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The qualitative and quantitative impact of copper and zinc applications on winter wheat cultivation

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The impacts of copper and zinc compounds were tested on winter wheat crop grown in calcareous chernozem soil in Hungary, Regöly, Tolna County, during the planting seasons of 2011 and 2013. This work aims to discover the effects of different doses of copper and zinc on wheat yield (t.ha^{-1}), in terms of gluten content (%), "Zeleny" number or sedimentation index and raw protein content (m%). Further, quality food crop of optimal chemical composition under sustainable farming conditions was also adopted. The primary criteria used for selecting the test area include: Soils with high phosphorus and lime content and those deficient of copper and zinc deficiency on the basis of soil analysis. Foliar treatments were applied on winter wheat (*Triticum aestivum L.*). The experiments were performed with a completely new sucrose copper compound that is not yet used in agriculture. The test compounds used in the ecological farming were applied twice in strip-plot design with area of 0.5 ha for each plot. The tests helped us to introduce the efficiency of the treatments throughout the three years. Zn increased the volume of the yield considerably. Maximum yields were achieved at 1.59 kg.ha^{-1} dose. Cu made each quality parameter improved more significantly. Maximum gluten contents were achieved at 1.38 kg.ha^{-1} Cu^{2+} doses. Cu of 1.50 kg.ha^{-1} produced the highest raw protein content and Cu of 1.52 kg.ha^{-1} showed the highest Zeleny values in sedimentation tests. This proves the highest efficiency of Zn and Cu applied at 1.4 to 1.6 kg.ha^{-1} doses.

Key words: Essential microelements, winter wheat, quality, yield, precision technology.

INTRODUCTION

The conditions of agricultural production have deteriorated all over the world. Since 1950, the population of the world has drastically grown from 3 to 7 billion. According to UNO's prognosis, this process is going to slow down in the coming (next) 40 years, but this number could reach 9.3 billion by 2050. Developing countries demand more food, including water and energy (United Nations, 2011).

The farming area, required to compete with the growing demands, is shrinking continuously. Agricultural production has the greatest challenges like conserving biodiversity and ensuring a liveable environment, as agricultural and technological development is not meant to damage biodiversity. Precision farming is one of the elements of sustainable agriculture. It makes us to achieve higher yields without increasing the quantity of energy, equipment and input materials (fuels, fertilizers,

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plant protection agents) significantly and helps us to meet the growing demands of food and the criteria of sustainable farming. Compared to traditional farming, which determines the field as the smallest unit of applications, precision farming divides the homogeneously treated area into smaller units. These units show different features within one field like soil, relief, water-supply, weed covering and humus content. In precision farming, those smaller units are considered and technological and agro-technical (site specific) intervention that considers field variations is applied (Neményi et al., 2002; Tamás, 2001; Tamás et al., 2005; Cambardella and Karlen, 1999).

Microelements are essential for our cultivated plants. This group of components includes first of all metal ions, whose primary role is to interact with the negative parts of molecules in living organisms (Schmidt et al., 1999). Both copper and zinc are essential factors for winter wheat (*Triticum aestivum L*.). Copper is indispensable for living organisms. Various quantities of copper can be found in different plants and in each plant's part. Among cereals embryonic tissues of cereal grains contain 2.5 as much copper as the endosperm. Several gene families and proteins that participate in the process of Cu-homeostasis have been identified. Experiments with different plants revealed that copper is strongly bound to the roots excluding most of the cations (Russell, 1986). It is transferred in plants bound to amino acids (histidine). Being an essential element it is indispensable for the adequate life processes. It activates the respiratory chain and forms a number of enzymatic activators and constituents (Shkolnyik, 1984).

It contributes to the development of yield quality and quantity and it also affects the protein and gluten content of cereals. Cereal crops often show copper deficiency even on soil well supplied with copper due to hindered transport processes. The majority of Hungary's soils appear to be deficient of copper and zinc deficiency. Mineral content of the soil forming rocks determines the total copper content of soils as in the case of Zn and Mg. Copper bounds clay minerals among cations the most, which reduces its availability and uptake by plants (Mitchel, 1955; Alloway, 1995; Ma and Dong, 2004).

Zinc is an enzyme component (carbonic anhydrase, alcoholic dehydrogenase, deoxyribonucleic acid (DNA**)** polymerase), and acts as an enzyme activator (e.g. enolase, aldolase, carboxypeptidase, lecithinase). It participates in the nitrogen metabolism through the activity of peptidases. By the interaction with manganese, it enhances auxin production which is essential for controlling plant growth (Várallyay et al., 2009). Zinc deficiency in plants may cause chlorosis, stunted growth, incomplete fructification, abnormal root formation and scald in fruits.

Zinc is responsible for stabilizing the structure of ribosomes, ribonucleic acid (RNA) and DNA as well as for the correct function of the immune system. It also influences the production and degradation of insulin, glucose and lipid metabolism and correct activity of sex hormones (Hotz and Brown, 2001). In case of deficiency the RNA and DNA polymerase activity reduces, and the oxidative damage of bio-membranes happens more easily (Hotz and Brown, 2001). Due to its role in life processes we should first of all eliminate zinc deficiency in plant organs. The tests showed a higher baking quality index and increased gluten and raw protein content (Szakál and Schmidt, 1996). Among foreign scientists Graham (1978), Loneragan et al. (1980), Peterson et al. (1983a, b, 1986), Han and Shepherd (1991), Owouche et al. (1994) and Mingdeng et al. (2009) tested copper on winter wheat. Yang et al. (2011), Lu et al. (2012) and Li et al. (2014) applied zinc in soil- and foliar fertilizers for the sake of replacement of different winter wheat varieties. Holloway et al. (2010) did a glasshouse study with zinc.

MATERIALS AND METHODS

Experiments on one of the varieties of winter wheat (Lupus III.) (*Triticum aestivum L*.) were launched in the fields with calcareous chernozem soil in Hungary in Regöly, Tolna over three years (2011, 2012 and 2013). Comparative tests with different doses of copper were carried out with ecologically approved sucrose copper compound using precision technology. These were done to produce a high quality food crop with optimal chemical composition in compliance with sustainable farming practices. The selection of the test areas relied on the results of precision soil analysis. Soil analysis was done by GPS-supported mechanical sampling using a diagonal sampling design, and a density of 1 ha at a depth of 0-30 cm according to the rules of crop land soil sampling. Drawing the sampling maps, the sample area was measured by Trimble GeoXT GPS with an accuracy of 20 to 30 cm. Then the coordinates of the area were transferred in an internally developed ArcGIS system and dividing the areas prepared for the net-sampling plans in *shp format. Those *shp files were imported into the field devices and based on those plans we proceeded diagonally and took the samples. One average model represented 1 ha, and was composed of 20 to 25 copper samples. The elements were analysed in the laboratory of sYnlab Umweltinstitut Ungarn Kft., Mosonmagyaróvár (Hungary). Soil reaction (pH), soil physical kind, that is, soil water capacity, total salt content, carbonic lime content, humus content, soluble phosphorus and potassium content, soluble Na-content, soluble nitrite- and nitrate –nitrogen as well as sulphate-sulphur, soluble Cu, Mn and Zn content were measured during the tests. Soil analyses results were obtained by a 3RP System programme both per field and per sampling area. Average values of the tested elements are summarized in Table 1.

Results of soil analyses are evaluated in tables and represented on maps. Digital data were interpolated in *dbf format by internal system. Laboratory data were ordered to the sampling sites and the nutrient supply maps were prepared. Selection of the test areas was based on the results of precision soil analyses, which showed the copper, zinc, lime and phosphorous content of the chosen fields. Further criteria were to launch the tests on almost plain fields. After choosing the fields, we had to mark plots within the fields. Based on the factors of soil analyses and relief, we marked the test plots with Trimble GeoXT GPS in *shp format. Then, they were assigned to the field boundaries and saved in the database of our server. In planning the plots, we had to consider the working width of the sprayer (24 m); therefore we adjusted the widths of all plots to that. Plots were treated in strip design with four replications.

Table 1. Soil physical-and chemical characteristics at Regöly 2010, IKR Co.

Parameter	Area (ha)	pH(KCl)	KA	Salt	$CaCO_3$	Humus	NO_3-N	P_2O_5	K_2O	Mg	Na	Zn	Cu	Mn	SO_4-$^{2-}$	Texture
				%		mg/kg										
65041, R12/1	29.2	7.58	36	0.53	17.72	1.96	52.2	129	182	153	47	0.6	0.84	17	14.6	1
65024, Bogaras II	22.1	7.36	38	0.02	6.59	2.85	12.6	171	211	137	35	0.8	0.96	58	14.1	1
65037, R3-4	79.2	7.5	37	0.114	6.44	1.99	156	144	218	152	34	0.6	1.49	90	15	1

Applications were done by a self-propelled Dammann sprayer, which was equipped with GPS. Therefore, we were able to record the applications and the applied quantities accurately. We applied foliar treatments, as cereal corps often show copper deficiency even on soils well supplied with copper – due to hindered transport processes. Applications were carried out in two phenological phases, at the time of shooting and flowering. Sucrose copper is a completely new compound - an acidic copper chloride, which has not been used in agriculture yet. We applied copper and zinc at doses of 0.1; 0.3; 0.5; 1.0; 2.0 kg.ha^{-1}. We harvested with a GPS controlled New Holland CR 9070 harvester equipped with yield and grain moisture meter. This way the yield data of each plot were available. At harvest we took samples from every treated and control plot. ICP was used to measure the element content of the crop. We also measured the Zeleny-index, the raw protein-, gluten- and starch content. The results were evaluated with the help of mathematical methods.

Yield data were marked on a map with the help of New Holland PFS software. As the yield meter records the data at harvest every second, we received a very dense mass of data. Since the sizes of the plots were smaller than 1 ha, we could not make any analyses, but had to average them. Then with an internally developed ArcGIS based system and spline method we interpolated the received yield data and analysed them (Figure 1). Spline means a curve described by parametric polynomial segments. The main point of the method is that the continuity of the approaching function is expected. A spline function is a polynomial segment, that is, a local method, but the change happens at the coefficients of the polynomial, because we fix them so that they ensure global smoothness for the approaching function (Stoyan and Takó, 2002). We also used the spline method to interpolate the parameters received by quality measurements, marked them on a map and finally analysed them with statistical methods (Figures 2 and 3).

RESULTS

We analysed the effect of copper and zinc separately. One-factor variance analysis was applied to show the effect of the applications at different doses (6 = control + 5 different applications, 3 different yearly replication usually in plant growth), in every case at 0.05% level of significance. To prove the average efficiency of the applications we carried out those measurements on the average of 3-year-data, because the average filters out the variations caused by the different environmental-climatic conditions of the basic data in 3 years. The different environmental-climatic conditions are in Table 2. To determine the optimal size of the applications on the averages made from the measured data, the production function depending on the applications (that is, we fitted a regression curve) was drawn. In the production function x_1 is for Cu, x_2 for Zn , y_{ij} is for the measured result variable: i = 1 Cu / i = 2 Zn application and j = 1 yield/ j = 2 gluten content/ j = 3 Zeleny number / j = 4 raw protein content. The best fitting curves (R^2>90%) were quadratic equations in every case, the maximum locations of which were calculated. Applying the correlation matrix, the closeness of the correlation between the measured result variables was calculated. The two types of applications (Cu /Zn) at different doses for the measured parameters (yield / gluten content / Zeleny number / protein content) were compared. Analyses were carried out in a traditional way, that is, the prior calculation results were visible besides the final results. Excel 7.0 was used as an aid to the calculations. In 2012, the Zeleny number (sedimentation index) was high compared to the other years due to the strong environmental impact on crop growth even in case of Cu and Zn applications, but the yield, gluten and raw protein content showed lower values.

Effect of copper applications

The differing environmental impact of each year resulted in a considerable nearly double -increase of the Zeleny number in both seasons of 2011 and 2013, whereas, in 2013, gluten and raw protein contents were highly increased.

Applications significantly increased the yields right from the doses of 0.5 kg.ha^{-1} (LSD = 0.22). The yield function ($y_{11} = -0.23x_1^2 + 0.77x_1 + 5.96$) achieved its maximum with the application of 1.67 kg Cu per hectare.

Based on the gluten analyses of the three years, applications showed a significant increase in the gluten content right from the doses of 0.3 kg.ha^{-1} (LSD = 1.38). The regression curve ($y_{12} = -2.49x_1^2 + 6.88x_1 + 28.63$) achieved its maximum with the application of 1.38 kg Cu per hectare. Measuring the Zeleny number the applications exposed a decrease in the sedimentation index up to the doses of 0.3 kg.ha^{-1}. Doses higher than

Table 2. Main soil and environmental properties of winter wheat in Hungary (Pepo and Sarvari, 2011).

Main soil and environmental properties of winter wheat in Hungary	
Soils	Can be thrown into all the soil of Hungary. Best soils: 1. Chernozem soil (calcareous, type, deep salty), 2. Leaner loess chernozem yarns, 3. The better yielding meadow soils, 4. Non-lime molds, 5. Better quality solitary solonetz soil.
Heat amount demand, Temperature range	2000 - 2200°C, -20 - 40°C
Water demand	Moderately water-demand plant: 420-460 mm
Irrigation	Critical periods (e. g. bloom) have a high water demand, but in Hungary evaporation is generally high. Therefore it is irrigated in many places (e g. Komárom-Esztergom county, Békés county) in Tolna county no irrigation was done

0.5 kg.ha^{-1} significantly increased (LSD = 2.91) the measured parameters. The Zeleny number function ($y_{13} = -4.56x_1^2 + 13.83x_1 + 31.25$) achieved its maximum at 1.52 kg.ha^{-1} doses of Cu.

Measuring the raw protein content of the 3 years, due to the applications the raw protein contents increased significantly (LSD = 0.22) right from the doses of 0.5 kg.ha^{-1}. The fitted regression curve ($y_{14} = -0.60x_1^2 + 1.80x_1 + 12.81$) achieved its maximum at Cu doses of 1.50 kg.ha^{-1} (Figure 4).

From experienced data the calculated correlation coefficient in pairs is tabulated in matrix (Table 3). The relation between the variables can be regarded as close, which is shown by the values of the correlation matrix. From the data of the correlation matrix it can be seen that:

1. There are strong connections between the examined values and the treatments as the correlation coefficients are between 0.7 and 0.9;
2. There are very strong connections between each factor as the correlation coefficients is higher than 0.9 in each case.

Effect of zinc applications

Based on the variance analysis (at 0.05% level of significance), applications were efficient in case of every measured factor.

Applications significantly increased the yields (LSD = 0.37) in the average of three years right from the doses of 0.5 kg.ha^{-1}. The yield function ($y_{21} = -0.46x_2^2 + 1.46x_2 + 6.00$) achieved its maximum at zinc doses of 1.59 kg.ha^{-1}. Measuring the gluten content we found that the quantities were significantly (LSD = 1.33) increased right from the doses of 0.5 kg.ha^{-1}. The yield function ($y_{22} = -1.45x_2^2 + 4.31x_2 + 28.47$) achieved its maximum at Zn doses of 133 kg.ha^{-1}.

Evaluating the Zeleny number found similarly with the copper applications, that the Zn applications reduced the value of this parameter until 0.3 kg.ha^{-1} have been reached, but doses higher than 0.5 kg.ha^{-1} significantly (LSD = 2.97) increased the measured parameter. The Zeleny number function ($y_{23} = -2.55x_2^2 + 7.61x_2 + 31.04$) reached its maximum at Zn doses of 1.49 kg.ha^{-1}.

Right from 0.5 kg.ha^{-1} doses the applications significantly (LSD = 0.45) increased the raw protein content. The fitted regression curve ($y_{24} = -0.49x_2^2 + 1.51x_2 + 12.50$) achieved its maximum at Zn doses of 1.54 kg.ha^{-1} (Figure 5).

From experienced data the calculated correlation coefficient in pairs is tabulated in matrix (Table 4).

The relation between the variables can be regarded as close, which is shown by the values of the correlation matrix. From the data of the correlation matrix, it is seen that:

1. There are strong connections between the examined values and the treatments as the correlation coefficients are between 0.8 and 0.9;
2. There are very strong connections between each factor as the correlation coefficient is higher than 0.9 in each case.

DISCUSSION

The drastic increase in the world population resulted in higher demand of food consumption paying attention to

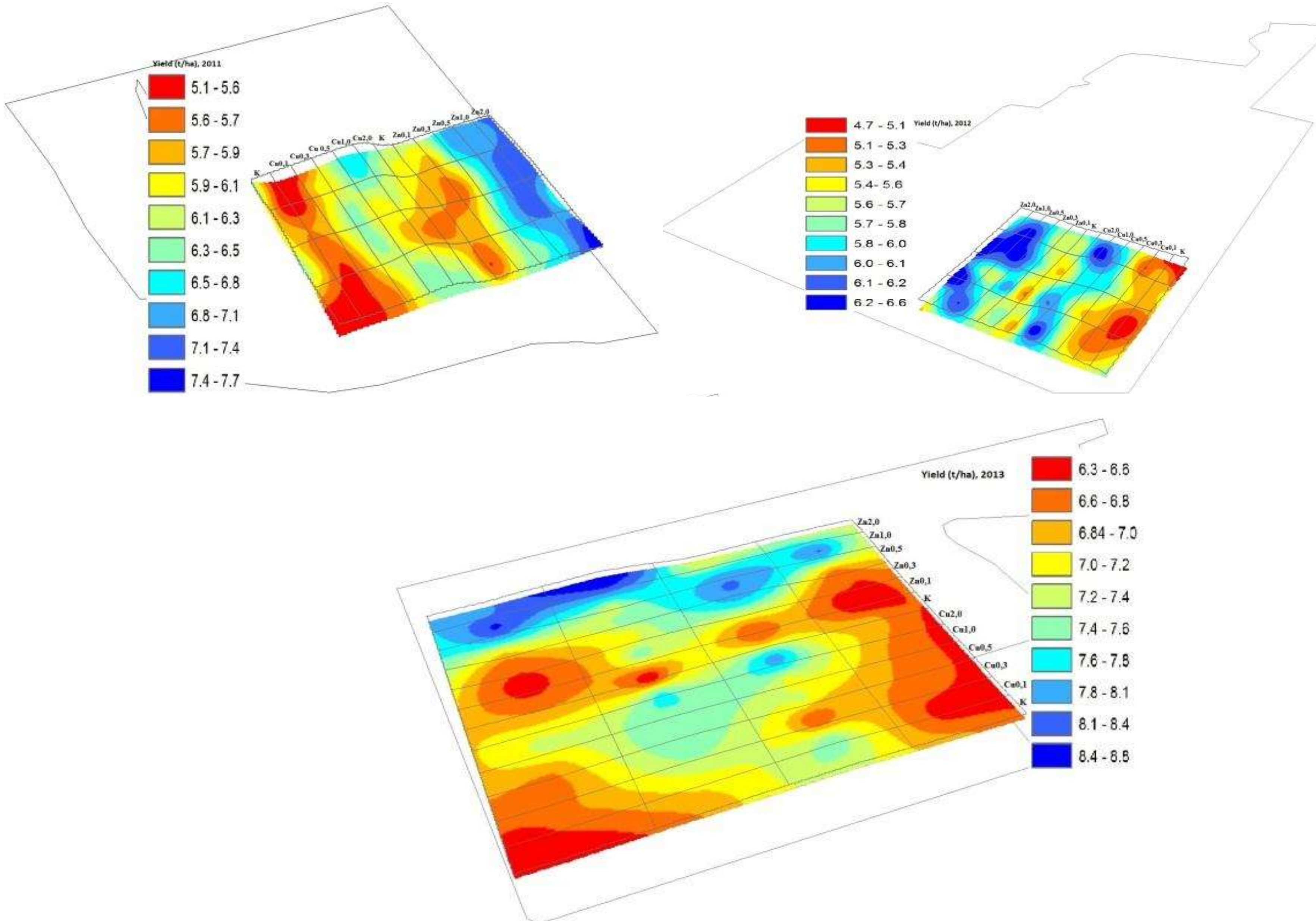

Figure 1. Yield map of winter wheat (2011, 2012 and 2013).

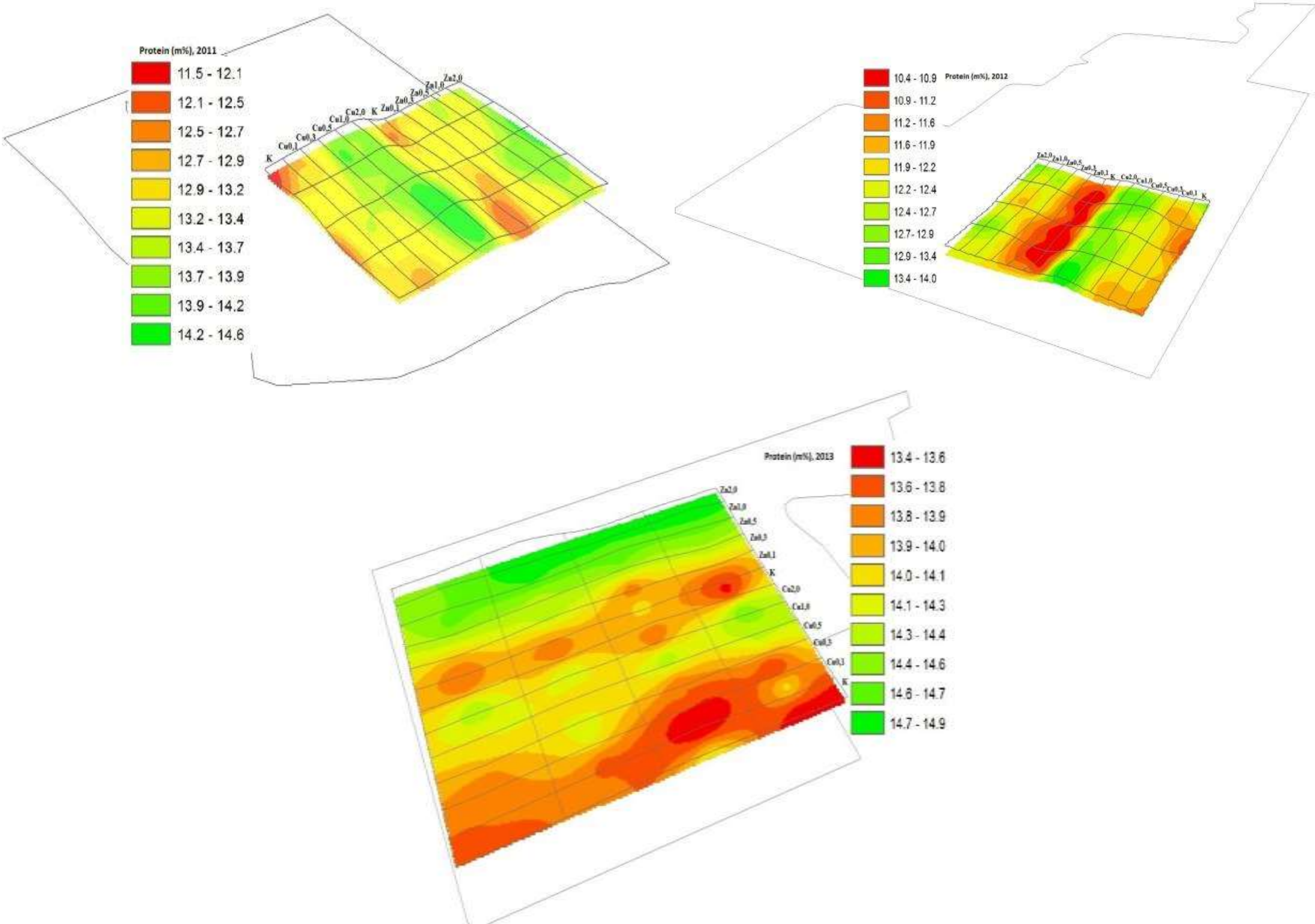

Figure 2. Protein content of winter wheat (2011, 2012 and 2013).

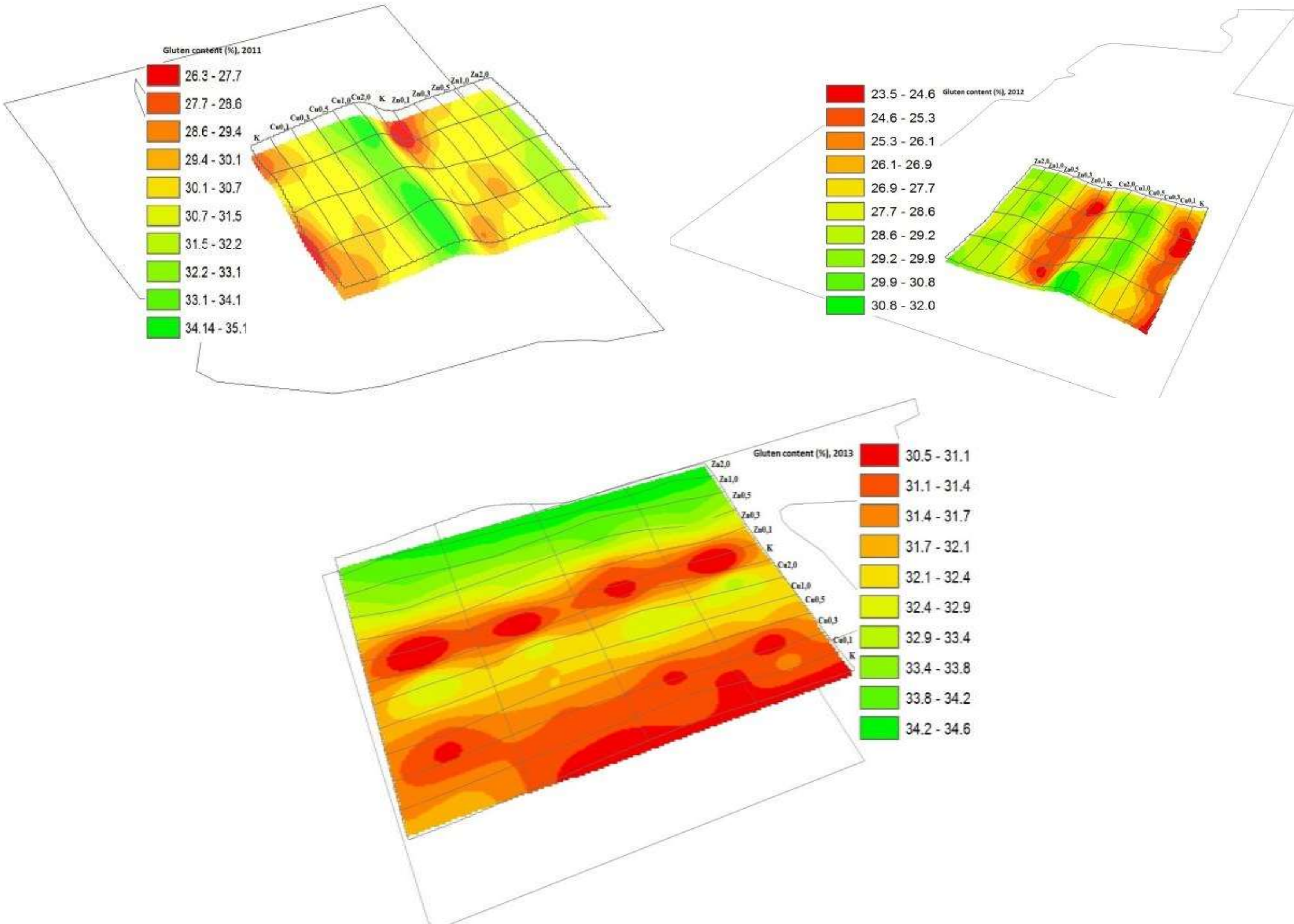

Figure 3. Gluten content of winter wheat (2011, 2012 and 2013).

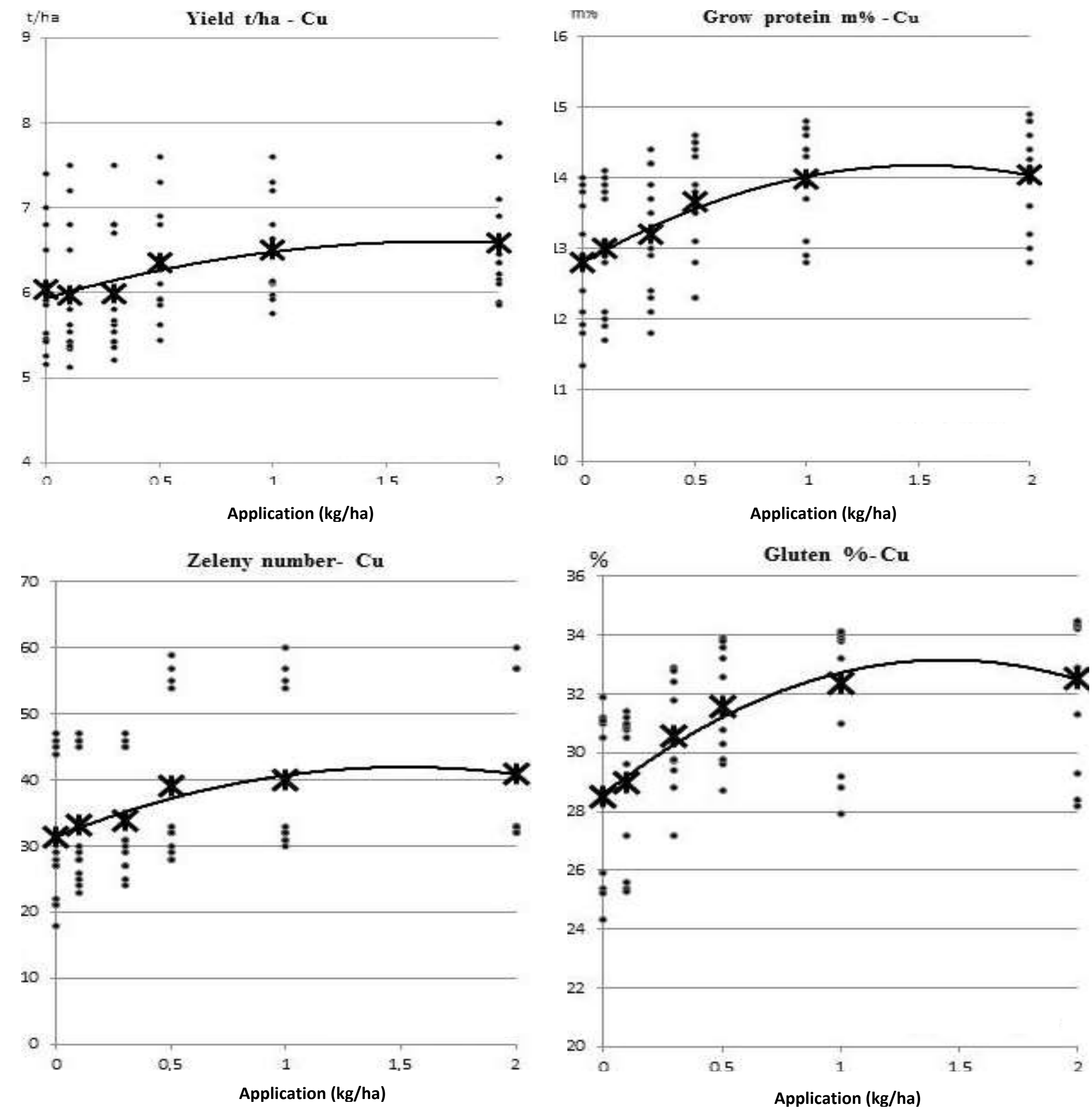

Figure 4. The effect of copper application yield, protein, Zeleny-number and gluten content.

Table 3. Correlation matrix of Cu.

Cu	Application (kg ha^{-1})	Yield (t ha^{-1})	Graw protein (m%)	Zeleny number	Gluten (%)
Application (kg ha^{-1})	1				
Yield (t ha^{-1})	0.90	1			
Graw protein (m%)	0.87	0.97	1		
Zeleny number	0.84	0.96	0.99	1	
Gluten (%)	0.77	0.92	0.96	0.97	1

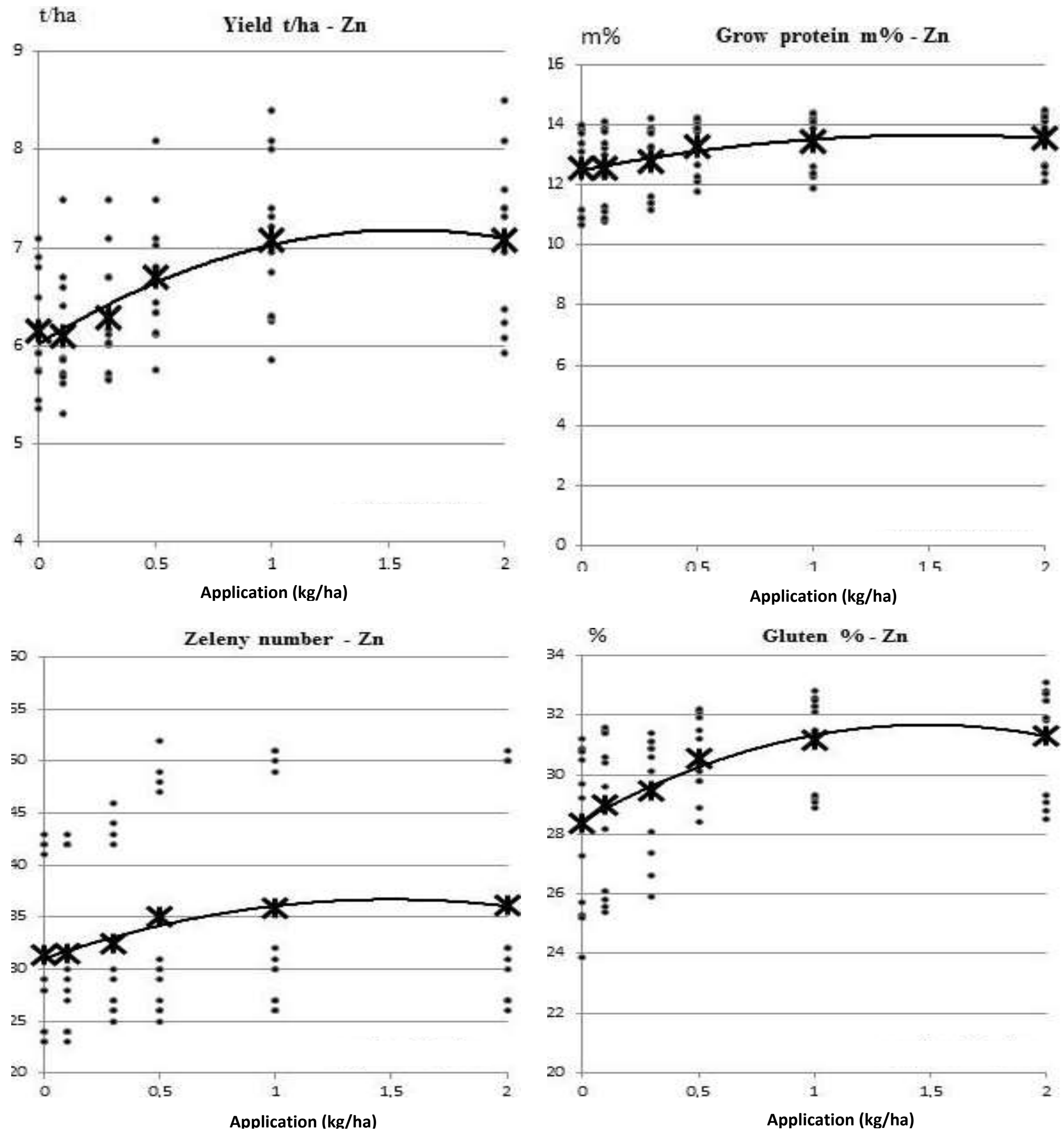

Figure 5. Effect of zinc application on the yield, protein, Zeleny-number and gluten content.

Table 4. Correlation matrix of Zn.

Zn	Application (kgha^{-1})	Yield (tha^{-1})	Graw protein (m%)	Zeleny number	Gluten (%)
Application (kgha^{-1})	1				
Yield (tha^{-1})	0.86	1			
Graw protein (m%)	0.85	0.99	1		
Zeleny number	0.86	0.98	0.99	1	
Gluten (%)	0.87	0.96	0.98	0.98	1

the quality of the produced food and the sustainability of biodiversity. Several research topics deal with the question: How can we harmonize the challenges of global environment conservation and food supply?

Considering the sustainable farming principles, the trials aimed to produce high quality food with optimal chemical composition and meeting the different administrative regulations on food produce and environment protection.

Our results revealed that the applications were efficient every year. Compared to the control areas the measured parameters increased and at doses higher than 0.5 kg. ha^{-1} significant difference could be shown in the measured quantities of yield, gluten content, Zeleny number and protein content.

Zn applications considerably increased the quantity of the yield, though some of the quality parameters increased more due to Cu applications. The highest effect of both elements is at doses of 1.4 to 1.6 kg ha^{-1}. A selection will be determined by the result variable (e.g. yield) that needs to be raised. As a summary, we can say that in the practice of soil quality, the actual availability or the price can influence the decision on Cu and Zn applications. The application of either element will definitely increase the wheat quantity and improve the quality of the crop, i.e. the value of the produce.

Previous studies have shown that nutritional values of winter wheat zinc and copper micronutrient supplementation need to be increased (Dudgeon and Bolland, 1916; Han and Shepherd, 1991; Owouche et al., 1994; Yang et al., 2011; Lu et al., 2012; Li et al., 2014; Cakmak et al., 1996; Cakmak, 2008), but high levels of zinc detected in the yield has not been studied so far, and zinc sulphate used during the treatments. Zinc carbonate complex compound was used in the experiments. Until now only small plot trials or glasshouse study (Holloway et al., 2010) has been reported in the literature. We performed all of the experiments in operational conditions with precision technology. The new copper sucrose complex has no scorching effect on cereals, they have better course availability and have favourable ligand effect.

Conclusion

The Hungarian soil is 70% zinc deficient, and 30% copper deficient. These minerals are essential to grow grain high in protein. Based on several years of research indicated, in the zinc deficient areas, 200 kg ha^{-1} of zinc agent should be replenished annually. In the case of copper, even in well-maintained areas, deficiencies may develop due to a hindered transport process. Obtaining an appropriate amount of replenishment necessary to remedy the deficiencies in the soil is difficult due to the decreasing quantity of available mineral resources and the increasing prices. So, we turned to waste materials that are becoming increasingly available in the world, as a possible source of mineral replenishment. Copper and zinc containing complex constituents were produced to serve as mineral replenishment. The materials we used in our experiment have met the standards of organic farming, so the technology used in sustainable agriculture is appropriate. The microelement replenishment in other zinc and copper deficient regions may also be effective for winter wheat growing, as well as the high fungicidal activity provided by the copper compound complex. One of the high challenges is that precision technology sometimes only makes slow progress relative to the increasing need. Successful application of the new technology is essential for proper soil and plant analysis and for the nutrition management consultancy (e.g., 3 RP System). The 3 RP System has been developed here and now is used in more than 100 000 ha. Based on these results, a stable and supportive agricultural policy can be developed to employ these newer technologies as evidenced by the increasingly large areas where the results of our work are being used year-by-year.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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