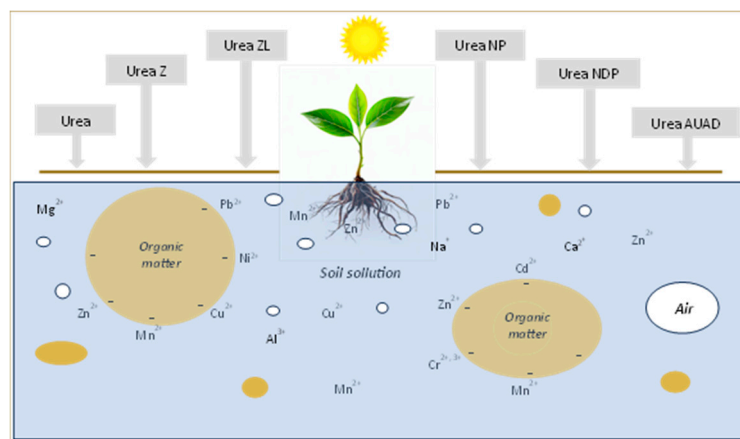


**Figure 2.** Experimental field location: 45°47′10.2″ N + 21°12′51.2″ E.

**Table 1.** General characteristics of the cambic chernozem.

Characteristic, M.U.	Value
pH in water	6.90
C/N ratio	13.7
Total nitrogen, %	0.171
Available phosphorus, mg·kg <sup>−1</sup>	75.7
Available potassium, mg·kg <sup>−1</sup>	205
CEC, me/100 g soil	32
CaCO <sub>3</sub> , % (0–60 cm)	9.8
Ni total, mg/kg	24.1
Cr total, mg/kg	68.5
Cu total, mg/kg	34.26
Mn total, mg/kg	527.6
Pb total, mg/kg	22.84
Zn total, mg/kg	51.34
Cd total, mg/kg	1.0

The field experiment was carried out in 6 variants, one for each type of urea fertilizer with inhibitors, and in 3 repetitions, applied to the wheat crop, based on a 13:13:13 complex fertilizer at a dose of 300 kg/ha. The tested fertilizer variants (Figure 3) are urea derivatives with nitrification and urease inhibitors, whose characteristics are presented in Table 2. The doses are equivalent to 50 kg of active nitrogen per hectare, applied at the beginning of March, when the soil temperature exceeded 5 °C.



**Figure 3.** Field experiences description.

**Table 2.** Fertilizer variants and characteristics of the applied fertilizer.

Variant	Type of Fertilizer	Extended Hydrolysis Period (Weeks)
G1-control	Urea—46% N	-
G2	Urea Z (urea—46% N with nitrification inhibitors (NI))	12—nitrification
G3	Urea ZL (urea—46% N with 2 urease inhibitors (UI)) (NBPT <sup>1</sup> + NPPT <sup>2</sup> )	2—ammonification
G4	Urea NP (urea—46% N with 1 urease inhibitor (UI) NBPT <sup>1</sup> )	2—ammonification
G5	Urea NDP (urea—46% N with 1 urease inhibitor (UI) NBPT <sup>1</sup> and 1 nitrification inhibitor (NI))	2—ammonification 12—nitrification
G6	Urea AUAD (urea—46% N with urease inhibitors (UI) (NBPT <sup>1</sup> + NPPT <sup>2</sup> ) and 1 nitrification inhibitor (NI))	2—ammonification 8—nitrification

<sup>1</sup>—N-(n-butyl) thiophosphoric triamide (NBPT). <sup>2</sup>—N-(n-propyl)-thiophosphoric triamide (NPPT).

## 2.2. Soil Sampling and Sample Preparation

To determine the influence of these new-generation urea-based fertilizers on soil pH, organic matter (OM), and the pseudo-total (TMe) and mobile metal content (MMe), soil samples were collected at a depth of 0–30 cm. Soil samples were collected before fertilization, and a second round of sampling was conducted as follows: for variants G1, G3, and G4, three weeks after application to align with the pick activity of the UI according to the fertilizers manufacturer's guidelines. For variants G2, G5, and G6, sampling took place in the fifth-week post-application to capture the effects of both UI and NI. After sampling, the soil was placed in polyethylene containers for laboratory determination of the monitored parameters. The samples were air-dried at room temperature (20 °C) for 14 days, then ground, and sieved through a 2 mm sieve to remove plant fragments.

## 2.3. Soil Sample Analysis

### 2.3.1. pH Determination

pH was determined using a conductometric method in an aqueous solution with a soil–water ratio of 1:2.5. A Mettler Toledo SevenEasy pH meter (Wien, Austria) was used.

### 2.3.2. Determination of Organic Matter (OM) Content

Organic matter (OM) content was determined using the gravimetric loss on ignition method, which estimates the amount of organic matter present in a soil sample. Samples were oven-dried and then heated to 400 °C, a temperature at which organic matter decomposes. By weighing the sample before and after decomposition, the OM content was estimated.

### 2.3.3. Determination of Pseudo-Total Metal Content—Aqua Regia Extractable, TMe (TCu, TCd, TNi, TPb, TCr, TZn, and TMn)

Pseudo-total metal content was determined using atomic absorption spectrometry (AAS) with a VARIAN 240FS spectrophotometer. About 0.5 g of the dried sample was subjected to mineralization with a 3:1 HCl–HNO<sub>3</sub> mixture in a TOPWAVE Analytic Jena microwave digester for 60 min at 360 °C to bring the metal ions into the solution. After cooling and filtration, the samples were brought to a 50 mL volumetric flask with deionized water. Merck grade acids were used. For calibration, standard solutions were used with a concentration ranging from 0.3 to 3 µg/L, prepared from multielement solution ICP Standard solution 1000 mg/L (Merck, Bucharest, Romania). Working conditions are specified in Table 3.

**Table 3.** Working conditions for Varian Spectra 240 FS spectrophotometer.

Metal	$\lambda$ (nm)	Lamp Current (mA)	Slit Width (mm)
Cu	324.8	4	0.5
Cd	228.8	4	0.5
Ni	232	4	0.2
Pb	283.3	10	1.2
Cr	357.9	7	0.2
Zn	213	5	1
Mn	279.5	5	0.2

Air–acetylene ratio 13.50:2. Nebulizer uptake rate: 5 L/min.

### 2.3.4. Determination of Mobile Metal Content MMe (MCu, MCd, MNi, MPb, MCr, MZn, and MMn)

Mobile metal content was determined using atomic absorption spectrometry (AAS) with a VARIAN 240FS spectrophotometer. Extraction of soluble metal forms was performed using 0.05 M NH<sub>4</sub><sup>+</sup>-EDTA (pH = 7)—an EDTA solution with the pH adjusted using NH<sub>4</sub>OH. About 0.5 g of soil was agitated for 2 h with 0.05M NH<sub>4</sub><sup>+</sup>-EDTA (at a soil–solution ratio of 1:10). After filtration and adjustment to a 50 mL volume, mobile forms were determined under the AAS operating conditions specified in Section 2.3.3.

## 2.4. Calculation of Pollution Indicators

Pollution indicators, a useful tool for assessing the degree of contamination, make a significant contribution to evaluating soil quality and predicting the future sustainability of the ecosystem [26]. Pollution indicators are calculated based on the mobile fraction of the soil, as this represents the actual bioavailable fraction.

- Enrichment factor—EF—an indicator used in determining the origin of heavy metals by estimating the anthropogenic impact on sediments [27].

$$EF = (C_x/C_{\text{ref sample}})/(C_x/C_{\text{ref background}}) \quad (1)$$

C<sub>x</sub>—content of heavy metal;  
C<sub>ref</sub>—content of reference metal.

The EF calculation was performed by referencing the iron concentration at the depth of the arable layer (0–25 cm). Soil quality can be divided into different classes based on the enrichment coefficient: EF < 2—minimal enrichment; 2 < EF < 5—moderate enrichment; 5 < EF < 20—significant enrichment; 20 < EF < 40—high degree of enrichment; and EF > 40—extremely high enrichment [26,27].

- Contamination factor—CF—determines which metal poses the greatest threat to the soil environment:

$$CF = (C_x \text{ sample}) / (C_x \text{ background}) \quad (2)$$

C<sub>x</sub> sample—content of heavy metal in soil;  
C<sub>x</sub> background—value of the geochemical background.

The enrichment factor (EF) and contamination factor (CF) are numerical indicators of soil pollution levels and are calculated based on the concentration of the exchangeable mobile form of heavy metals.

- Geoaccumulation Index—GI—allows for the assessment of soil contamination with heavy metals based on their content in the A or O horizon:

$$GI = \lceil \log \rceil 2 (C_x \text{ sample}) / (1.5 \cdot C_x \text{ background}) \quad (3)$$

C<sub>x</sub> sample—concentration of individual heavy metal;  
C<sub>x</sub> background—value of the geochemical background.

### 2.5. Data Analysis

Statistical analysis was performed using Microsoft Excel, SPSS 12.0, and OriginPro 2024b. Differences resulting from the application of different fertilizer variants were estimated using analysis of variance (ANOVA) and a two-sample t-test assuming equal variances, with  $p < 0.05$  considered statistically significant. Correlations between MMe and soil factors (pH, OM) were performed using regression analysis. Principal component analysis (PCA) with varimax rotation was performed for dimensionality reduction to provide a concise and optimal description of the data.

## 3. Results

### 3.1. Pseudo-Total Content of Heavy Metals in Soil

In the soils of the study area, heavy metals are primarily found within the structure of primary and secondary minerals, as well as bound through physicochemical and chemical adsorption mechanisms by soil mineral colloids. Significant quantities of heavy metals are associated with soil organic matter in forms relatively accessible to plants. The pseudo-total metal content (TMe), determined in soil samples before the application of fertilizer variants under wheat cultivation, is presented in Table 4 as the arithmetic mean of three replicates  $\pm$  SD.

Analyzing the average values, the proportion of TMe is as follows: Mn (1518 mg/kg) > Cr (82.18 mg/kg) > Zn (76.74 mg/kg) > Cu (50.72 mg/kg) > Ni (37.38 mg/kg) > Pb (29.23 mg/kg) > Cd (1.627 mg/kg).



**Table 4.** Pseudo-total metal content determined in soil samples before application of the six fertilizer variants under wheat cultivation (means  $\pm$  SD)—values are expressed in mg/kg.

	G1	G2	G3	G4	G5	G6
Cu	51.548 $\pm$ 2.632	55.090 $\pm$ 1.162	57.846 $\pm$ 1.326	56.224 $\pm$ 1.340	51.934 $\pm$ 1.224	55.604 $\pm$ 1.210
Cd	1.467 $\pm$ 0.038	1.676 $\pm$ 0.047	1.478 $\pm$ 0.007	1.756 $\pm$ 0.039	1.514 $\pm$ 0.024	1.520 $\pm$ 0.022
Ni	36.081 $\pm$ 0.254	36.523 $\pm$ 0.362	37.046 $\pm$ 0.198	38.392 $\pm$ 0.200	37.534 $\pm$ 0.750	40.718 $\pm$ 1.979
Pb	34.52 $\pm$ 0.764	32.78 $\pm$ 0.895	32.94 $\pm$ 0.448	33.92 $\pm$ 0.147	25.22 $\pm$ 0.315	28.41 $\pm$ 0.260
Cr	81.38 $\pm$ 1.496	76.96 $\pm$ 1.078	78.64 $\pm$ 1.000	81.74 $\pm$ 2.062	97.40 $\pm$ 2.232	82.86 $\pm$ 3.543
Zn	74.72 $\pm$ 0.897	83.38 $\pm$ 0.440	82.33 $\pm$ 0.815	86.58 $\pm$ 0.535	78.45 $\pm$ 0.481	85.62 $\pm$ 0.370
Mn	1210.87 $\pm$ 26.2	1163.27 $\pm$ 44.2	852.22.5 $\pm$ 19.7	793.20 $\pm$ 29.01	1256.53 $\pm$ 42.5	834.12.25 $\pm$ 48.6

Before fertilization, the pseudo-total copper (TCu) content ranged from 48.638 to 59.129 mg/kg, with the maximum value found in variant G3. These values fall within the range typical of cambic chernozems in western Romania. For cadmium (Cd), cambic chernozems in the study area generally have low contents, typically between 1 and 1.5 mg/kg. However, multi-year fertilization, particularly with phosphate fertilizers, has contributed to its accumulation in the soil. The determined values ranged from 1.422 to 1.800 mg/kg, with the maximum value in variant G4. TNi values ranged from 35.789 to 42.800 mg/kg, with the maximum value in G6, and pseudo-total lead (TPb) ranged from 24.875 to 35.000 mg/kg, with the maximum value in G1. TCr is relatively high, attributable to the chromium-rich parent material from which this soil type formed. It ranged from 75.60 to 86.82 mg/kg, with the maximum value in variant G5. Similarly, for TZn (74.05 to 87.01 mg/kg, maximum in G4) and total manganese (TMn) (793.20 to 1256.56 mg/kg, maximum in G5), the determined values exceed the averages typical for cambic chernozems.

The pseudo-total metal content determined after the application of the six fertilizer variants is presented in Table 5, expressed as the arithmetic mean of three replicates  $\pm$  SD. The results of the ANOVA test and the t-test (assuming equal variances) performed to determine statistically significant differences at a 95% confidence level, compared with the control, are expressed using letters.

**Table 5.** Total metal content determined in soil samples after application of the six fertilizer variants under wheat cultivation (means  $\pm$  SD)—values are expressed in mg/kg.

	G1	G2	G3	G4	G5	G6
Cu	44.82 $\pm$ 0.832	40.36 $\pm$ 1.172 <sup>a</sup>	40.18 $\pm$ 0.712 <sup>a</sup>	32.86 $\pm$ 1.002	47.318 $\pm$ 0.720	42.46 $\pm$ 1.020 <sup>a</sup>
Cd	1.693 $\pm$ 0.050 <sup>a</sup>	1.876 $\pm$ 0.031 <sup>b</sup>	1.691 $\pm$ 0.020 <sup>a</sup>	1.944 $\pm$ 0.0443 <sup>b</sup>	1.719 $\pm$ 0.021 <sup>a</sup>	1.680 $\pm$ 0.023 <sup>a</sup>
Ni	38.16 $\pm$ 1.698 <sup>a</sup>	41.17 $\pm$ 1.312 <sup>a</sup>	41.63 $\pm$ 0.920 <sup>a</sup>	44.47 $\pm$ 1.177 <sup>b</sup>	41.22 $\pm$ 1.655 <sup>a</sup>	45.19 $\pm$ 1.173 <sup>b</sup>
Pb	29.93 $\pm$ 1.220 <sup>a</sup>	36.86 $\pm$ 0.738 <sup>b</sup>	31.82 $\pm$ 1.260 <sup>ac</sup>	34.61 $\pm$ 1.231 <sup>bc</sup>	27.83 $\pm$ 1.105 <sup>a</sup>	29.66 $\pm$ 1.498 <sup>ac</sup>
Cr	84.14 $\pm$ 3.190 <sup>a</sup>	73.67 $\pm$ 3.710	87.76 $\pm$ 6.186 <sup>ab</sup>	71.38 $\pm$ 3.256 <sup>c</sup>	82.26 $\pm$ 11.262 <sup>abc</sup>	87.58 $\pm$ 3.550 <sup>ab</sup>
Zn	148.22 $\pm$ 10.033 <sup>a</sup>	112.64 $\pm$ 10.271 <sup>b</sup>	136.02 $\pm$ 7.014 <sup>ac</sup>	116.13 $\pm$ 14.292 <sup>bc</sup>	206.14 $\pm$ 18.219	145.62 $\pm$ 19.507 <sup>abc</sup>
Mn	1231 $\pm$ 110.527 <sup>a</sup>	1148.50 $\pm$ 57.95 <sup>a</sup>	956.0 $\pm$ 74.80 <sup>b</sup>	808.50 $\pm$ 54.500 <sup>b</sup>	1243.0 $\pm$ 77.25 <sup>a</sup>	867.00 $\pm$ 104.75 <sup>b</sup>

There are no statistically significant differences between the means of variants with the same letters at a 95% confidence level.

Analyzing the average values of TMe after fertilization, it is observed that the proportion remains the same as before fertilization.

Differences in TMe content before and after application of the fertilizer variants are shown in Figure 4.



**Figure 4.** Differences in pseudo-total metal (TMe) content before and after application of the fertilizer variants: (a) TCu, (b) TCd, (c) TNi, (d) TPb, (e) TCr, (f) TZn, and (g) TMn.

Following fertilization with urea-containing urease inhibitors (UIs) and nitrification inhibitors (NIs), a decrease in total copper (TCu) content was observed in all fertilizer vari-



ants, with a minimum decrease of 32.86 mg/kg in variant G4, 41.5% lower than the unfertilized variant. For total lead (TPb) and total chromium (TCr), values varied compared to the unfertilized variants depending on the fertilizer variant. For lead, decreases in TPb content were recorded in the control variant (G1) by 13% and in variant G3 (UI) by 3%. In the other variants, an increase of 2–12% in the content of TPb was recorded. For chromium, a reduction of 4–15% in TCr content was observed in three of the experimental variants (G2, G4, and G5). In the other variants, a small increase in chromium content was recorded, ranging from 2.76 (G1) to 6.02 (G3) mg/kg.

The content of pseudo-total TCd, TNi, and TZn increased in all experimental variants. TCd slightly exceeds the normal values for the soil studied (with an average of 1.5 mg/kg Cd). TNi increased following fertilization by 2.08 (G1) to 6.08 mg/kg (G4). The largest increases compared to G1—control are for TZn, with values ranging from 112.64 mg/kg (G2) to 206.14 mg/kg (G5).

The content of pseudo-total manganese (TMn) underwent very small changes following fertilization. Increases in TMn values were determined in four of the variants: variant G1 by 1.6%, G3 by 10.8%, G4 by 1.8%, and G6 by 3.8%. Variants showing a decrease in Mn content were G2 and G5, which had the highest pH (G2—7.17, G5—7.15) and the highest OM content (G2—10.63%, G5—10.11%).

### 3.2. Pollution Indexes

The geochemical index (GI) allows us to analyze natural fluctuations in the content of a particular heavy metal in the soil and to detect even a very small anthropogenic influence. The obtained GI values indicate moderate to severe contamination with zinc (Zn) ( $2 < GI < 3$ ), moderate contamination with Cu, Cd, Pb, and Mn ( $1 < GI < 2$ ), and that there is no contamination with Cr and Ni ( $GI < 1$ ) [26].

In the analyzed soil, regardless of the fertilizer variant, the EF values are uniform, ranging between 0 and 2. Values of  $0.5 \leq EF \leq 2$  indicate that the heavy metal concentration in the soil originates from mineral weathering processes [26–28], while EF values  $> 2$  indicate that a significant portion of the heavy metal concentration may originate from various pollution sources [26], such as, in our case, the application of mineral fertilizers. Analyzing the EF values, it can be concluded that the heavy metal causing higher contamination is zinc (Zn), with EF values  $> 1$  determined in all experimental variants, despite the fact that fertilizers containing this metal were not applied. Analyzing the EF values, it can be concluded that there is an accumulation of Zn in the soil; for this heavy metal, EF values  $> 1$  are determined in all experimental variants, even though fertilizers containing this metal were not applied (Table 6).

**Table 6.** Pollution indexes.

Index	Cu	Cd	Ni	Pb	Cr	Zn	Mn
EF	1.01	0.86	0.80	0.73	0.57	1.64	0.86
CF	2.11	1.76	1.25	1.28	1.18	3.43	1.81
GI	1.65	1.40	0.91	1.17	−0.002	2.33	1.41

The contamination factor (CF) has values ranging from 1.18 for Cr to 3.43 for Zn. CF values between 1 and 3 indicate moderate contamination for Cu, Cd, Ni, Pb, Cr, and Mn, while values between 3 and 6 indicate significant contamination, as is the case for Zn [29,30]. The increased accumulation of Zn may be related, on the one hand, to the antagonism between P and Zn, with phosphate fertilization hindering Zn uptake by the plant, and on the other hand, to the high content of OM, as this element has a high affinity for soil organic matter [31,32].

The accumulation of heavy metals in soil is closely related to the composition of the solid and liquid phases, the presence and behavior of other metallic cations, mobility, transformation, and accumulation processes in the ecosystem, the type of bedrock from which the soil is formed, the degree of weathering, pH, redox conditions, and soil temperature [33]. Furthermore, the accumulation of heavy metals depends on the presence and quantity of OM, their adsorption/desorption at the level of soil colloids, and microbiological activity—factors influenced by the application of urea with urease inhibitors (UIs) and nitrification inhibitors (NIs).

### 3.3. Soil Reaction and Organic Matter

The values determined for pH and organic matter (OM) content before and after fertilization are presented in Table 7.

**Table 7.** pH values and organic matter (OM) content determined before and after application of the fertilizer variants.

Status		G1	G2	G3	G4	G5	G6
Before	pH	6.98	6.88	6.93	6.84	7.02	6.95
	OM(%)	7.69	8.18	8.53	8.86	8.59	8.91
After	pH	7.05	7.17	7.12	7.07	7.15	7.09
	OM(%)	9.63	10.63	10.25	8.82	10.11	9.03

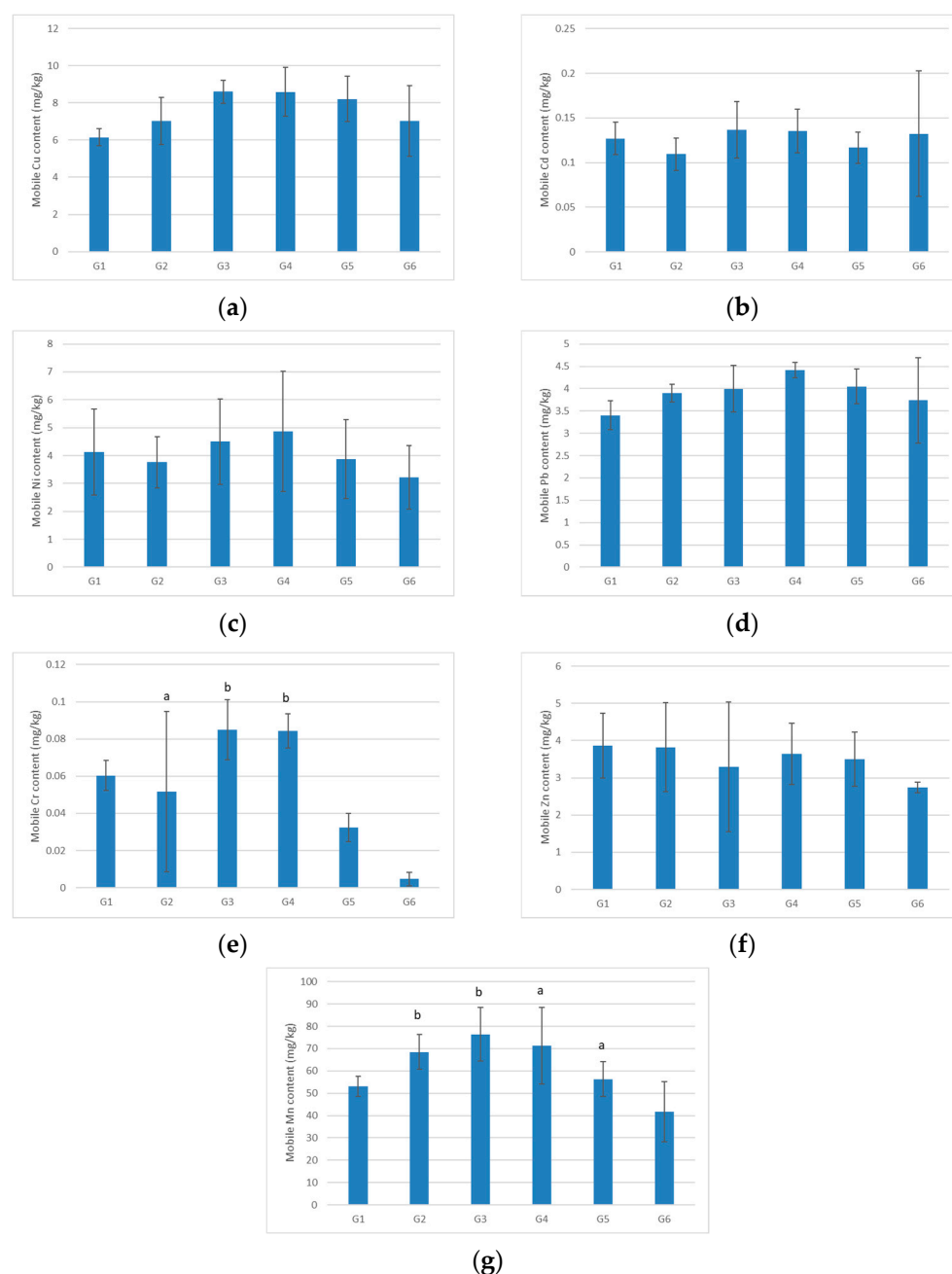
pH is one of the main factors determining the mobility in soil and plant availability of heavy metals, with several studies indicating a negative correlation between pH and heavy metal availability [2,34]. The pH determined both before fertilization (pH: 6.84–7.02) and after (pH: 7.05–7.17) indicates a neutral soil. After fertilization, pH values increased in all experimental variants, with a maximum of 7.17 determined in the variant fertilized with urea with NI (G2), followed by the variant in which urea with UI and NI was applied (G5, pH = 7.15) and the variant with urea and two urease inhibitors (G3, pH = 7.12). In the variants with one UI (G4) and two UIs + NI (G6), the pH values are slightly lower and closer, pH = 7.07 and pH = 7.09, respectively. The lowest pH value was determined in the variant fertilized only with urea without inhibitors (pH = 7.05).

The values of organic matter (OM) content determined before and after the application of the fertilizer variants are presented in Table 7. Analysis of the data shows that in the vast majority of cases, the OM content increased following fertilizer application, with the most pronounced increases determined in variants G1 (25%), G2 (30%), G3 (20%), and G5 (17%). The only variant showing a constant OM content is G6, where urea with two UIs + NI was applied. The highest OM content was recorded in the variant with the highest pH, G2 (10.63%), fertilized with urea and NI. Urea with two UIs led to a greater amount of OM (10.25%) compared to urea with one UI (OM = 8.82%). In the case of the presence of both inhibitors, variant G5 with one UI + NI has a higher OM content (10.11%) compared to variant G6 with two UIs + one NI (9.03%).

### 3.4. Mobile Heavy Metal Content

The values determined for the mobile forms (MMe) of the investigated metals are presented in Figure 5. Following ANOVA and t-tests, it was observed that there are no significant differences at a 95% confidence level for the following metals: copper (Cu) ( $p = 0.145$ ), cadmium (Cd) ( $p = 0.994$ ), nickel (Ni) ( $p = 0.809$ ), lead (Pb) ( $p = 0.312$ ), and zinc (Zn) ( $p = 0.602$ ), where  $p$  is the probability level (ANOVA).

To determine the degree of correlation between the studied parameters, Pearson correlations were performed, the results of which are presented in Table 8.



**Figure 5.** Differences in mobile metal (MME) content before and after application of the fertilizer variants: (a) MCu, (b) MCd, (c) MNi, (d) MPb, (e) MCr, (f) MZn, and (g) MMn. There are no statistically significant differences between the means of variants at a 95% confidence level.

**Table 8.** Pearson correlation coefficient matrix. Blue color and its shades indicate a positive correlation; red color and its shades indicate a negative correlation.

	pH	OM	MCu	MCd	MNi	MPb	MCr	MZn	MMn
pH	1.000								
OM	0.569	1.000							
MCu	0.247	−0.564	1.000						
MCd	−0.698	−0.845 *	0.439	1.000					
MNi	−0.302	−0.882 *	0.574	0.510	1.000				
MPb	0.250	−0.428	0.886 *	0.294	0.544	1.000			
MCr	−0.181	−0.772	0.434	0.356	0.939 **	0.386	1.000		
MZn	0.047	−0.084	−0.163	−0.414	0.487	−0.009	0.568	1.000	
MMn	0.281	−0.523	0.616	0.113	0.775	0.594	0.876 *	0.461	1.000

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed).

Significant positive correlations were observed between pH, OM, and mobile forms (MMe) at a statistical significance level of  $\alpha = 0.01$  for MCr–MNi ( $p = 0.005$ ), and significant positive correlations for MCr–MMn ( $p = 0.022$ ) and Pb–Cu ( $p = 0.018$ ). Significant negative correlations were observed for OM–MCd ( $p = 0.033$ ) and OM–MNi ( $p = 0.020$ ) at a statistical significance level of  $\alpha = 0.05$ .

An increase in mobile copper (MCu) content was observed following the application of all forms of urea, ranging from  $6.15 \pm 0.47$  to  $8.59 \pm 0.51$  mg/kg. The application of all forms of urea led to an increase in mobile copper (MCu) content, which ranged from  $6.15 \pm 0.47$  to  $8.59 \pm 0.51$  mg/kg. The most significant increases compared to the control (urea only) were observed in variants G4 and G3 (36%), as well as G5 (33%). In G3 and G4, where UI inhibited urease activity, MCu values were nearly identical:  $8.59 \pm 0.61$  mg/kg (G3) and  $8.58 \pm 1.32$  mg/kg (G4). Similar MCu values were found in variant G2 with NI ( $7.01 \pm 1.27$  mg/kg) and in variant G6 with two UIs + one NI ( $7.02 \pm 1.90$  mg/kg). These values could not be correlated with pH ( $r = 0.247$ ), but a negative correlation was found between organic matter (OM) and MCu ( $r = -0.564$ ).

Mobile cadmium (MCd) levels ranged from  $0.109 \pm 0.018$  to  $0.136 \pm 0.031$  mg/kg. The highest values were determined in variants where urea + UI was applied,  $0.136 \pm 0.031$  mg/kg in G3 and  $0.135 \pm 0.024$  mg/kg in G4. Lower MCd values than the control variant (G1,  $0.127 \pm 0.018$  mg/kg) were found in G2 ( $0.109 \pm 0.018$  mg/kg) and G5 ( $0.116 \pm 0.017$  mg/kg). There is a negative correlation between pH and MCd ( $r = -0.698$ ), and a significant negative correlation ( $p < 0.05$ ) between organic matter (OM) and MCd ( $r = -0.845$ ).

The highest MNi content was observed in G4 (4.87 mg/kg) and G3 (4.50 mg/kg), where the pH values were slightly higher than the control variant (7.07 for G4 and 7.12 for G3), and the OM content was lower (8.82% for G4 and 9.04% for G3). Variant G2, urea + NI, had the highest pH value (7.17) and the highest OM content (10.63%), resulting in the lowest MNi content (3.23 mg/kg). This confirms the reduction in  $\text{Ni}^{2+}$  availability with increasing pH. Similar to MCd, there is a significant negative correlation between OM and Ni, with a correlation coefficient of  $r = -0.882$  ( $p < 0.05$ ).

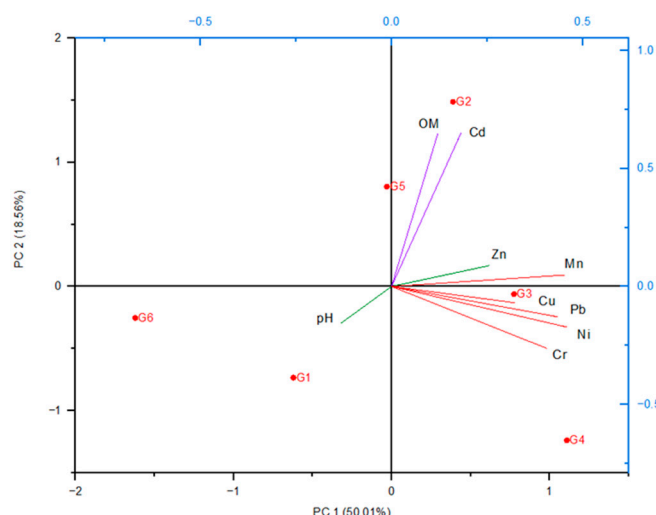
Mobile lead (MPb) content ranged from  $3.40 \pm 0.32$  mg/kg (G1) to  $4.41 \pm 0.16$  mg/kg (G4). The variation in pH influenced lead bioavailability, significantly altering the ratio between easily mobile and exchangeable lead forms. The highest MPb value was found in variant G4 and the lowest was determined in variant G2 ( $3.899 \pm 0.20$  mg/kg Pb) and in G6 ( $3.73 \pm 0.96$  mg/kg Pb). The correlation coefficient between pH and MPb was low ( $r = 0.250$ ), while a negative correlation was found between OM and MPb ( $r = -0.428$ ), consistent with literature data [35,36]. Additionally, a strong significant correlation between MPb and MCu was observed ( $p < 0.05$ ).

MCr values range from 0 to  $0.084 \pm 0.009$  mg/kg, with the highest in variants fertilized with urea + UI; these were the only variants, where MCr levels increased compared to the control. In variants with NI, MCr levels decreased compared to the control. The research shows a very low correlation between pH and MCr ( $r = -0.181$ ) and a strong, but negative, correlation with organic matter (OM) ( $r = -0.778$ ). Additionally, there was a significant correlation between MCr and MNi ( $r = 0.939$ ,  $p < 0.01$ ).

Mobile zinc (MZn) content ranged from  $2.74 \pm 0.14$  mg/kg to  $3.87 \pm 0.87$  mg/kg, with the highest value observed in G1. MZn decreased across all experimental variants, regardless of the type of inhibitor applied. As for MMn content, it ranged from  $41.71 \pm 13.4$  mg/kg (G6) to  $76.32 \pm 12.0$  mg/kg (G3). Variants fertilized with urea and UI had high MMn levels (G3— $76.32$  mg/kg, and G4— $71.43$  mg/kg), which were 36–43% higher than in the control variant (G1). Application of urea with NI resulted in an MMn content of  $68.43$  mg/kg, 29% higher than in G1. Our study found that the influence of pH on MMn was low ( $r = 0.281$ ),

while the influence of OM was stronger but negative ( $r = -0.523$ ). A significant correlation was observed between MMn content and MCr ( $r = 0.876$ ,  $p < 0.05$ ).

Principal component analysis (PCA) extracted three components with eigenvalues  $> 1$ . PC1, contributing 50% to the total variance, is strongly correlated with five of the initial variables: the mobile forms of manganese (Mn), copper (Cu), lead (Pb), chromium (Cr), and nickel (Ni). The PCA result indicates that the mobile forms of Mn, Cu, Pb, Ni, and Cr are most influenced by the fertilizer variants. This suggests that these variables vary together. PC2 accounts for the next 18.56% and is correlated with OM and Cd, while PC3 is correlated with pH and Zn. Analyzing Figure 6, it is observed that fertilizer variant G3 influences the mobile forms of Mn, Cu, Pb, Ni, and Cr, while G2 and G5 influence the content of OM and mobile Cd.



**Figure 6.** The influence of fertilization with different variants of urea on soil pH, OM, and MME.

## 4. Discussion

### 4.1. Pseudo-Total Content of Heavy Metals in Soil

The urea used for wheat fertilization does not contain copper; however, the presence of urease inhibitors (UIs) and nitrification inhibitors (NIs) in the soil can alter pH values, affecting the sorption and desorption processes of heavy metals. The reduction in total copper (TCu) content after fertilization can be attributed to plant uptake, as wheat requires copper for root development and early growth [37]. Additionally, some soil copper may be lost through leaching, especially in areas with shallow groundwater. Although soil organic matter (OM) is the strongest sorbent for copper, it can also bind with other heavy metals, which compete with copper for available binding sites. Consequently, copper binding to OM is lower in soils with groundwater rich in carbonates and salts, causing copper leaching [38,39].

The high porosity and permeability of the analyzed chernozem, along with its high  $\text{HCO}_3^-$  content, contributed to the accumulation of Ni and Pb in the soil. This type of chernozem is also rich in  $\text{S}^{2-}$ , which precipitates Ni and Pb ions, thereby retaining them in the soil. According to Yu et al. (2023) [40], ammonium nitrogen fertilizer can reduce the pH of the soil, enhance Pb activity in the rhizosphere, and promote Pb uptake by plants. In variant G3, the presence of UI temporarily blocks urea hydrolysis, causing a temporary increase in pH and a reduction in Pb content. In other experimental variants, TPb increased after fertilizer application. However, there are no statistically significant differences between the means of variants with the same letters at a 95% confidence level, as confirmed by Tu et al. (2000) [41], which showed that applying urea at a rate of 200 mg N/kg significantly reduced the soluble plus exchangeable fraction. The increase in TNi content in

the fertilized variants is not influenced by the application of urea with inhibitors, with no statistical differences observed between fertilizer variants. NPK fertilizers and some soil amendments are significant sources of Ni in the soil, and the increase in Ni content is attributed to phosphate rock used in phosphate fertilizers; multi-year application leads to the accumulation of this cation in the upper soil layers [41].

The reduction in TCr content after applying urea with inhibitors in variants G2, G4, and G5 is linked to pH and organic matter (OM). It is known that there is a negative correlation between TCr content in the soil and pH and a positive correlation between TCr and OM [42]. The increase in TCr values in variants G1, G3, and G6 can be attributed to the annual application of complex NPK fertilizers, which causes accumulation in the soil. However, no statistically significant differences are observed between the experimental variants at a 95% confidence level. The small variations are due to the high mobility of chromium in the soil, as well as the differences in pH and organic matter values [43].

Various studies have shown that urea fertilization can influence TZn in soil by changing the distribution between exchangeable and soluble zinc. The ammonium ion can displace zinc from exchange sites or soil colloids through ion-exchange processes [44]. Additionally, long-term application of phosphate (P) fertilizers affects the equilibrium of different Zn forms in the soil and their bioavailability. High doses of phosphate fertilizers can hinder zinc uptake by plants because both nutrients compete for the same adsorption sites in the soil. Their interaction with adsorbent surfaces is complex [45,46]. The co-adsorption of phosphorus and zinc onto iron oxides may explain the retention of zinc in the soil and its reduced uptake by plants [47,48].

Since TMn includes forms of Mn not part of the silicate matrix but rather retained through adsorption or complexation with organic matter, its content is primarily influenced by pH, soil oxygen levels ( $pO_2$ ), microorganisms, and organic matter (OM). Various studies indicate that the type of nitrogen fertilizer (nitrate or ammonium) directly affects Mn forms in the soil [49]. When urea without a nitrification inhibitor (NI) is applied, the resulting  $NH_4^+$  from urea hydrolysis is oxidized to  $NO_3^-$  with the release of  $H^+$  ions. These  $H^+$  ions, released during nitrification, reduce manganese from  $MnO_2$ , an insoluble compound, to  $Mn^{2+}$ , which plants can absorb [49]. In variants with only NI, TMn decreased, a finding supported by research showing that the prolonged presence of  $NH_4^+$  in the soil and the lack of  $H^+$  release prevent the reduction in insoluble Mn [50]. When urea is applied with urease inhibitors (UIs) and NIs, TMn levels vary based on pH and OM. In variants where TMn decreased after fertilization, higher pH and OM content, which can bind Mn through complexation, were observed. Additionally, applying complex phosphorus fertilizers leads to phosphorus accumulation in the soil, increasing Mn adsorption onto oxide surfaces [51]. The reduction in Mn content is likely due to its translocation into plants.

#### 4.2. Soil pH and Organic Matter

Long-term application of urea negatively impacts the chemical and biological properties of the soil, leading to acidification. Contrary to expectations, pH values increased in all experimental variants. According to [52], the extent of pH changes after applying urea and urea with inhibitors depends on the soil's pH buffering capacity (pHBC). During urea hydrolysis, the presence of urease in the soil leads to  $(NH_4)_2CO_3$  formation, which dissociates into  $NH_4^+$  and  $CO_3^{2-}$ , causing a temporary pH increase near the hydrolysis zone [8]. In variant G2, the presence of NI halted the oxidation of  $NH_4^+$  to  $NO_3^-$ , preventing the release of  $H^+$  and subsequent pH decrease, which would have increased heavy metal mobility [11]. In variants G3 and G4, where urea with UI was used, the extended hydrolysis period and gradual  $NH_4^+$  release led to increased pH values. Since nitrification is slower than urea hydrolysis [53], variant G3 had a higher pH. The increase in pH with urea and UI



+ NI may be due to prolonged  $\text{NH}_4^+$  release and absorption by wheat plants, with minimal nitrification and neutralization of released  $\text{H}^+$  by the soil's high buffering capacity. Similar findings are reported in [49,54]. As soil pH increases, the adsorption capacity for heavy metal cations also rises due to negative soil surface charges, reducing their mobility [10,27].

The availability of cations in the soil is strongly affected by the organic matter content. Negative charges, which mainly result from the dissociation of carboxyl and phenolic groups of soil OM, allow the retention of cations in non-leachable but exchangeable forms, from which they can be accessible to plants. The retention of metal ions is primarily electrostatic but can also be partially covalent, especially when the negative charges are neutralized by the cations of transition metals ( $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Ni}^{2+}$ ) [16]. The percentage of organic matter in the soil controls the geochemical activity of heavy metals, influencing their mobility and bioavailability through physicochemical adsorption processes, coordination, and chelation. The mobility of heavy metals is limited by retention in solid organic matter; however, dissolved organic matter, which contains active radicals such as N and O, can combine with heavy metals, increasing their mobility [6]. These contradictory results are due to different soil types, experimental conditions, types, and doses of fertilizers, making it difficult to obtain consistent conclusions [2].

In the presence of one UI, the formation of the  $\text{NH}_4^+$  ion began more rapidly compared to the variant with two UIs. This rapid formation may explain the unchanged OM content in G4, possibly due to the toxic effect of  $\text{NH}_4^+$  on soil microorganisms and enzymes, as noted by [55]. In G3, the combined action of two UIs prevented the formation of  $\text{NH}_4^+$ , thus allowing microbial activity on OM decomposition to continue unaffected. In the presence of NI in G2, the formed  $\text{NH}_4^+$  is not oxidized to  $\text{NO}_3^-$ , leading to an increase in pH in the urea hydrolysis zone and enhancing enzymatic activity, which favors OM decomposition. In setups using both urease inhibitors (UIs) and nitrification inhibitors (NIs), differences in organic matter (OM) content arise from varying  $\text{NH}_4^+/\text{NO}_3^-$  ratios, which impact soil pH and microbial activity [56]. Geng et al. (2021) [57] found that  $\text{NH}_4^+$  significantly reduces organic carbon content in the soil's organic layer, whereas  $\text{NO}_3^-$  affects both organic carbon content and the density fractions of the mineral layer [58].

#### 4.3. Mobile Metal Content

Following the application of urea with UI, the low organic matter content of this experimental variant determined a weak copper binding. This weak binding released copper from the chelates formed with soil OM, increasing its bioavailability for wheat plants [59]. Similar findings were observed in solonetz soil with reduced urease activity [9,60]. Kamrun et al. demonstrated in experiments on paddy soils that there is a negative correlation between the nitrification rate and the concentration of MCu [61]. Consequently, inhibiting the nitrification process increases the bioavailable copper content [11], as confirmed by our research, which shows an increase in MCu in the presence of UI and NI.

Although NPK fertilizers are applied annually and phosphate rock is the main anthropogenic source of cadmium in agricultural soils [12,13,17], the determined MCd content remains low across all fertilizer variants. Unlike other HM, cadmium is less strongly retained in the soil; it is relatively soluble and behaves similarly to calcium ( $\text{Ca}^{2+}$ ), but it interacts more strongly with OM and soil sulfides. As a result, cadmium's mobility in soil and its availability to plants are relatively low [62]. In the G2, the presence of NI allowed urea hydrolysis only up to the formation of the  $\text{NH}_4^+$  ion, preventing its oxidation to  $\text{NO}_3^-$  and causing an increase in soil pH [20]. Consequently, a decrease in MCd content was noted, as it is known that an increase in pH enhances cadmium sorption capacity [19]. Additionally, the highest OM content in this variant showed a greater tendency to reduce cadmium mobility due to its sorption [14,18]. Similar MCd behavior was observed in G5,

where the application of urea with UI and NI led to increased pH and OM content, resulting in decreased MCd content. The presence of UI alone in the soil causes a decrease in OM content and a slight increase in MCd due to the reduced number of organic matter functional groups that can retain  $\text{Cd}^{2+}$ . Our results are confirmed by research conducted by [61] and reflect the complex interplay between pH, OM, and the presence of inhibitors in determining cadmium mobility and availability in soil [11].

pH is the main factor controlling Ni adsorption in the soil; its availability decreases significantly in soils with high pH [63,64]. Other factors affecting nickel mobility in soil and the activity of free Ni cations in the soil solution are the adsorption sites of oxides, competing cations in the soil solution, and OM content [65–67]. Miśkowiec et al., 2020 [68] found a significant correlation (Pearson coefficient higher than 0.50) between urease activity and the residual form of Ni and a lack of a strong correlation between urease activity and the content of Ni bound to OM. In the variants where urea was applied with only UI, urease activity was slowed, and due to the high OM content, nickel binds to organic matter, increasing its mobility in the soil. The presence of NI caused a decrease in MNi content in the soil compared to the control; the higher urease activity probably caused Ni to bind in a residual form, i.e., tightly bound to the mineral structures in the soil and not released under natural conditions.

With the application of urea with inhibitors, an increase in  $\text{Pb}^{2+}$  content is observed compared to the control (G1), with all variants having a higher pH than the control, but a variable OM content. According to [69,70], increasing soil acidity can enhance the solubility of Pb. However, this mobilization process is generally slower than the accumulation of Pb in the OM-rich layer. Our research indicated the highest MPb content in the variant with one UI, where the pH and OM content are the lowest. Fifi et al. (2013) [71] demonstrated that the mobility of HM in soils is restricted due to competition for adsorption sites, with Pb being preferentially adsorbed over Cu and Cd. The significant correlation between Pb and Cu suggests that their coexistence decreases their propensity to be adsorbed onto the soil's solid phases. This finding is also supported by research conducted by [72].

Ertani et al., 2017 [73] showed that soil pH determines the chemical form of Cr in the soil solution and controls the equilibrium between Cr solubility, adsorption, and desorption in the soil. Also, OM plays an important role in determining Cr bioavailability in the soil through oxidation/reduction and adsorption/desorption [74]. In the variants fertilized with urea with NI, and NI and UI, respectively, the MCr content is lower than in variant G1; in all these variants, the OM content is higher than in G1. OM has a higher cation-exchange capacity (CEC) and can form simple organic molecules and humic substances with Cr ions [75,76], which can increase Cr mobility in the soil.

Numerous studies indicate that zinc solubility increases as soil pH decreases [77,78]. This is evident from the results, where the highest mobile zinc (MZn) value was observed in variant G1, which had the lowest pH (7.05). According to [79], a one-unit increase in pH leads to a 100-fold decrease in zinc solubility. Therefore, applying urea with urease inhibitors (UIs) and nitrification inhibitors (NIs), which raise the pH, will reduce MZn in the soil. Organic matter (OM) content also affects MZn levels. While zinc–humic acid compounds are not as stable and insoluble as copper–humic acid compounds, soluble forms of zinc, such as organic complexes, can make zinc more mobile in the soil [80].

Although the mobile manganese (MMn) content in soil generally decreases as pH rises above 6.2, Benton (2012) [64] found that in some soils, this decline only occurs at pH levels greater than 7.5. The high MMn values observed in certain variants could be attributed to their low organic matter (OM) content. For instance, variant G2 (urea with NI) has an MMn content of 68.43 mg/kg, the highest pH value, and higher OM content compared to G3 and G4. Research by [81–83] indicates that high OM content tends to

reduce available manganese in the soil. Further studies [15,84] suggest that combining urease UI and NI with urea, particularly DCD inactivating NBPT/NPPT, slows down the nitrification process. This leads to the accumulation of  $\text{NH}_4^+$ , resulting in increased pH levels. In turn, this rise in pH, coupled with high OM content, reduces MMn in the soil. By examining these factors, it is clear that the interaction between pH, OM, and the presence of inhibitors significantly influences manganese availability in soil.

## 5. Conclusions

While urea is a highly effective and widely used fertilizer, its use, especially when combined with urease inhibitors (UIs) and nitrification inhibitors (NIs), comes with certain disadvantages. Urease and nitrification inhibitors slow down the formation of ammonium and its conversion to nitrate, which helps reduce nitrogen losses. However, this process can also disrupt natural microbial activities crucial for the cycling of heavy metals (HMs), potentially leading to long-term negative effects on soil fertility and plant health.

Blocking the hydrolysis process at the  $\text{NH}_4^+$  ion formation stage or inhibiting nitrification causes changes in soil pH and OM, affecting the adsorption and desorption processes of heavy metals. Continuous use of UIs and NIs may lead to the accumulation of certain heavy metals in the soil. Our study shows that applying urea with UI and NI indirectly influences the mobility of heavy metals by altering pH and organic matter. These inhibitors shift the balance between organically bound, exchangeable, and bioaccessible forms of heavy metals. The highest concentrations of mobile metals (MMe) were observed when urea with UI was applied, in the order:  $\text{Mn} > \text{Cu} > \text{Pb} > \text{Ni} > \text{Cd} > \text{Cr}$ . However, the presence of both UI and NI resulted in a reduction in mobile zinc (MZn) levels.

While these inhibitors are designed to be more environmentally friendly, their activity in soil depends on soil type, experimental conditions, doses of applied fertilizers, and plant species, so further research is needed to avoid potential negative impacts on soil health.

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