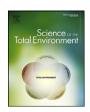
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The assessment of soil availability and wheat grain status of zinc and iron in Serbia: Implications for human nutrition



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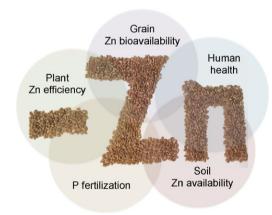
HIGHLIGHTS

- The first report of Zn and Fe status in soils, wheat grain and flour in Serbia
 No Fe deficient soils; 13% of soil sam-
- ples are Zn deficient
 Grain and flour levels of Zn and Fe are
- low and inadequate.

 Excessive P fertilization of calcareous
- soils further decreases grain Zn.

 A high risk of latent Zn deficiency in hu
- A high risk of latent Zn deficiency in human nutrition is expected.

GRAPHICAL ABSTRACT



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ABSTRACT

The deficiency of zinc (Zn) and iron (Fe) is a global issue causing not only considerable yield losses of food crops but also serious health problems. We have analysed Zn and Fe concentrations in the grains of two bread wheat cultivars along native gradient of micronutrient availability throughout Serbia. Although only 13% of the soil samples were Zn deficient and none was Fe deficient, the levels of these micronutrients in grain were rather low (median values of 21 mg kg $^{-1}$ for Zn and 36 mg kg $^{-1}$ for Fe), and even less adequate in white flour. Moreover, excessive P fertilization of calcareous soils in the major wheat growing areas strongly correlated with lower grain concentration of Zn. Our results imply that a latent Zn deficiency in wheat grain poses a high risk for grain quality relevant to human health in Serbia, where wheat bread is a staple food.

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1. Introduction

Zinc (Zn) and iron (Fe) are essential microelements for plants and also important nutritional and health factor in humans. Deficiency of

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these two microelements is widespread in humans, affecting nearly half of the world population (Hotz and Brown, 2004; Welch and Graham, 2004). Inadequate intake of Fe is well-documented to cause anaemia (Hotz and Brown, 2004), while Zn deficiency has been estimated to be responsible for approximately 4% of the worldwide cases of morbidity and mortality in young children, and a loss of nearly 16 million global disability-adjusted life years (Gibson, 2012). Zinc malnutrition is manifested in severe health complications, including impairments of physical growth, immune system, brain function and learning ability, DNA damage and cancer development. Zinc cannot be stored in human body, and, unlike other micronutrients, the major factor associated with development of Zn deficiency is inadequate dietary intake. Deficiency of plant available fractions of Zn and Fe is widespread in arable soils of semiarid regions and their bioavailability is strongly affected by high soil pH, presence of bicarbonates and drought (for review see Römheld and Nikolic, 2006; Alloway, 2008). Moreover, high application of phosphorus (P) fertilizers can exacerbate Zn deficiency in crops (Loneragan and Webb, 1993). Nearly 50% of the cereal-grown areas in the world have soils with low plant availability of Zn and Fe (Cakmak et al., 2004; Cakmak, 2008). These soils are mostly distributed in lowincome developing countries, where wheat provides even up to 70% of the daily calorie intake (Cakmak et al., 2004; Hotz and Brown, 2004). In general, wheat grains have inherently low levels of Zn and Fe, and most of these micronutrients are lost during processing to white flour. Moreover, wheat is rich in the compounds that further decrease availability of Zn and Fe in a human body, such as phytate and fibres (Welch and Graham, 2004; Zhang et al., 2010; Wessells et al., 2012; Kumssa et al., 2015). As a consequence of poverty-driven cereal based diet, Zn and Fe deficiency rank as the 5th and 6th major causes of illness and diseases in many low-income countries (Cakmak, 2008). Deficiency of Zn and Fe in staple food crops is globally recognized not only as a problem of reduced yields, but also as a severe public health problem with considerable societal costs (Rengel et al., 1999; Cakmak et al., 2004; Cakmak, 2008; Hotz and Brown, 2004). Increasing the bioavailable concentrations of Fe, and particularly of Zn in edible parts of field crops has become a national priority strategy in many countries (e.g. Cakmak et al., 1999, Cakmak, 2009; Rengel et al., 1999), and biofortification of staple food with these micronutrients is being recognized as a global challenge for plant breeders, agronomists and policy makers (e.g. White and Broadley, 2005; Graham et al., 2007; Cakmak, 2008; Palmgren et al., 2008; Prasad et al., 2014; Velu et al., 2014).

In Serbia, however, the public awareness on this issue is still lacking. Wheat is the staple food and the second most important crop in terms of cultivated area and total production. The average annual per capita consumption of wheat bread made of white flour is about 106 kg (Statistical Office of Serbia, 2015), what is three times more than the average consumption in the EU. The average grain yield of wheat in the country is rather low, under 5 t ha^{-1} (Statistical Office of Serbia, 2015). About two thirds of the arable soil area in Serbia is moderately acidic, while calcareous soils prevail in the northern part of the country (flatland of the Vojvodina Province; Manojlovic and Singh, 2012), where the levels of inputs of mineral fertilizers in wheat production are the highest. However, despite the importance of wheat in the diet of the vast majority of the population, no systematic survey of the availability of Zn and Fe in soils and in wheat grains has been undertaken in Serbia so far. The present study is the first report of the status of micronutrients Zn and Fe in soils, grains and flour throughout the major bread wheat production areas in the country.

2. Materials and methods

2.1. Wheat grain sampling and growing conditions

We sampled grains of the two new high-yielding winter bread wheat (*Triticum aestivum* L.) cultivars Simonida and NS 40S throughout the major cereal producing regions in Serbia (Fig. 1). Both examined

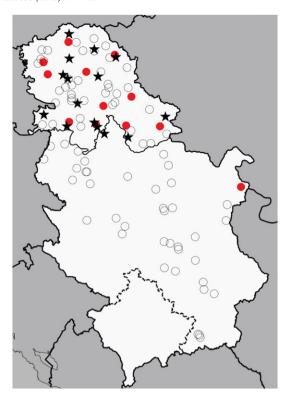


Fig. 1. Location of the sampling sites. The study included 157 samples of bread wheat grain from 93 locations throughout Serbia (circles) and 15 flour samples from the major wheat producing region of Vojvodina (stars). Soil samples were Zn deficiency was detected ($<0.5~{\rm mg~kg^{-1}}$ of DTPA-extractable Zn) are shown as filled circles.

cultivars, released by the Institute of Field and Vegetable Crops (Novi Sad, Serbia) in the last decade, are becoming very popular with the Serbian farmers and their use is increasingly growing, The study included 157 samples from 93 locations throughout Serbia (Fig. 1); 51 locations were in Vojvodina (the region accounting for about 52% of the wheat growing area in the country, Statistical Office of Serbia, 2015), and 42 were distributed through the rest of the country. All the surveyed fields, as demonstration test plots of the Institute of Field and Vegetable Crops, had comparable management, i.e. no irrigation, similar weed control, autumn fertilization with 100–400 kg ha⁻¹ of NPK (15:15:15) followed by spring addition of 60–100 kg N ha⁻¹, and no application of micronutrient-based fertilizers. The grain samples were collected at harvest 2013.

2.2. Flour sampling

In this study we also analysed samples of commercially available white wheat flour from the major wheat production region in Serbia (Vojvodina). About 60% of wheat in the country is produced in Vojvodina, with the average grain yields by about 30% higher than in the rest of the country (Statistical Office of Serbia, 2015). Commercially available flour is a composite of white flour obtained from different bread wheat cultivars (including Simonida and NS 40S) grown in the area near the mill. This work included white wheat flour from 15 major mills distributed throughout the Vojvodina Province (Fig. 1).

2.3. Grain and flour analysis

Grains of the two wheat cultivars were cleaned from glumes, awns and palae, washed with deionized water, air dried at 70 $^{\circ}$ C and grinded. Grain and flour samples were digested with a mixture of conc. HNO3 and H2O2 (3:2) in a microwave oven (Speedwave MWS-3+; Berghof Products + Instruments GmbH, Eningen, Germany) and the samples were subjected to multi-elemental analyses by inductively coupled

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