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Effects of Sulfur Application on Soil pH and Uptake of Phosphorus, Iron and Zinc in Apple Trees

Mohammad Reza Dilmaghani[1*], Syavash Hemmaty[1] and Lotfali Naseri[2]

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[1]Academic Center for Education, Culture and Research (ACECR), Urmia Branch,Urmia University, Urmia, Iran
[2]Department of Horticulture, Urmia University, Urmia, Iran
[*]Corresponding author email: mrdh1970@gmail.com

Abstract
The main reasons for the fixation of some elements and consequently nutrient deficiencies, especially phosphorus, iron and zinc, are high levels of calcium (calcareous soils) and soil pH. Two sulfur fertilizers (sulfur alone and sulfur + organic material + *Thiobacillus spp.*) with two rates (2 and 4 kg sulfur/tree) were used to decrease soil pH and availability of P, Fe and Zn in apple trees during the growing season. A control treatment with no sulfur was also included in the experiment. The experiment was based on randomized complete blocks design with three replications and was carried out in the apple orchards of the West Azarbaijan province. Results showed that the sulfur treatments had significant effects on soil pH, chlorophyll content of leaves and phosphorus, iron and zinc concentrations of leaves and fruits in apple. On the average, applying sulfur decreased pH of soil and increased the chlorophyll content of leaves, and iron, zinc and phosphorus concentration of fruits in apple by17.09%, 4.8%, 30.24%, 11.61% and 18.76%, respectively, as compared to the control. Regarding the soil pH and other criteria about the nutrients availability and concentration, it seems that the application of 2 kg/tree sulfur fertilizer would be beneficial for apple orchards located in the highly alkaline soils of the area under study.

Keywords: Apple tree, Nutrients, Soil pH, Sulfur

Introduction
Iran is located in the arid and semi-arid region of the world. Furthermore, a large proportion of the cultivated lands in the country consist of calcareous soils. Such soils have high levels of calcium and pH that cause nutrient deficiencies. Sulfur oxidation in soils is an effective process in the reclamation of saline soils in addition to providing the sulfur needs of plants. Sulfuric acid is an appropriate substance to reduce the pH of soil and irrigation water, but since its handling needs special precautions, application of sulfur itself is recommended. The solubilizing effect of elemental sulfur (S) on P, Fe and Zn and other micronutrients in the intimate contact with them has been demonstrated in soil (Friesen *et al.* 1987). This effect depends on the presence of microorganisms, particularly *Thiobacillus* spp. Applying acidifying materials such as elemental sulfur is regarded as a possible and economic way to improve nutrient availability and plant growth in calcareous and alkali soils (Kalbasi *et al.* 1988; Singh and Chaudhari 1997; Kaplan and Orman 1998).

The objectives of this research were to study the effect of sulfur fertilizers and rates to decrease pH of calcareous soils and to increase uptake of phosphorus, iron and zinc in apple orchards.

Material and Methods
Experimental site
The study was conducted at the apple orchards in West Azarbaijan province, Iran (37˚ 39´ 5˝ N, 45˚

4′ 28″ E, altitude 1313m) during the two growing seasons, 2006 and 2007.

Laboratory analysis

Soil samples were prepared from the depths of 0-30 cm and 30-60 cm of orchards, mechanically crumbled and passed through a 2 mm sieve for the chemical and physical analyses and 0.5 mm sieve for the organic carbon analysis. The percent of organic carbon, the amounts of available phosphorus, potassium, Fe, Zn, Mn, Cu and sulfur (mg/kg) were determined according to A.O.A.C (1980) methods. EC and pH were measured with Eutech 6500 bench-top pH and Conductometer (Eutech Instruments, USA). Soil texture was determined with a hydrometer (Hydrometer G/L ASTM-E 100, Germany). Leaves were collected in the early July and during the growing season from around the trees then washed and dried at 70^{o}C. Fruits were harvested in the early October (conventional harvesting time). Leaves and fruits were randomly selected and analyzed for their mineral contents using the A.O.A.C. (1980) methods.

Experimental design and statistical analysis

The experiment was carried out as randomized complete blocks design with three replications. Four sulfur treatments from the combination of two types of sulfur fertilizers (sulfur alone and sulfur + organic material + *Thiobacillus spp.*) at two rates (2 and 4 kg/tree) were applied on the experimental plots. A control treatment with no sulfur was also included in the experiment. The fertilizer that consisted of sulfur plus organic material and *Thiobacillus spp*. was produced by Farin Missagh Saderat Co Ltd, Iran. Ingredients of this fertilizer were S (40-50%), organic material (60-50%) and *Thiobacillus spp.* inoculum. Sulfur and other fertilizers were applied in autumn after fruit harvesting or in late March to early April in pits around the trees. Statistical analyses were performed by the MSTATC software. Least Significant Difference (LSD) test was used for comparing different means.

Results and Discussion

Soil and water properties

The soil of the experimental site was calcareous (fine loamy, mixed, mesic Typic Calcixerept) and contained 200 mg/kg potassium in the depth of 30 cm. Calcium carbonate content in this soil was relatively high (12%) and organic matter and other nutrients were low to moderate (Table 1). Chemical analysis of water indicated that there was no specific limitation in water quality except for high bicarbonate concentration (HCO_3^-), which requires proper management to maintain soil quality at the optimum level (Table 2). The concentration of Cl, Ca, Mg and Na in the irrigation water were 1.4, 2.7, 4 and 0.6 mmol/l, respectively. The concentration of calcium + magnesium in the irrigation water was relatively high (6.7 mmol/l).

Due to the calcareous parent material and high level of HCO_3^- in the irrigation water, the pH of soil was high (above 8.1). Such conditions can enhance the deficiency of Ca and Fe. High pH in the soil may result in the absorption of HCO_3^- by the roots and its transportation via the xylem to the stem and leaves. The absorbed HCO_3^- may cause alkalinization of the apoplast resulting in the inactivation of translocated nutrients (Ca and Fe). In the long term, it is necessary to acidify the irrigation water or decrease the pH in the soil by the addition of sulfur, organic matter and *Thiobacillus* bacteria.

Table 1. Some important physicochemical characteristics of the soil in the apple orchard under study

Depth (cm)	pH[a]	EC[b] (dS/m)	SP	T.N.V	O.C	P[c]	K[d]	Fe[e]	Mn[e]	Zn[e]	Cu[e]	Clay (%)	Soil ttexture[f]
			(%)		(mg/kg)								
0-30	7.7	0.47	47	12	1.28	17.2	200	8	5.5	0.78	1.4	25	L
31-60	7.7	0.45	41	12	0.83	1.2	80	7.5	4.3	0.65	1.3	28	C.L

a. Determined in 1:2.5 soil: water solution
b. Determined in saturated water extract
c. Determined by the Olsen method
d. Extracted in 1.0 N ammonium acetate
e. Extracted in DTPA solution
f. Determined by the hydrometer method

Table 2. Chemical characteristics of the water in the apple orchard under study

EC (µS/m)	pH	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	SAR	B (mg/l)	Class
		(meq/L)									
653	7	-	6.1	1.4	0.1	2.7	4	0.6	0.79	0.2	C_2S_1

Chlorophyll content

The main physiological processes of plants (intensity of photosynthesis, amount of chlorophyll and accumulation of organic matter) are strongly affected by mineral nutrition of plants. Nitrogen, copper, zinc, iron and manganese are the important elements in synthesis of chlorophyll. Application of 2 kg/tree of sulfur + organic material + *Thiobacillus spp.* significantly increased (10.4%) the chlorophyll content of leaves over the control (Table 3). Regarding all sulfur treatments, this superiority was on the average 4.8%. Other researchers indicated that the application of elemental sulfur increased the sulfur content of leaf tissues and reduced the cell-sap pH, either by increasing sulfur content or by decreasing calcium content (Saroha and Singh 1979; Saroha and Singh 1980).

Table 3. Means of different sulfur treatments for leaf chlorophyll content and soil pH in the apple orchard

Sulfur rate	Sulfur fertilizer	Chlorophyll ($\mu g/ cm^{-2}$)	Soil pH
0 kg (control)	-	40.39	7.9
2kg sulfur/tree	Sulfur	41.34	6.4
	Sulfur + organic material	44.59	6.7
4kg sulfur/tree	Sulfur	40.67	6.5
	Sulfur + organic material	42.75	6.6
LSD (0.05)		2.57	0.36

Application of sulfur was more effective in increasing pseudo-stem length, circumference (cm), number of green leaves at bunch shooting (data not shown) in both seasons. These effects might be due to the enhancement of nitrogen in soil by decreasing soil pH and, also increasing plant metabolism (Mostafa and Abd El-Kader 2006). Interveinal chlorosis of the youngest leaves toward shoot tips is one of the first signs for high pH of the soil. This chlorosis is caused by the lack of chlorophyll production in the leaves. Although the plant may exhibit iron deficiency signs, but the main reason is the high soil pH. To solve this problem, growers can apply a foliar spray of iron chelate, but the permanent solution is to apply 224 kg/ha elemental sulfur, twice a year, to the soil under the plants. In a study, application of sulfur treatments, either basal or foliar, significantly increased the chlorophyll a, chlorophyll b and total chlorophyll contents of leaves in all stages of the crop growth. The deficiency of S not only inhibited synthesis of S-containing amino acids, but also inhibited chlorophyll synthesis in a similar manner by causing a shortage of the proteins required for chlorophyll formation (Marschner 1995).

Soil pH

In this study, the soil pH decreased significantly at all sulfur treatments (on the average, 17.09%) as compared with the control. However, no significant differences were observed among sulfur treatments (Table 3). This may have resulted from the reduction of the root zone pH (Saroha and Singh 1979). These results were in concordance with the reports of Besharati *et al*. (2000). The change of acidity in the soil depends on various soil and plant factors, such as soil buffering capacity, soil moisture level and aeration, CO_2 production by plant roots and microorganisms, microbial acid production, root exudation of carboxylates, plant genotype and nutritional status of the plant (Marschner 1995). Sulfur application in certain soils has been reported to reduce pH levels and to increase SO_4 content (Abrol 1990; Lopez- Aguirre *et al*. 1999; Stamford *et al*. 2002). Excess uptake of anions over cations leads to the net removal of protons in the soil and cause an increase in soil pH. In contrast, excessive uptake of cations is charge balanced by a net-release of protons and consequently leads to soil acidification. In soils with high pH many plants respond by releasing hydrogen (H^+ ions) from the root tips to acidify the soil surrounding the root and by the release of electrons to reduce oxidized ferric iron to the more soluble ferrous ion. Although this was best studied in relation to iron deficiency, the pH-lowering component of this response results in greatly increased solubility of all trace elements. However, the ability of plants to decrease the soil pH is strongly inhibited by the presence of bicarbonate which buffers the soil pH. In many soils with the zinc deficiency there are frequently extremely high levels of extractable zinc which are apparently not available to apple (Crowley 1997). This inability to mobilize soil zinc may be due partly to the relatively weak root stress response in plants. However, the trace metal efficient plants would normally acidify the soil and increase the solubility of iron and zinc (Manthey and Crowley 1997).

Concentration of nutrients in soil

Effect of sulfur on ability of nutrients absorption in the soil zone is presented in Table 4. The results showed that sulfur had significant effect on absorption of nutrients. The highest rate of available phosphorous was obtained when 2 kg sulfur + organic material + *Thiobacillus* bacteria was used, which was 53.9 percent more than the control treatment. However, this increase may not be attributed to the association of *Thiobacillus* with organic matter in this treatment because these bacteria are mainly obligate chemolithotrophs which use CO_2 as the major carbon source under all growth conditions and organic materials seem not to play an important role in their metabolism (Kuenen and Veldkamp 1973). Other types of sulfur oxidizing bacteria in the soil may have interacted with the organic materials existed in the fertilizer. Therefore, the fertilizers comprising only sulfur + *Thiobacillus* may have more fruitful results. Basharati *et al*. (2000) also indicated that sulfur alone significantly caused an increase in the availability of phosphorus, but adding *Thiobacillus* doubled the positive effect of sulfur.

Table 4. Means of different sulfur treatments for the concentration of phosphorus and iron in the soil of apple orchard

Sulfur rate	Sulfur fertilizer	Phosphorus (mg/kg)	Iron (mg/kg)
0 kg (control)	-	99.93	16.170
2kg sulfur / tree	Sulfur	138.16	19.297
	Sulfur + organic material	153.78	20.792
4kg sulfur / tree	Sulfur	122.98	21.692
	Sulfur + organic material	140.96	23.367
LSD (0.05)		4.325	3.687

There were significant differences among treatments for the absorption of iron. Most of the sulfur treatments had significantly higher absorption of iron that the control. Highest amount of iron availability belonged to the treatment of 4 kg sulfur + organic material +

Thiobacillus bacteria but it was not significantly different from the application of 4 kg sulfur alone and also 2 kg sulfur + organic material + *Thiobacillus* bacteria. Treatment of 2 and 4 kg sulfur + organic material + *Thiobacillus* and 4 kg sulfur alone increased availability of iron by 28.6, 44.5 and 34.15%, respectively. The micronutrient availability is controlled by pH. Improving of the deficiency of zinc, iron, manganese and copper is expensive. It is easier to apply sulfur or sulfuric acid to the soil before planting which can improve the problem quickly. Irrespective of the sulfur sources, soil acidification seems to increase nutrient availability in neutral and alkaline soils particularly under conditions of P, Fe and Zn deficiency (Römheld 1987; Neumann 2000; Liping *et al*. 2005). The processes involved in the uptake of nutrients are affected by alterations in soil pH, apart from the effects on nutrient availability in soils. Generally, cation uptake is declined with decreasing pH, whereas anion uptake is inhibited when the external pH increases. This may be attributed to (1) competition of H^+ and OH^- (HCO_3^-) with other cations or anions, respectively, (2) external pH effects on the electrochemical potential gradient which provides the driving force for nutrient uptake and (3) pH-induced alterations of root metabolism and function (Marschner 1995). These effects may be superimposed by the pH effects on nutrients availability. As an example for the effect of pH on ion uptake by modification of the dissociation equilibrium, P uptake is stimulated at low external pH due to enhanced formation of monovalent $H_2PO_4^-$, which is the preferential form for P uptake by higher plants (Marschner 1995).

Nutrients concentration of leaf and fruit

Regarding to the effect of sulfur on nutrients concentration of leaves and fruits, significant differences were found among treatments and all sulfur sources had significantly higher concentration of phosphorous in leaves and fruits (Tables 5 and 6). Although the highest concentration of leaves and fruits' phosphorous were obtained with the use of 4 kg sulfur + organic material + *Thiobacillus,* but there were no significant differences among the treatments comprising sulfur. The increase in the concentration of leaf phosphorous over the control treatment ranged from 28.26% (2 kg sulfur alone) to 39.86% (4 kg sulfur + organic material + *Thiobacillus*) in leaves and from 16.35% (4 kg sulfur alone) to 22.24% (4 kg sulfur + organic material + Thiobacillus) in fruits (on the average, 34.42% and 18.76%, respectively).

There were significant differences among treatments ($P \leq 0.05$) for the iron content in leaves and fruits. All treatments comprising sulfur had significantly higher amount of iron in leaves and fruits than the control treatment. Although the highest amount of available iron was obtained by the application of 4 kg sulfur + organic material + *Thiobacillus* bacteria, but the differences among sulfur treatments were not significant. The increase in the iron content range from 26.18% (2 kg sulfur alone) to 35.05% (4 kg sulfur + organic material + Thiobacillus) in leaves and from 24.84% (4 kg sulfur alone) to 41.29% (4 kg sulfur + organic material + Thiobacillus) in fruits (on the average, 30.19% and 30.24%, respectively). The acidification of soils with acid-producing materials increases the availability of Fe to plants (Maribela *et al*. (2003). Oxidation of elemental S can create an acidic environment in the rooting media that facilitate reduction of Fe (III) to Fe (II) (Romheld 1987). Thus, combining ferrous sulfate or other sources with acidic products may maintain the added Fe source in an available form for a longer period.

Table 5. Means of different sulfur treatments for phosphorus and iron concentration of the apple leaves

Sulfur rate	Sulfur fertilizer	Phosphorus (%)	Iron (mg/kg)
0 kg (control)	-	0.138	382.3
2kg sulfur / tree	Sulfur	0.177	482.4
	Sulfur + organic material	0.182	493.7
4kg sulfur / tree	Sulfur	0.190	498.5
	Sulfur + organic material	0.193	516.3
LSD (0.05)		0.0219	43.11

Table 6. Means of different sulfur treatments for phosphorus and iron concentration of the apple fruits

Sulfur rate	Sulfur fertilizer	Phosphorus (mg/100gr FW)	Iron (mg/100gr FW)
0 kg (control)	-	12.05	0.310
2kg sulfur / tree	Sulfur	14.41	0.398
	Sulfur + organic material	14.08	0.392
4kg sulfur / tree	Sulfur	14.02	0.387
	Sulfur + organic material	14.73	0.438
LSD (0.05)		1.402	0.07

Sulfur fertilizers significantly increased the zinc concentration of leaves and fruits (on the average, 12.26% and 11.61%, respectively) (Figures 1 & 2). The highest concentration of leaf zinc was recorded when 2 kg sulfur alone was used. This treatment increased the zinc concentration of leaves over the control treatment by 12.82%. However, the differences among sulfur treatments were not significantly different (Figure 1). Furthermore, the highest concentration of zinc in fruits was obtained by the the application of 2 kg sulfur + organic material + *Thiobacillus* which showed a 20% increase over the control treatment; however, it was not significantly different from that of 2 kg sulfur alone (Figure 2). Cui *et al.* (2004) reported that the elemental sulfur acidified the soil and increased shoot concentration of Zn and Cd. According to Wild (2003), the deficiency of micronutrients in soil, such as Zn, limit the crop growth but can be corrected by the application of fertilizers containing the required elements. Zn concentration in the leaf at the tillering and panicle initiation stages increased when S powder together with Zn sulfate was applied as compared with the control. The maximum Zn content in the leaves at both stages was attained when NPK, S and Zn were applied together.

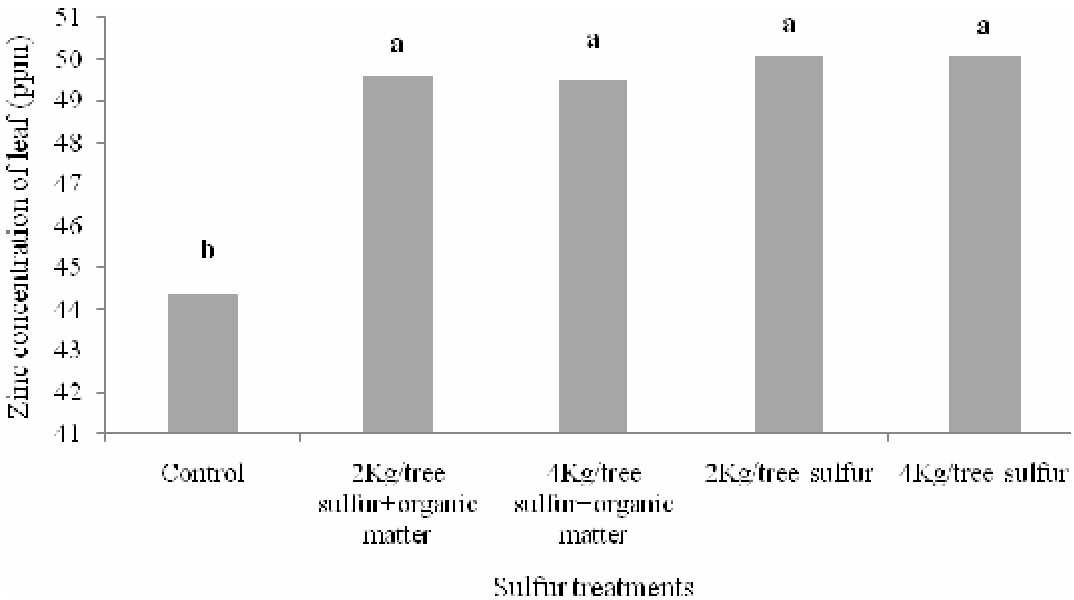

Figure 1. Effect of different sulfur treatments on zinc concentration of apple leaves.

Means with different letters are significantly different at the 5% probability level

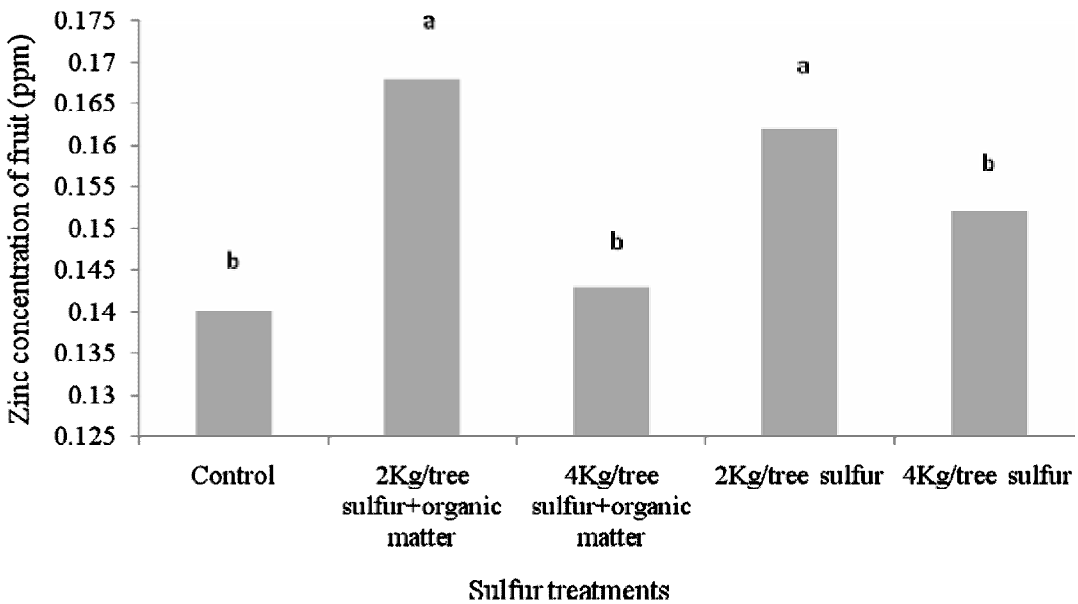

Figure 2. Effect of different sulfur treatments on zinc concentration of apple fruits.

Means with different letters are significantly different at the 5% probability level

Conclusion

Various studies have shown that sulfur fertilization in calcareous and alkaline soils reduces pH and improves soil properties. In calcareous soils the intake of nutritional elements by the plant are impaired which result in the reduction of yield and productivity. Furthermore, the plants do not efficiently benefit from the fertilizers applied to the soil. In this study, sulfur fertilization improved soil properties and intake of the nutrients. Thus, regarding the soil pH, concentration of phosphorus and iron in the soil, chlorophyll content in the leaves, the concentration of P, Fe and Zn in the leaves and fruits, it seems that the application of 2 kg/tree sulfur fertilizer would be beneficial for apple orchards in the highly alkaline soils of the area under study.

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