IOŚ-PIB OCHRONA ŚRODOWISKA I ZASOBÓW NATURALNYCH
ENVIRONMENTAL PROTECTION AND NATURAL RESOURCES 2014
DE GRUYTER OPEN
VOL. 25 NO 4(62): 23–27
DOI 10.2478/oszn-2014-0020

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Biofortification of wheat grain with copper through soil fertilization[1]

Biofortyfikacja ziarna pszenicy miedzią poprzez nawożenie doglebowe

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Keywords: wheat grain, copper concentration, soil application, pot experiment
Słowa kluczowe: ziarno pszenicy, zawartość miedzi, nawożenie doglebowe, doświadczenie wazonowe

Abstract
In recent years, in the literature, there have been frequent reports of insufficient amounts of copper in the diet of various groups of the inhabitants of our country. This is disturbing as the adequate input of copper is significant from the point of view of prevention of cardiovascular diseases. At the same time, grain of wheat cultivated in Poland is characterized by low content of this element. Considering that the main source of Cu is bread and cereal preparations, the important issue is to increase the content of Cu in the grain of wheat. If the deficiency in the diet is accompanied by the deficiencies in the soil, biofortification through fertilization is a favourable solution. Pot experiments have shown the possibility to significantly increase Cu content in the grain of wheat as a result of soil fertilization with copper. It was also found that a small difference between the deficiency and surplus of Cu in the grain may lead to some copper excess content, especially on the soils with low organic matter content. For this reason, biofortification of wheat with copper requires a precise determination of soil fertilization doses under the conditions of field experiments.

Streszczenie
W ostatnich latach w literaturze pojawiają się częste doniesienia o niewystarczającej ilości miedzi w diecie różnych grup mieszkańców naszego kraju. Jest to niepokojące, ponieważ odpowiednia podaż miedzi jest istotna z punktu widzenia profilaktyki chorób sercowo-naczyniowych. Jednocześnie ziarno pszenicy uprawianej w Polsce charakteryzuje się niską zawartością tego pierwiastka. Biorąc pod uwagę, że głównym źródłem Cu jest pieczywo i przetwory zbożowe, istotnym zagadnieniem jest zwiększenie zawartości Cu w ziarnie pszenicy. W sytuacji, gdy niedoborom w diecie towarzyszą niedobory w glebie, korzystnym rozwiązaniem jest biofortyfikacja poprzez nawożenie. W przeprowadzonych badaniach w warunkach doświadczenia wazonowego wykazano możliwość znaczącego zwiększenia zawartości Cu w ziarnie pszenicy na skutek nawożenia doglebowego miedzią. Stwierdzono również, że mała różnica pomiędzy niedoborem a nadmiarem Cu w ziarnie może prowadzić do wystąpienia jej nadmiernych zawartości, szczególnie na glebach o niskiej zawartości materii organicznej. Z tego powodu biofortyfikacja ziarna pszenicy miedzią wymaga precyzyjnego ustalenia doglebowych dawek nawozowych w warunkach doświadczeń polowych.

1. INTRODUCTION

More than 40% of people in the world suffer from micronutrient deficit, which is the reason for various health problems [Murphy et al. 2008, Progress report 2000]. While in the world, there are mostly deficiencies of iron and zinc [White and Zasoski 1999], in Poland, the additional problem is an insufficient amount of copper in food and animal feed. It is connected with a considerable amount of soils with low content of this element in our country [Bombik et al. 2004]. According to the latest studies of Polish agrochemical laboratories, up to 34% of the arable soils in Poland have deficiencies of copper available to plants [Lipiński 2013].

An adequate supply of copper from the diet is important from the point of view of prevention of cardiovascular disease (CVD) [Terlikowska et al. 2013]. Copper is a part of the antioxidant enzymes (e.g. superoxide dismutase), and its deficiency in the diet can lead to disorders of the antioxidant system in the body. This is connected with the occurrence of oxidative stress, which can lead to CVDs [Grygiel-Górniak 2010, Klevay 2000, Suliburska 2010, Uriu-Adams and Keen 2005]. It is estimated that CVD causes nearly 50% of all deaths in Europe. According to the European Cardiovascular Disease Statistics, coronary heart disease is the cause of 21% of deaths, stroke – 12.5%, while other CVDs cause 13.5% of deaths among the residents of Europe [Nichols et al. 2012].

An insufficient amount of copper in the diet can be dangerous not only for people but also for farm animals. Cu deficiencies lead to anaemia, reduce growth and fertility, cause bone breakability and nervous system disorders [Kluczek 2006, Sikora 2007, Zatta and Frank, 2007].

In recent years, many authors have reported insufficient amount of copper in the diet of the inhabitants of our country. Bronkowska and Karcz [2007] recorded a very low consumption of copper (36.5% of normal) in the test group of 100 women with professional low physical activity. A similar low consumption (40.4% of normal) was observed in a group of 100 women in perimenopause [Bronkowska et al. 2009]. Staniek et al. [2006] have shown, on the basis of the menu of the orphanage in Trzemeszno and the home of social assistance in Skubarczewo, that the daily food diets of

[1] The work has been prepared as part of task 2.6 in the IUNG-PIB Long-term Programme.

the elderly and children provide too little of Cu. According to Jaworska and Bazylak [2007], copper requirements of female pharmacy students in Bydgoszcz, estimated for 73 persons, were satisfied only in 44–49%. The analysis of the Cu content in hair during perimenopause has shown the possibility of copper deficiencies in a relatively large part of the population of older women [Wlaźlak et al. 2007]. Bolesławska et al. [2009] have shown a too low level of copper intake in the randomly selected group of 723 men and women. On the basis of studies among 78 students of Warsaw schools, the average content of Cu in the diet of young people was only 50–60% of the recommended daily supply [Dybkowska et al. 2011]. According to Wielgos et al. [2012], the intake of copper in daily diets of 157 students from randomly selected schools from Cracow and Skawina generally did not meet the requirements for this element.

The main source of copper in the diet of Poles is bread and cereals [Dybkowska et al. 2011, Seidler et al. 2013], i.e. grain cereals. Thus, a low Cu content in the grain of Polish wheat is a concerning issue. According to previous studies on the national scale, it amounted to an average from 2.6 to 3.4 mg•kg^{-1} for the country [Gembarzewski et al. 1995, Report 2002, Wojciechowska et al. 1995, Wróbel 2000]. Currently, there are no studies covering the whole country, but frequent reports of shortages of this element in the diet suggest that it is still insufficient.

It should be noted that the content at the level of 2.6–3.4 mg•kg^{-1} is also insufficient from the point of view of nutritional requirements of farm animals. According to Polish authors, the standard copper content of the animal feed is 10 [Falkowski et al. 2000, Kruczyńska 1985], and according to American National Research, it ranges from 3 to 16 mg•kg^{-1} Cu depending on the species and age group [USA NRC 1989, 1994, 1998, 2000, 2001].

In the case of deficiencies of micronutrients in the diet, biofortification of cereal grains seems to be the most cost-effective solution [Bouis et al. 2011]. Different strategies of biofortification, such as application of fertilizers, traditional breeding and genetic engineering, are widely discussed in the world literature [Bashir et al. 2013, Cakmak 2008, Velu et al. 2014]. When the deficiencies in the diet are accompanied by the deficiencies in the soil, biofortification through fertilization seems to be the most advantageous solution.

The aim of this study was to examine the possibility of increasing copper content in winter wheat grain through soil fertilization with this element.

2. MATERIALS AND METHODS

In 2012–2013, at the Experimental Station of IUNG-PIB in Jelcz-Laskowice near Wrocław, a 2-year pot experiment with soil fertilization of spring wheat with copper was conducted. The experiment was established in a completely randomized design in eight replicates, the four of which were collected in the beginning stage of shooting and the other four in the stage of full grain maturity. The experiment included two types of soils – S1 and S2, which were previously sieved through a sieve with a diameter of 1 cm and limed at a dose of 12 g of $CaCO_3$ per pot. Soil S2 was obtained by adding organic matter in the form of de-acidified peat to soil S1 in the ratio of 1:8. The description of the soils used in the experiment is presented in Table 1.

The experiment was performed in Wagner pots filled with 9 kg of soil. Copper was introduced into the soil in the first year of the experiment for a month before sowing of wheat in the following doses: 0, 5, 10, 30 and 60 mg Cu per pot in the form of $Cu_2SO_4 \cdot 5H_2O$. In addition, a micronutrient medium with the composition of 1.6 mg Mo, 4.1 mg B, 8.4 mg Zn, 23.3 mg Mn and 62.5 mg Na per pot was applied once in all the treatments before the sowing.

The test plant was wheat of Jasna variety. Initially, 23 grains of wheat were sown per pot, and after reaching the three-leaf stage, 17 plants per pot were left. Every year, the plants were fertilized with nitrogen before sowing and during the beginning of shooting, in a total dose of 1.8 g N in the form of NH_4NO_3 per pot. Soil moisture was maintained at 60% of field water capacity.

In both years of the study (2012 and 2013), samples of whole above ground parts in the beginning stage of shooting, cut 5 cm above the ground, and samples of grains in the stage of full maturity were collected for the determinations of copper in plant material . Soil samples were collected three times: in spring 2012 after liming and mixing the soil with peat and before Cu fertilization – for performing the characteristics of the experimental soils, and after harvest in 2012 and 2013 – for the determinations of available copper.

The copper content in the plant samples was determined by FAAS method. The analyses were performed after prior ashing of ground, air-dry samples in a muffle furnace and dissolving them in hydrochloric acid. Copper in the soil was determined by AAS method after prior extraction in 1 M HCl. Furthermore, the soil texture was determined by sieve-aerometric method, pH in 1 M KCl and the content of organic carbon by oxidation method.

Available phosphorus and potassium were determined by Egner-Riehm's method in 0.04 M $Ca(CH_3CHOHCOO)_2 \cdot 5H_2O$ and available forms of magnesium were determined according to Schachtschabel' method in 0.0125 M $CaCl_2$.

ANOVA calculation was performed with the use of AWAR program [Filipiak and Wilkos 1995]. The comparisons of differences between the means for treatments were made using the multiple Tukey's test. All the presented results are the average of 2 years of the research.

3. RESULTS AND DISCUSSION

In the experiment, wheat was grown on two soils: S1 and S2, which differed mainly in terms of organic matter content and pH level (Table 1). Both of these features of the soil affect the availability of Cu to plants and an increase of them limits the bioavailability of this element. This relationship was also observed in our study. Wheat growing in the soil S2 without Cu (S2Cu0 treatment) contained by about 10% less copper in the shoots and by 40% in the grain compared with the wheat of S1Cu0 (Figs 1 and 2).

In the beginning stage of shooting, the wheat from the treatments without Cu growing on S1 contained 6.2 mg•kg^{-1}, and on S2 – 5.7 mg•kg^{-1} of copper (Fig. 1). It is difficult to assess whether these values were sufficient for its growth and development since different authors give different critical limits for this stage. According to Bergmann [1992], the deficiency is below 7 mg•kg^{-1}, while according to Shnug and Haneklaus [2008], it is below 4 mg•kg^{-1}. Cu content below 3 mg•kg^{-1} in the grain is insufficient for correct yielding of wheat and too low to meet the needs of farm animals

Table 1. Characteristics of experiment soils

Soil	Soil type	Fraction <0.002 mm (%)	pH	C org (%)	P	K	Mg	Ca	Cu
					(mg•kg^{-1})				
S1	Loamy sand	1.4	6.2	0.7	165	116	100	1564	2.2
S2	Loamy sand	1.4	6.6	2.3	253	99	120	2187	2.3

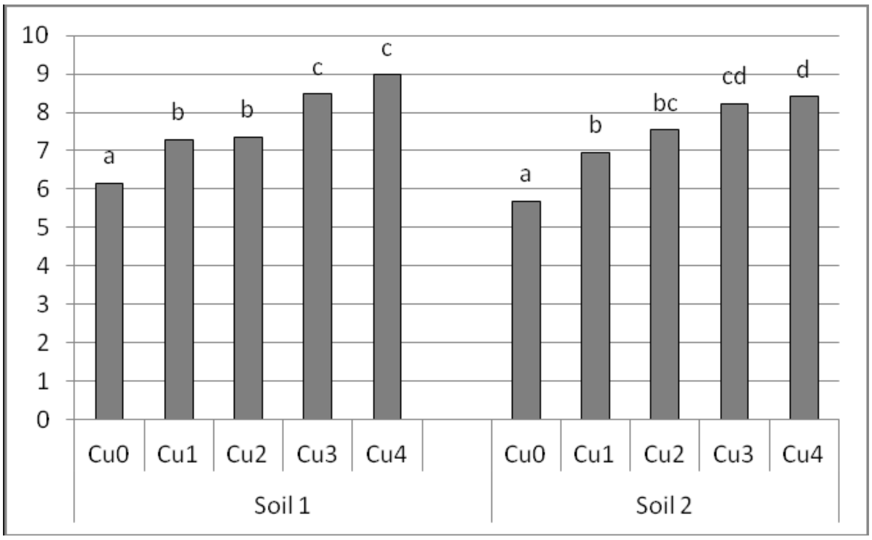

Fig. 1. Copper concentration in shoots (mg • kg^{-1})

[Kirchmann et al. 2009]. Copper deficiency is a serious problem for ruminants in many parts of the world [McDowell 2003].

At the same time, the content above 6 mg • kg^{-1} is dangerous from the point of view of possible surplus of copper in the diet [Kabata-Pendias and Mukherjee 2007, Raport 2002]. The optimal range of 3–6 mg • kg^{-1} Cu in the grain is rather narrow. There is some risk of excessive increase of Cu amount in the grain under the influence of high doses of fertilizer.

In our experiment, the copper content in the grain of control treatment was on the verge of deficiency in the soil S1 (3.0 mg • kg^{-1}) or well below this limit in soil S2 (1.9 mg • kg^{-1}) (Fig. 2). It should be noted that the content in S2 was significantly lower than that reported by other Polish authors. In general, the Cu content in wheat grain grown on Polish territory was at the level of 2.6–3.4 [Gembarzewski et al. 1995, Raport 2002, Wojciechowska et al. 1995, Wróbel 2000]. However, the majority of Polish soils are characterized by both a significantly lower content of C org, as well as by lower levels of pH than the soil S2, which may be the reason for the greater availability of copper and its higher contents in wheat plants in the country. This indicates that the wheat grown on limed soils with above average organic matter content may require fertilization with copper.

Soil fertilization with increasing doses of copper resulted in an increase in its content in the shoots and grains of wheat. The largest increase in its content occurred at the highest dose of Cu4 and amounted to 45% for S1 and 48% for S2 in the shoots, and 88% for S1 and 125% for S2 in the grain (Figs 1 and 2). For soil S1, all the applied doses of Cu raised its content in the grain above 3 mg • kg^{-1}, which is the limit of deficiency. It should be noted that the highest dose Cu4 caused a high increase in the content, close to 6 mg • kg^{-1}. This situation did not occur in the soil S2. In contrast to soil S1, the doses of Cu1–Cu2 did not counteract the deficiencies. Only the doses of Cu3 and Cu4 provided sufficient copper content in the grain, while at the same time not approaching to the excess limit.

In soil S2, an increase in the copper amount in the grain under the influence of fertilization corresponded with increasing amounts of copper in the soil (Fig. 3). Similarly like in the grain, only the doses of Cu3 and Cu4 significantly raised the content of available Cu in the soil to the level 8.6–9.1 mg • kg^{-1} (Fig. 3). In soil S1, Cu content in the soil increased steadily in the range of 2.2–8.2 mg • kg^{-1}.

The obtained results indicate the possibility of biofortification of wheat grain with copper by soil fertilization. Foliar fertilization often does not allow for biofortification of grains with microelements.

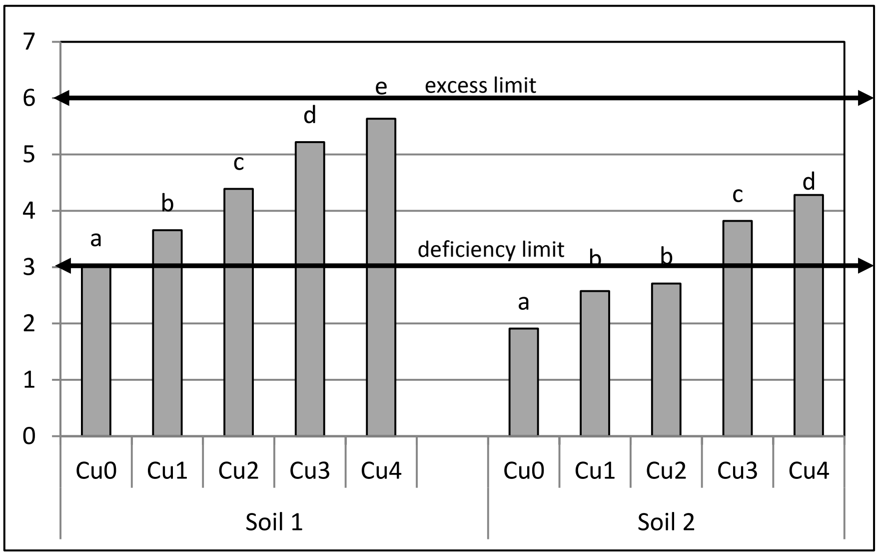

Fig. 2. Copper concentration in wheat grain (mg • kg^{-1})

Earlier studies of these authors showed that doses in foliar fertilization are too small to cause Cu increase in the grain [Korzeniowska and Stanisławska 2011]. In the soils with copper deficiencies, fertilization causes an increase in yields, which in the case of insufficient doses leads to the dilution. It prevents the increase in copper content in the grain.

In the world literature, there is a lack of research on biofortification of grain with copper. This is most likely the result of a rare deficiency of this element in the diet of people from other regions of the world. Many authors investigate the problem of grain biofortification with zinc [Hussain et al. 2012, McGrath et al. 2012, Phattarakul et al. 2012], iron [Aciksoz et al. 2011] and selenium [Lyons et al. 2005].

4. SUMMARY

Soil fertilization with copper can significantly increase the content of that element in the grain of wheat in the case of its deficiencies. The soil with higher pH and organic matter content requires the use of higher doses of copper than poorer and more acidic soils. Due to the small difference between deficiency and excess of copper in the grain the possibility of Cu excess must be taken under consideration in the case of its inappropriate doses. In particular, it concerns the soils with low organic carbon content. Biofortification of wheat grain with copper through soil fertilization can be effective; however, it requires a precise determination of the dose of fertilizer depending on the type of soil under the conditions of field experiments.

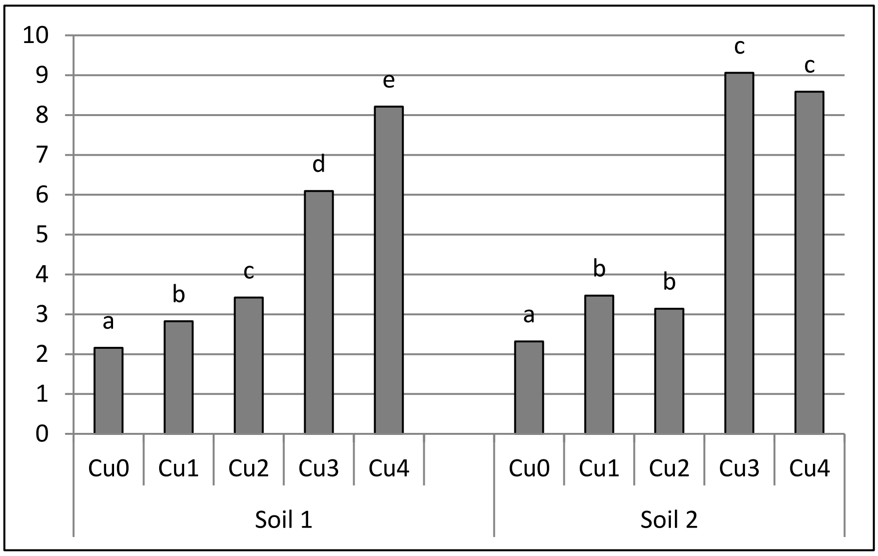

Fig. 3. Available copper concentration in soil after harvest (mg•kg^{-1})

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