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*Full Length Research Paper*

# Liming increases alfalfa yield and crude protein content in an acidic silty loam soil

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**A three year field trial examined the effect of three hydrated lime (HL, $Ca(OH)_2$) rates (0, 1.5 and 3 Mg HL $ha^{-1}$) on yield and quality characteristics of alfalfa on an acid soil ($pH_{water\ 1:2.5}$ 4.7) in Western Serbia. Lime was applied only once. Total dry mass yields of a new planting alfalfa (*Medicago sativa* L.) increased up to 6500% by the treatments of HL, compared with the untreated control. Lime application significantly ($P \leq 0.05$) increased the crude protein content of alfalfa, in comparison to the no limed control. Liming also significantly increased Ca, K, and P concentrations but decreased Mg, Fe, Mn, Zn, and Al concentrations in alfalfa tissue, compared with the control treatment. Alfalfa yield increase was attributed to the increase of Ca, P and K uptake. Furthermore, the low (1.5 Mg HL $ha^{-1}$) and high (3 Mg HL $ha^{-1}$) lime treatments increased soil pH even further by 0.9 and 1.5 pH units, respectively. The results suggest that an initial application of hydrated lime at a rate of 3 Mg HL $ha^{-1}$ may ameliorate soil acidity and increase the yield and quality characteristics of alfalfa at least over a 3-year period.**

**Key words:** Alfalfa, soil acidity, liming, herbage yield, elemental composition.

## INTRODUCTION

Soil acidity is a major factor that affects the growth of many crops throughout the world. In the Republic of Serbia, soil acidity is a serious problem because nearly three million hectares of agricultural land (approximately 60% of total arable land) is estimated to be extremely to moderately acidic ($H_2O$ pH 4.5 to 6.0). Silty loam planosol is a typical type of acid soils, predominating in both Serbia and the wider region. It occupies a surface area of >500,000 ha (approximately 18% of the total arable land of Serbia). This soils present considerable management problems. Soils with high silt content have properties which often cause soil structure problems such as surface crust formation at plant establishment. Planosols in West Serbia are often low in organic matter and poorly drained. Furthermore, aluminium toxicity and phosphorus deficiency are common problems. The very low pH is a limiting factor for the yield formation of crops on some acidic planosols in Serbia (Gajić et al., 2007). In acid soils (pH < 5.5), there is a need for liming to increase soil pH and, hence, create a suitable environment for the nutrient uptake by the plant. Many silt loam acidic planosols in West Serbia require application of more than just NPK fertilizers and liming, and their low fertility level may prove difficult to correct.

Soil pH decreases with time due to rainfall, leaching, organic matter decay, harvest of crops and surface-applied acidic N fertiliser, especially in no-till systems (Li et al., 2001). In acid soils, poor plant growth may result from phytotoxic substances such as soluble Al and Mn, nutrient deficiencies (P, Ca and Mg), and reduced uptake

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**Abbreviations: HL,** Hydrated lime [HL, $Ca(OH)_2$]; **CP,** crude protein.

**Table 1**. Selected characteristics of the arable layer (0 to 25 cm) of the soil at the start of the study.

| Property | Value |
|---|---|
| Sand (2-0.05 mm) (%) | 11 |
| Silt (0.05-0.002 mm) (%) | 67 |
| Clay (<0.002 mm) (%) | 22 |
| Soil texture | Silty loam |
| pH (1:2.5 soil/water) | 4.7 |
| Organic matter C (%) | 1.64 |
| Total N (%) | 0.184 |
| C/N | 8.7:1 |
| Available $P_2O_5$ (mg $kg^{-1}$) | 65 |
| Available $K_2O$ (mg $kg^{-1}$) | 79 |
| Exchangeable Al (cmol$^+$ $kg^{-1}$) | 2.1 |
| CEC (cmol$^+$ $kg^{-1}$) | 10.8 |

CEC: cation exchange capacity.

of nutrients (Awadi et al., 1976; Beckie and Ukrainetz, 1996).

Liming has long been recognized as the main strategy of improving the productivity of acidic soils. Positive crop response to liming are due to the ameloration of one or more of the above-mentioned factors (Aitken et al., 1998; Materechera and Mkhabela, 2002; Prado et al., 2007; Álvarez et al., 2009). Grewal and Williams (2003) reported that liming of acid soils increased alfalfa yields. Alfalfa yields were increased by agricultural lime application in acidic silty loam soils in USA (Chen et al. (2001); but according to Raun et al. (1999), alfalfa yields were not affected by dolomitic limestone application in silty loam soils in Oklahoma. The differences in crops response to liming are likely to be due to the different soil and fertilization conditions, to type and rate of liming materials, and fertilization conditions to type and rate of liming materials, or to environmental and genetic (cultivars) factors. These findings suggest the need to improve the understanding of liming effects on crops yield and quality grown in acid soils.

According to Hauptvogel (2003), alfalfa is one of the crops most susceptible to soil acidity. The most suitable soils for alfalfa production supply sufficient nutrients and have a neutral to weakly alkaline pH with values ranging from 6.6 to 7.5 (Brauer et al., 2002; Peters et al., 2005). Therefore, the management of soil acidity is essential for successfully growing alfalfa on acid soils. With high biomass yield and nutrition, alfalfa is one of the most important forages in the world (Ren et al., 2010). Alfalfa has been used for centuries as an animal feedstuff and is gaining much attention as a human food component, consumed as a garnish, leaf protein concentrate, or food nutritional supplement (Stochmal et al., 2001). So, maximizing crude protein content is important in alfalfa production. The objective of this study was to identify how alafalfa responded to liming in an acidic silty loam soil in Western Serbia. Hydrated lime $Ca(OH)_2$, which is more effective and fast-acting than agricultural lime, was applied in this study because there is no local source of agricultural lime.

## MATERIALS AND METHODS

### Site description, soil properties and weather conditions

The study was carried out over a 3-year period (April 2008 to September 2010) on an acid soil in Krnule, West Serbia (44° 34' N, 19° 46' E, altitude 170 m a.s.l). The soil parent material was quaternary sediment, and the soil is classified as a planosol (FAO, 2006). The soil is typical to those used by the majority of small-scale farmers. Its limiting factors to crop production are principally P deficiency and acidity. The main general parameters of the arable layer are shown in Table 1. The region had a 20 year average annual rainfall of 719 mm, and the mean annual air temperature of 12°C. The basic meteorological data during the growing season (April to September) in the three years of experimentation are shown in Figure 1.

### Experimental design, treatment application and plant culture

The field experimental design was a randomized complete block with three replications. The size of a basic plot was 10.0 $m^2$ (5 × 2 m). Treatments consisted of three rates of lime application (0 to control plots, 1.5 and 3 Mg of HL $ha^{-1}$). Alfalfa (cultivar "NS Banat ZMS II") seed was inoculated with *Rhizobium meliloti* just before sowing, which is the normal procedure used in sowing alfalfa on farms in West Serbia for effective nodulation. Seed was broadcasted in rows at 0.5 to 1 cm depth, with a space of 0.20 m between rows. Seeding rate was 18 kg $ha^{-1}$. Date of sowing was 6th April, 2008. The field was previously in a corn-wheat rotation with wheat grown in 2007. The liming material was hydrated lime ($Ca(OH)_2$), with 99% of particles of < 0.42 mm and a neutralizing value (or calcium carbonate equivalence, CCE) of 136%. Hydrated lime powder was applied to soil by uniform surface application only once, in October 2007, and incorporated to a 20 cm depth. All plots received 400 kg $ha^{-1}$ of NPK fertilizer (15:15:15) at sowing. To compensate for the relatively low organic matter content of these soils, nitrogen (N) fertilizer was applied at the time of seeding to provide supplemental nitrogen to seedling alfalfa until nodulation. No fertilizer was applied after sowing. Annual grasses and broadleaf weeds were controlled with imazethapyr 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridine carboxylate at a rate of 0.1 kg $ha^{-1}$. *Phytodecta fornicata*, *Phytonomus* spp., and *Halticinae* were controlled with fenitrothion O,O-Dimethyl O-4-nitro-m-tolyl-phosphorothioate at a rate 0.75 kg $ha^{-1}$.

### Soil sampling and analysis

Before the application of hydrated lime (HL), a composite soil sample consisting of 25 cores was taken with a 2.5 cm diameter soil probe, to a 20 cm depth across the experimental area to determine particle size distribution, pH, soil organic mater content, plant available P, plant available K, and total N (Table 1). After applying HL, five soil cores (2.5 cm diameter) from the upper 20 cm were collected from the experimental plots to determine pH in distilled water (1:2.5 soil : water slurry). The cores from the five sampling points were mixed thoroughly to provide a single composite sample per plot. After visible plant materials were remowed, all soil samples were air-dried at room temperature (21°C), and ground to pass

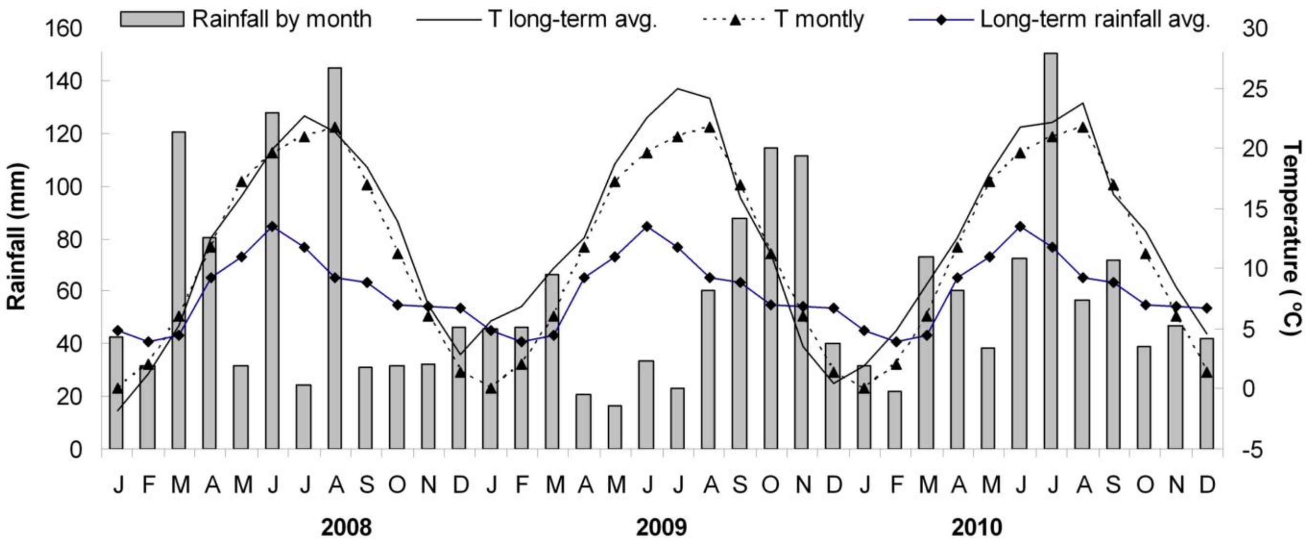


**Figure 1.** Mean air temperatures and rainfall by month for 2008, 2009, and 2010, along with the long-term (25 years) average temperatures and rainfall at Krnule, Western Serbia.

through a 2 mm mesh, and physical and chemical soil properties were measured. Soil analyses were carried out by using the standard methods of the soil testing laboratory (van Reeuwijk, 1995), except that plant available P and plant available K were determined by the method of a Egnér et al. (1960) after extraction with acid ammonium lactate.

#### Plant harvest and analysis

Alfalfa was harvested three times in 2008 and four times in 2009 and 2010; because of financial constraints, the second harvest of the post-seeding year were analyzed for Ca, P, K, Mg, Fe, Mn, Zn, and Al content, but samples for second harvest in 2008 to 2010 were used to analyze crude protein content. Yields were determined by clipping a randomly selected 1 $m^2$ area from each plot to a 5 cm stubble height when approximately 10% alfalfa bloomed. Alfalfa yields were determined on a dry mass basis. 1000 g subsample was dried at 60°C for 48 h and subsequently used for chemical laboratory analysis. Prior to chemical analysis, oven-dried forage samples (500 g) of each treatment were ground to pass a 0.75 mm screen using a shear mill (ZM100, Retsch). Forage samples of each treatment were analyzed for moisture (135°C for 2 h) and crude protein (Kjeldahl nitrogen × 6.25) according to the standard procedures of the Association of the Official Analytical Chemists (1990). To measure K, Ca, Mg, Zn, Fe, and Mn, subsamples of ground, dried herbage were digested according to the method of Nelson and Sommers (1980). The concentration of the elements in the digest was measured by atomic absorption spectroscopy. Plant phosphorus was analyzed colorimetrically using the vanadomolybdophosphoric yellow color method (Jackson, 1958).

#### Statistical analysis

The effects of lime treatment on nutrient concentration and alfalfa yield were assessed by analysis of variance (ANOVA) using the SAS package (SAS, 2001). The least significant difference (LSD) test was used to detect differences between means at probability level $P \leq 0.05$.

## RESULTS AND DISCUSSION

### Soil characteristics and rainfall

Average annual rainfall at this site was 719 mm, and the majority of the total (427 mm) was received during the growing season months of April to September. Rainfall from 2008 to 2010 was generaly lower than normal for this site, except for 2008 (745 mm). Early season precipitation in the seedling year was abundant for establishment of alfalfa. During the course of this experiment, rainfall departure from normal values for April through September were +26, -53, and -14 mm from 2008, 2009, and 2010, respectively. The differences in mean temperature among the 3 years were not substantial. Monthly average temperatures and monthly rainfall in the three years, and the long-term average rainfall are summarised in Figure 1. During the experimental period, soil pH increased for all treatments (Figure 2). As expected, this increase in pH was more pronounced for treatment with higher HL rate. Averaged across the years, soil pH significantly increased by 0.9 and 1.5 in the 1.5 and 3 Mg HL $ha^{-1}$ treatments respectively, in comparison to the un-amended control (pH 4.7). The treatment with the highest HL rates increased most rapidly, while the lowest treatment increased pH the least. Raun et al. (1999) reported optimal soil pH above 6.0 for alfalfa production.

### Dry matter herbage yield

Total dry mass yields of newly established alfalfa field for 2008 through 2010 harvest are presented in Figure 3. Increasing application rates of the HL significantly

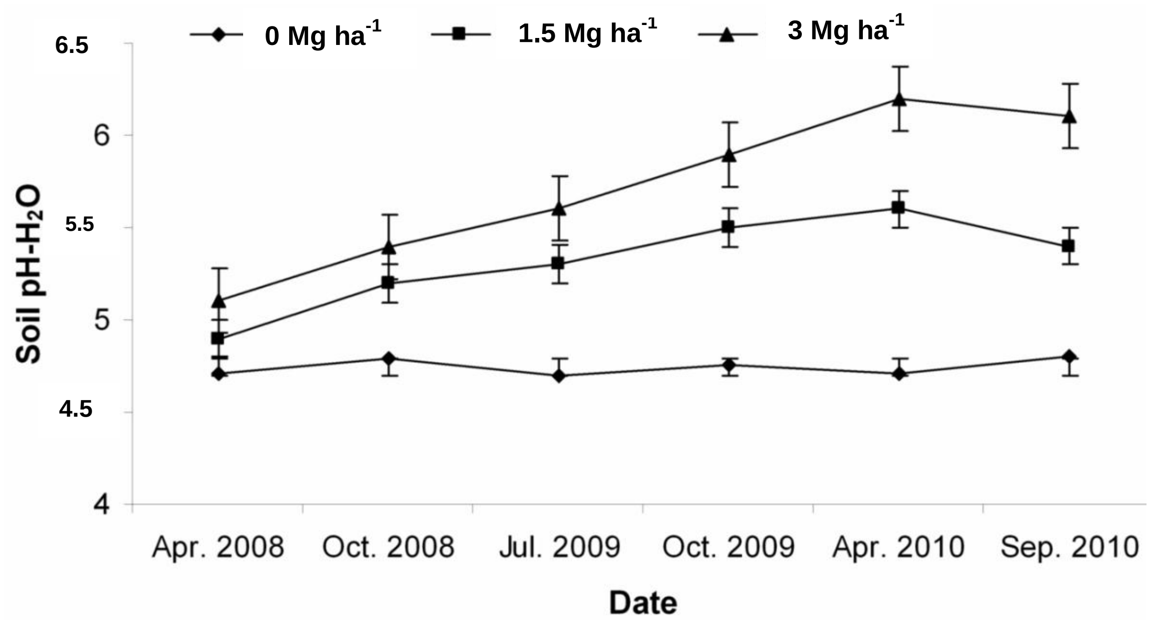


**Figure 2.** Soil pH changes over time for three lime treatments (Mg HL ha$^{-1}$).

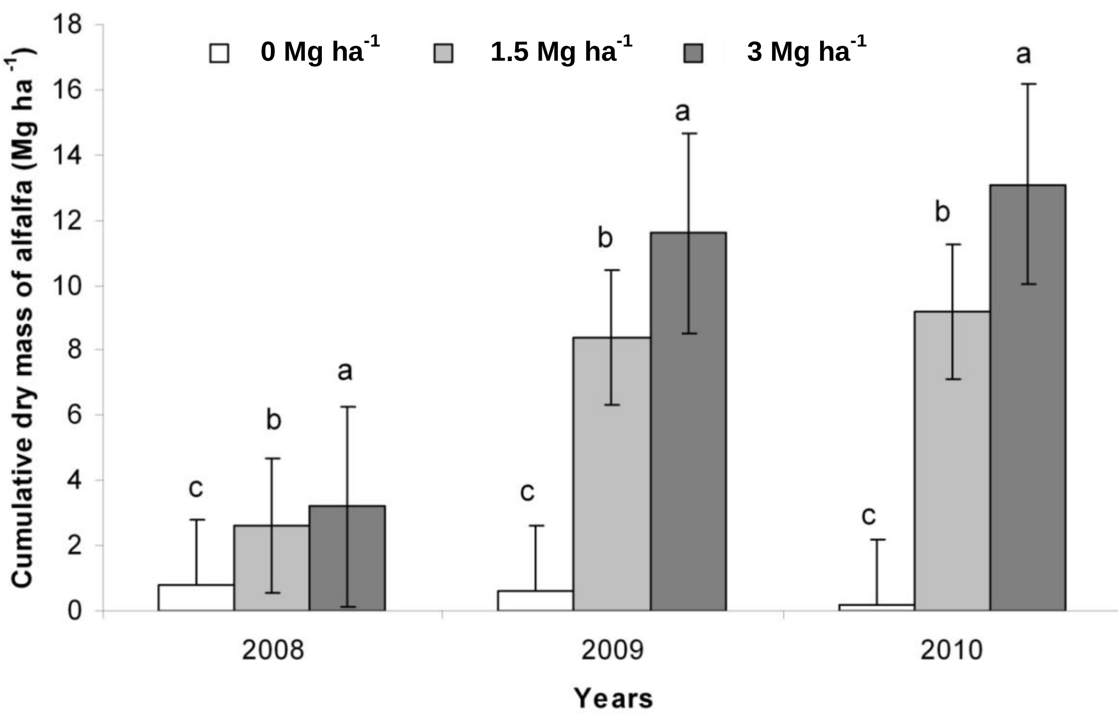


**Figure 3.** Effect of lime application amount on cumulative dry mass of alfalfa (obtained by combining all harvests in year). Different letters over each bar represent a significant difference at P ≤ 0.05.

increased mean alfalfa yields (P ≤ 0.05). The data show that the soil that received the highest rate of HL had the greatest yield (Figure 3). The response of alfalfa dry matter yield to liming was consistent and tended to increase in successive years. Greatest yields were obtained with the 3 Mg ha$^{-1}$ rate of HL (3.2, 11.6, and 13.1 Mg ha$^{-1}$, in 2008, 2009, and 2010, respectively). The increases in total alfalfa yields associated with the HL treatments were significantly greater than the control plots by 325 to 400% in 2008 and 1400 to 1933% in

**Table 2.** Effect of liming on crude protein (CP) concentrations.

| HL amount ($Mg\ ha^{-1}$) | Season | | |
|---|---|---|---|
| | 2008 (%) | 2009 (%) | 2010 (%) |
| 0 | 15.8±2.1[a] | 16.6± 0.5[c] | 16.0±0.9[c] |
| 1.5 | 19.1±1.9[ba] | 21.0±1.0[b] | 20.4±0.3[b] |
| 3.0 | 21.7±1.3[a] | 25.9±3.4[a] | 22.2±0.4[a] |

Within the column, data followed by different letters, differ significantly at P ≤ 0.05. *n* = 3.

2009. In 2010, the third year after treatment, alfalfa yields in plots treated with HL were approximately 4600 to 6550% greater, compared with the unamended control. Forage yield and response to lime significantly changed from year to year (Figure 3). The low first-year yield is somewhat typical because the plants had not fully developed their root systems. The considerably higher yields in 2010 may be attributed to more favorable climatic conditions in late spring and summer (that is, higher and more favorably distributed rainfall) (Figure 1), which reduced the traditional drought period in 2009 and limited alfalfa growth. These more favorable conditions allowed a positive response of alfalfa growth to HL addition (that is, higher dry mass yields with increasing HL rate). These results are in agreement with the studies of Grewal and Williams (2003), who applied lime at the rates of 2 Mg $ha^{-1}$ in brown sandy clay loam with a soil pH of 4.8 (1:5 calcium chloride) in Australia. They found that alfalfa yields were significantly (P ≤ 0.05) incresed by lime addition, but alfalfa cultivars differed markedly in response to lime application. Similarly, Chen et al. (2001) found that the alfalfa yield was increased by the addition of agricultural lime to an acidic soil (Wooster silt loam, USA). These results contradict with those in Oklahoma on a Grant silt loam soils (Raun et al., 1999) where dry masses of alfalfa were not affected by dolomitic limestone (4480 kg $ha^{-1}$) application. The reason why dry masses of alfalfa were not affected by dolomitic limestone application could be accounted for by possible differences in climate, soil and growth conditions, which might affect the chemical components and growth rate of the plants.

## Crude protein concentration in shoots

Applied HL increased crude protein in alfalfa. Averaged across years, crude protein levels was increased (P ≤ 0.05) by 21 to 56% in the treatments that received 1.5 and 3 Mg HL $ha^{-1}$, respectively, compared to the unamended treatments (Table 2). Increasing the application rate of the HL significantly increased crude protein level. Average crude protein concentration was greater in 2009 than in the other two years. A similar trend in increased crude protein of alfalfa was reported by Stout et al. (1997) in a field experiment that amended clayey Gleyed Gray Luvisol (Canada) with 2.3 to 5 Mg $CaCO_3$ $ha^{-1}$. In contrast, Raun et al. (1999) reported that the application of dolomitic limestone did not produce a significant response in protein concentration of alfalfa. This difference was possibly due to the different cultivars and different ecological conditions from their study.

## Concentration of nutrient elements in dried shoots

Liming significantly increased the calcium (Ca), phosphorus (P), and potassium (K) concentration of alfalfa shoots (Table 3). The plant essential elements in alfalfa tissue varied, depending on applied rate of HL. Concentrations of Ca in alfalfa were significantly increased by 33 to 48%, as liming quantity increased from 0 to 3 Mg HL $ha^{-1}$. These results are in agreement with those reported by Grewal and Williams (2003), but inconsistent with those of Chen et al. (2001), who found that concentrations of Ca in alfalfa were not significantly affected when soil was treated with agricultural limestone. Averaged across treatments, concentrations in alfalfa of P and K were affected by application of HL and significantly increased by 29 to 61% and 24 to 62%, respectively, when compared with the untreated control. Application of the higher level of HL appeared to have the best effect on P and K accumulation in alfalfa plants. In contrast to our results, Grewal and Williams (2003) reported that liming slightly improved P concentration of alfalfa tissue.

According to Whitehead (2000) and Nilsson (2003), low soil pH in combination with low CEC may result in low nutrient availability for plant production; because investigated soils are acidic, phosphorus fixation is dominated by Al and Fe compounds. Phosphorus availability is low in strongly acid soils because of the formation of Fe and Al phosphates, from which phosphorus is very slowly available to plants (Donahue et al., 1971). Phosphorus is an important plant nutrient, it is indispensable for phospholipids, nucleic acids synthesis and adenosine triphosphate (ATP) and therefore a deficiency can limit plant growth (Schachtman et al., 1998). Maintaining soil pH between 6 and 7 will generally result in the most efficient use of phosphate. Potassium is generally not a limiting element within agricultural systems, but it is a major nutrient for crop quality and yield in intensive grass or alfalfa production (Whitehead, 2000). Donahue et al. (1971) reported that liming acid

**Table 3.** Effect of liming on P, K, Ca and Mg concentrations of alfalfa at second cutting of 2009.

| HL amount ($mg\ ha^{-1}$) | P | K | Ca | Mg |
|---|---|---|---|---|
| | $g\ kg^{-1}$ on dry matter basis | | | |
| 0 (control) | 2.8±4.4[c] | 18.4±0.3[c] | 8.9±2.4[c] | 3.2±2.0[c] |
| 1.5 | 3.6±3.4[b] | 22.8±0.3[b] | 11.8±4.5[b] | 2.8±1.4[b] |
| 3.5 | 4.5±3.6[a] | 29.9±1.3[a] | 13.2±5.9[a] | 2.1±2.6[a] |

Within the column, data followed by different letters, differ significantly at P ≤ 0.01. *n* = 3.

**Table 4.** Mean concentrations of selected plant essential trace elements and potentially toxic elements in alfalfa grown in soil treated with lime in 2009.

| HL amount ($Mg\ ha^{-1}$) | Fe | Mn | Zn | Al |
|---|---|---|---|---|
| | $mg\ kg^{-1}$ on dry matter basis | | | |
| 0 (control) | 202.7±1.1[a] | 73.7±2.5[a] | 34.4±1.6[a] | 90.1±1.1[a] |
| 1.5 | 151.6±2.0[b] | 60.1±1.9[b] | 28.1±0.8[b] | 49.4±0.7[b] |
| 3.5 | 107.4±0.9[c] | 52.5±2.8[c] | 24.7±3.3[c] | 41.9±2.3[c] |

Within the column, data followed by different letters, differ significantly at P ≤ 0.01. *n* = 3.

soils makes phosphorus more available and makes potassium more efficient in plant nutrition. Furthermore, liming adds essential calcium to acid soils for greater plant growth. Concentration in alfalfa of Mg significantly decreased by 14 to 34% when acidic soil was treated with HL compared to the untreated control. Chen et al. (2001) found that the concentrations of Mg slightly decreased when soil was treated with agricultural limestone compared to the untreated control. According to Marschner (1995), the rate of Mg uptake is known to be depressed by other cations such as Ca, K, and Mn. Plant essential trace elements in alfalfa tissue are shown in Table 4. The concentrations of Fe, Zn and Mn in dried herbage significantly decreased with increasing HL addition by 34 to 89, 23 to 40 and 22 to 39%, respectively, when compared to the untreated control.

Similary, Chen et al. (2001) found that the concentrations of Fe, Mn and Zn in alfalfa were greatly decreased by the addition of agricultural lime. In contrast, Grewal and Williams (2003) reported that liming significantly reduced the Fe and Mn concentration of alfalfa shoots, while there was a little effect on Zn concentration of alfalfa shoots. Concentration of Al, which is often toxic to plants in acid soil, was generally significantly decreased in the alfalfa grown in plots treated with HL (Table 4). Aluminum concentration in alfalfa tissue was decreased by 82 and 100% as liming quantity increased from 0 to 3 Mg HL $ha^{-1}$. Previous studies showed that liming decreased significantly Al concentration in alfalfa tissue (Chen et al., 2001; Grewal and Williams, 2003).

## Conclusion

The results of this 3-year experiment on an acid soil suggest that a single initial application of hydrated lime in the first year at rates of 1.5 and 3 Mg HL $ha^{-1}$ may significantly increase the herbage yield and crude protein content of alfalfa up to 3 Mg HL $ha^{-1}$. The study has shown that liming on acid soil not only increases yield of dried herbage, but also improves Ca, P, and K content in alfalfa; liming reduced accumulation of Mg, Al, Mn, and Fe in alfalfa tissue. The present study clearly indicates that application of lime, particularly for heavy application, markedly increased the pH of an acid soil, compared with an unamended control. Increased alfalfa yield may be attributed to the reduction of soil acidity, and the increased tissue concentrations of P, K, and Ca. On acidic silty loam soils like of West Serbia, application of lime, to increase soil pH and yield, is a relevant measure to be taken when growing alfalfa.

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