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## MACRONUTRIENTS CONTENT IN WHEAT STRAW AND ITS YIELD AS AFFECTED BY BACTERIAL AND MINERAL FERTILIZATION

Aleksandra STANOJKOVIĆ-SEBIĆ<sup>1\*</sup>, Dragutin A. ĐUKIĆ<sup>2</sup>, Leka MANDIĆ<sup>2</sup>, Violeta MANDIĆ<sup>3</sup>, Aleksandar STANOJKOVIĆ<sup>3</sup>, Radmila PIVIĆ<sup>1</sup>

<sup>1</sup>Department for Soil Reclamation, Institute of Soil Science, Belgrade, Serbia

<sup>2</sup>University of Kragujevac, Faculty of Agronomy, Čačak, Serbia

<sup>3</sup>Institute for Animal Husbandry, Belgrade - Zemun, Serbia

\*Corresponding author: astanojkovic@yahoo.com

### Abstract

Regardless of their major role in crop productivity, increased and long-term use of mineral fertilizers in agricultural production has raised concerns causing numerous environmental problems. This could be overcome by partial replacement of mineral fertilizers using bacterial inoculants, which could lead to the improvement of physical, chemical and biological soil properties, as well as help to ensure that the supply of nutrients contributing to optimized yield of crops. The study evaluated the effect of application of different rates of composite mineral fertilizers and their combination with bacterial inoculants (N-fixing *Klebsiella planticola* and *Enterobacter* spp.) on macronutrients content in straw of winter wheat and its yield. Unfertilized treatment was used as a control. The contents of nitrogen, phosphorus, potassium and crude proteins in plant samples were determined at the beginning of tillering and in full grain maturity stage, at the end of the wheat vegetation. Measuring of the straw yield was carried out at the end of the vegetation. The results of the study showed that the use of high rates of composite mineral fertilizers and their combination with bacterial inoculants resulted in increased contents of nitrogen, phosphorus, potassium and crude proteins in the wheat straw, which was noticeably observed in the stage of tillering. The highest increase in the straw yield was obtained by the same mentioned treatments, although the combination of bacterial inoculants and low rates of mineral fertilizers resulted in higher yields comparing to the use of solely low rates of mineral nutrients.

**Keywords:** *Wheat straw, yield, chemical composition, bacterial inoculants, composite mineral fertilizers.*

### Introduction

Wheat (*Triticum aestivum* L.) is grown on every continent thanks to the large polymorphism. Along with maize, wheat represents a major crop in agricultural production in Serbia, where its cultivation in the period 2000-2009 occupied an average area of 600,171 ha with an average grain yield of 3.47 t ha<sup>-1</sup> (Jocković *et al.*, 2010; Mladenov *et al.*, 2011). The yield and quality of wheat grain depend mainly on the balanced mineral nutrition, which is correlated with the type of soil, climate factors in the region and other agroecological factors. Nevertheless, creating an optimal conditions for growing wheat in order to achieve higher grain yields is primarily related to application of nitrogen fertilizers (Đekić *et al.*, 2017). Similar could be said for the wheat straw, whose yield, besides mineral nutrition, depends on variety, soil and climate conditions. Wheat straw is the agricultural product obtained from different parts of wheat plant like stem, leaves etc., after the grain and chaff have been removed. Straw makes up about half of the yield of wheat. It has many uses, primarily as fuel, livestock bedding and fodder. An accurate content of macro and micronutrients in wheat straw can vary from cultivar to cultivar, stages of plant growth, climatic conditions and the nature of soil and fertilizers used (Safdar *et al.*, 2009; Yasin *et al.*, 2010; Saleem Khan and Mubeen, 2012).

Regardless of their major role in soil fertility and wheat productivity, increased use of mineral fertilizers in agricultural production can cause environmental contamination (Acosta-Martinez and Tabatabai, 2000; Alizadeh and Ghadei, 2006). The problems concerned can be overcome by partial replacement of these fertilizers by application of microbial inoculants, in order to inhibit or stimulate certain cellular processes, including mineralization ones, thus leading to the improvement of physical, chemical and biological soil properties (Milošević *et al.*, 2003; Pešaković *et al.*, 2008). Regarding the preceding comments, the main purpose of this study was to evaluate the effect of different rates of composite mineral NPK fertilizers (N:P:K=15:15:15) and their combination with selected bacterial inoculants on macronutrients content in wheat straw and its yield.

### Material and methods

The study was conducted on Mladenovac experimental station of Institute of Soil Science, located about 55 km south-east from Belgrade in Serbia, in 2006/2007. Average monthly air temperatures and precipitation sums for the investigated period along with multi-annual average (1990-2007) are presented in Table 1. Monthly air temperatures during the vegetation period of wheat (October 2006 - July 2007) were considerably higher in comparison with multi-annual average. The distribution of precipitation from the period January - June in 2007 was favorable, except in April, when it was measured only 3.8 mm of precipitation.

Table 1. Average monthly air temperatures and precipitation sum for the period 2006/2007 and multi-annual average.

Month	Year				Average (1990-2007)	
	2006	2007	2006	2007		
	Temperature (°C)		Precipitation (mm)		Temperature (°C)	Precipitation (mm)
January	-0.5	7.6	43.2	49.3	1.7	41.8
February	1.9	7.2	59.1	56.0	3.5	38.4
March	6.5	10.2	104.4	99.6	7.9	40.8
April	13.7	14.9	97.0	3.8	12.7	55.7
May	17.4	19.5	42.3	79.0	18.1	50.8
June	20.2	23.8	137.8	107.6	21.5	97.7
July	24.7	25.8	23.3	17.5	23.2	66.8
August	20.9	24.2	120.6	72.5	23.1	60.9
September	19.2	16.2	24.3	84.1	17.6	63.5
October	15.2	11.8	20.9	103.6	13.0	55.8
November	8.9	5.2	24.5	131.5	7.3	55.9
December	4.3	1.1	51.9	34.5	2.2	60.5
Average	12.8	14.0	-	-	12.7	-
Total	-	-	749.3	839.0	-	688.6

The studied type of soil was Eutric Cambisol (WRB, 2014). The experiment was set up in a randomized block design on 9 x 6 m<sup>2</sup> plot size, with three replications, based on the following variants: control (Ø, non-fertilized soil); 60 kg ha<sup>-1</sup> N and P<sub>2</sub>O<sub>5</sub>, and 40 kg K<sub>2</sub>O ha<sup>-1</sup> (N1); 120 kg ha<sup>-1</sup> N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O (N2); *Enterobacter* sp. strains + 60 kg ha<sup>-1</sup> N and P<sub>2</sub>O<sub>5</sub>, and 40 kg K<sub>2</sub>O ha<sup>-1</sup> (ES+N1); *Enterobacter* sp. strains + 120 kg ha<sup>-1</sup> N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O (ES+N2); *Klebsiella planticola* + 60 kg ha<sup>-1</sup> N and P<sub>2</sub>O<sub>5</sub>, and 40 kg K<sub>2</sub>O ha<sup>-1</sup> (KP+N1); *Klebsiella planticola* + 120 kg ha<sup>-1</sup> N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O (KP+N2). Winter wheat (cv. Evropa 90) was used as a test plant in the trial and sown and harvested in optimal terms (1<sup>st</sup> decade of November in 2006, 3<sup>rd</sup> decade of June in 2007, respectively). Mineral fertilization was carried out manually for each experimental plot using composite NPK fertilizer in relation N:P:K = 15:15:15. Nitrogen (N) fertilizer was applied in the form of urea with 46% N, phosphorus (P) - in the

form of monoammonium phosphate (MAP) with 52%  $P_2O_5$  and 11% N, and potassium (K) - as a 40% potassium salt (potassium chloride, KCl). The established amounts of potassium and 1/3 of nitrogen fertilizer were applied in the fall 2006 before sowing, and the remaining 2/3 of nitrogen fertilizer was applied during March in 2007 at the stage of tillering. The procedure for obtaining the bacterial inoculums for fertilization was previously described in study of Stanojković *et al.* (2012). Bacterial fertilization was carried out using plastic haversack sprinkler with  $300.00\text{ cm}^3\text{ m}^{-2}$  of diluted liquid bacterial inoculums made from the pure cultures of associative N-fixing bacteria *Klebsiella planticola* (strain TSHA-91) and *Enterobacter* strains KG-75 and KG-76 and tap water. The first soil and foliar fertilization with bacterial inoculums was carried out in the stage of 2-3 formed leaves, and the second - in the tillering stage, a few days after fertilizing with mineral nitrogen. *K. planticola* was obtained from the stock culture of the Microbiology Laboratory of Faculty of Agronomy (Čačak, Serbia) and cultivated for 24 h at  $28^\circ\text{C} \pm 1$  on the slanting nutrient medium, while *Enterobacter* strains were obtained from the stock culture of the Microbiology Laboratory of the Center for Small Grains (Kragujevac, Serbia), where they have been isolated from the rhizosphere of wheat. These strains were cultivated for 48 h at  $28^\circ\text{C} \pm 1$  on the slanting nutrient medium. The preliminary analysis of the study soil, after the samples were air-dried and passed through a sieve, included the following chemical parameters: soil acidity (pH in  $H_2O$  and 1M KCl, v/v - soil: $H_2O$ =1:5, soil:1M KCl=1:5) was analyzed potentiometrically, using glass electrode (SRPS ISO 10390, 2007); total nitrogen (N) was analyzed on elemental CNS analyzer Vario EL III (Nelson and Sommers, 1996); available phosphorus ( $P_2O_5$ ) and potassium ( $K_2O$ ) were analyzed by Al-method according to Egner-Riehm (Riehm, 1958), where  $K_2O$  was determined by flame emission photometry and  $P_2O_5$  by spectrophotometer after color development with ammonium molybdate and stannous chloride; humus content was determined using Tiurin's method, modified by Simakov (Ostrowska *et al.*, 1991). The yield of wheat straw without grain and chaff from each experimental plot was measured and determined directly on plots at the end of the vegetation and calculated into  $t\text{ ha}^{-1}$ . The content of macronutrients in wheat samples was determined at the beginning of tillering (vegetation stage I, S1) and at the end of the vegetation period (vegetation stage II, S2). The content of phosphorus (P) and potassium (K) was determined by so called "wet" combustion, i.e. they were heated to boiling with the mixture of concentrated sulfuric ( $H_2SO_4$ ) and perchloric ( $HClO_4$ ) acids. In the obtained solution, P was determined by spectrophotometer with molybdate, and K - by flame emission photometry (Jakovljević *et al.*, 1985). The content of nitrogen (N) was analyzed using elemental CNS analyzer, Vario model EL III (Nelson and Sommers, 1996), while the content of crude proteins was calculated on the basis of N content according to Licitra *et al.* (1996), using the following formula: crude proteins (%) = N (%) x 6.25 (factor for conversion of nitrogen content to crude protein). The obtained data on soil properties were presented as arithmetic means of three replicates, standard deviation values and intervals. The effects of different fertilization treatments on chemical parameters tested and yield were evaluated using ANOVA (SPSS 20.0, Chicago, USA), followed by Duncan's Multiple Range Test (DMRT). Significant differences between means were tested by the LSD test at  $P = 0.05$  and  $P = 0.01$ .

### Results and discussions

The main chemical characteristics of the study soil are presented in Table 2. According to the reference values (Šestić *et al.*, 1969), the soil is characterized by very acid reaction with pH in 1M KCl 4.06, high available potassium, medium to high available phosphorus, and medium humus and total nitrogen supply.

Table 2. Main chemical characteristics of the studied Eutric Cambisol

Chemical parameter	Value (means $\pm$ standard deviation)	Intervals
pH in H <sub>2</sub> O	4.90 $\pm$ 0.03	4.87-4.92
pH in 1M KCl	4.06 $\pm$ 0.05	4.00-4.10
Total N (%)	0.136 $\pm$ 0.005	0.132-0.141
Humus (%)	2.19 $\pm$ 0.01	2.18-2.19
Available P <sub>2</sub> O <sub>5</sub> (mg 100g <sup>-1</sup> )	15.73 $\pm$ 0.31	15.51-16.09
Available K <sub>2</sub> O (mg 100g <sup>-1</sup> )	25.30 $\pm$ 0.30	25.08-25.65

By analyzing the content of macronutrients in plant material at the end of the wheat vegetation period it was determined that the content of total nitrogen, phosphorus, potassium and proteins significantly depended on the fertilization treatment applied (Table 3).

Table 3. Effect of the fertilization treatments on macronutrients content in wheat straw and its yield (in t ha<sup>-1</sup>).

Treatments	Total N (%)		P <sub>2</sub> O <sub>5</sub> (%)		K <sub>2</sub> O (%)		Proteins (%)		Yield
	Vegetation stage								
	S1	S2	S1	S2	S1	S2	S1	S2	S2
Ø	0.72± 0.007g	0.52± 0.005g	0.75± 0.003g	0.44± 0.003f	2.15± 0.003e	0.86± 0.009f	4.48± 0.041g	3.27± 0.028g	1.78± 0.012f
N1	0.85± 0.006f	0.63± 0.004f	0.89± 0.004f	0.58± 0.004e	2.34± 0.008d	1.19± 0.003e	5.29± 0.034f	3.90± 0.024f	3.96± 0.004e
N2	2.26± 0.005c	0.79± 0.006d	0.99± 0.004c	0.69± 0.005c	2.97± 0.057b	1.59± 0.001b	14.15± 0.031c	4.98± 0.034d	5.55± 0.285b
ES+N1	2.02± 0.005d	0.66± 0.003e	0.92± 0.004d	0.60± 0.003d	2.68± 0.009c	1.31± 0.005c	12.59± 0.029d	4.14± 0.019e	4.95± 0.003c
ES+N2	2.84± 0.004a	1.43± 0.003a	1.09± 0.001a	0.78± 0.003a	3.04± 0.005a	1.62± 0.016a	17.73± 0.025a	8.86± 0.161a	6.43± 0.004a
KP+N1	1.63± 0.006e	0.89± 0.006c	0.91± 0.004e	0.60± 0.003d	2.65± 0.006c	1.28± 0.009d	10.16± 0.038e	5.54± 0.039c	4.43± 0.007d
KP+N2	2.37± 0.007b	0.98± 0.003b	1.02± 0.002b	0.71± 0.002b	3.07± 0.010a	1.62± 0.003a	14.82± 0.041b	6.11± 0.021b	6.48± 0.005a
P value	***	***	***	***	***	***	***	***	***
LSD (0.05)	0.009	0.009	0.005	0.006	0.039	0.014	0.061	0.116	0.188
LSD (0.01)	0.013	0.013	0.007	0.008	0.054	0.020	0.080	0.160	0.262

LSD - least significant differences at P=0.05 and P=0.01; \*\*\* indicates statistical significant differences at the P<0.05, P<0.01 and P<0.001 levels, respectively; DMRT was used to compare different variants at P $\leq$ 0.05, where values followed by the same letter in a column are not significantly different.

Application of high rates of mineral NPK fertilizers and their combination with bacterial inoculants has caused a significant increase in the share of nitrogen, phosphorus, potassium and crude proteins in the plant material compared to the other tested variants, which was noticeably observed in the stage of tillering, the vegetation period in which the plant growth and accumulation of nutrients are the most intensive (Čurić, 1982). This excess of microbiologically fixed nitrogen along with higher amounts of mineral nitrogen influenced positively on the accumulation of macronutrients in the study plant material, which is in accordance with previous researches (El-Sirafy *et al.*, 2006; Behera and Rautaray, 2010).

The present study also indicated a little increase in the share of macronutrients in plant material in variants where microbial inoculation of soil and plant along with low rates of mineral NPK fertilizers was applied comparing to the application of solely low rates of mineral fertilizers. Similar results were obtained in the study of Cvijanović *et al.* (2007). The analysis of the yield of wheat straw (based on Duncan's test) showed highly significant yield differences between the applied fertilization treatments (Table 3). The highest increase in

yield was obtained by combined application of bacterial inoculants used and high rates of mineral NPK fertilizers. This was also observed in treatments with combined usage of bacterial inoculants and low rates of mineral NPK fertilizers where it was obtained higher yield comparing to the treatments with solely low rates of mineral fertilizers. Behera and Rautaray (2010) also found higher straw yields in biofertilizers + 50% NPK treatments than in treatments with solely 50% NPK. According to Josipović *et al.* (2005), the character of the applied fertilizers effects on the yield of crops also depends on the weather conditions specific to each year. It is assumed that good distribution of rainfall and temperature in the period November 2006 - June 2007 contributed largely to the achieved yields of wheat straw in the investigated agro-ecological conditions.

### Conclusion

The present study demonstrated the significant positive effects of combined application of bacterial inoculants studied (*Klebsiella planticola* and *Enterobacter* spp.) and high and low rates of the composite mineral fertilizers on the yield of wheat straw (without grain and chaff). The same results were obtained regarding the effects of the mentioned applied combinations on the contents of total nitrogen, phosphorus, potassium and crude proteins in the analyzed plant material, which was noticeably observed in the stage of tillering. These data suggest that the studied bacterial inoculants can be used in further studies as the potential agents of new biofertilizers for improved wheat production and other agriculture crops.

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