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Full Length Research Paper

Manganese analysis of sorghum hybrid (Sorghum bicolor (L) Moench) on different lamp current using an atomic absorption spectrophotometer

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Several studies on the effect of water treatment sludge (WTS) have been conducted on trace metal contents of sorghum hybrid (Sorghum bicolor (L.) Moench) grown on a mountainous Kumsan district in the Republic of Korea. The objective of the present study was for measuring manganese (Mn) content in sorohum hybrid on an appropriate lamp current with an atomic absorption spectrophotometer. Four treatments were used in this experiment namely: a) without fertilizer (control), b) fermented alum sludge (Compost), c) alum sludge+nitrogen, phosphorus, potassium (Alum+NPK), and d) fermented alum sludge+nitrogen, phosphorus, potassium (Compost+NPK). After sorghum's harvest, the Mn content was investigated with a spectrophotometer on the conditions of three lamp currents; 4, 5, and 6 mA. Several ratios between the background and the absorbance values might be a very useful index for deciding the condition of the Mn analysis. The three ratios, (mean of backgrounds)/(standard deviation of absorbances), (standard deviation of backgrounds)/(Standard deviation of absorbances), and (2 x Standard deviation of backgrounds)/(mean of absorbances), were considered to be good tools for appropriate Mn analysis. As a result, 5 mA current was favorable for the analysis, therefore, the Mn content was calculated on 5 mA lamp current. There were significant differences of Mn contents among the four treatments: (Alum+NPK) < control, compost or (compost+NPK). The Mn contents of the sorghum for all the four treatments were higher than 40 mg/kg DM (dry matter), and they were considered over-optimum level for the ruminants' feed.

Key words: Absorbance, alum sludge, atomic absorption spectrophotometer, background, lamp current, manganese, ratio, sorghum hybrid.

INTRODUCTION

Water treatment sludge (WTS) was utilized as fertilizer (Awwarf and Kiwa, 1990) for sorghum hybrid (Kim et al.,

1997). Fribourg (1985) described that summer annual grasses, for example sorghum, provide high quality feed

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in summer when well managed, but poor management results in misuse of land resources. little regrowth later in the season, and hungry livestock. Trace metal ions play important roles in a wide spectrum of areas. Manganese is a very important one in plant metabolism (Kanwar and Youngdahl, 1985) in soil (Yanai et al., 2012), and in water (Sharaf and Alharbi, 2013). However, it is toxic at higher levels, and chronic manganese poisoning affects the central nervous system (Baytak and Turker, 2004). Like iron (Fe). Mn relates in the phenomenon of oxydoreduction in the level of the life of cell. And manganese exists in the form of labile and not labile. Also, it seemed to constitute the reserve (Pinta et al., 1980). For ruminants, manganese (Mn) was considered to be necessary more than copper (Cu), cobalt (Co), or iodine (I) among trace elements as follows: Iron (Fe) (30 - 200 mg/kg DM)> zinc (Zn) (50 mg)> Mn (40 mg)> Cu (5 to 10 mg)> I (0.12 to 0.80 mg)> Co (0.1 mg) (McDonald et al., 1985). The amount of manganese present in the animal body is extremely small. But Mn is important in the animal body as an enzyme activator and resembles magnesium in activating a number of phosphate transferases and decarboxylases, notably those concerned with the tricarboxylic acid cycle (McDonald et al., 1985). At the present, we investigated how to measure the Mn content of forage. The oldness of the lamp might be a reason of the decreased intensity of the radiation with atomic absorption spectrophotometer (Pinta et al., 1979). Ansari et al. (2004) analyzed the trace metals of medicinal plants on the lamp current from 7.5 to 15.0 mA. In this report, we chose three different electric currents of hollow cathode lamp for the conditions of the Mn analysis. Finally, we intended to find an index in order to analyze Mn on a better condition with atomic absorption spectrophotometer.

MATERIALS AND METHODS

Experiment 1

The experimental field was situated at a mountainous site with an altitude of 260 m in Joongbu University, at Kumsan-gun in ChungcheongNam-do. The period of field experiment was from May to November of 1993. The field was newly established on May 26, 1993. On a randomized block design, there were four treatments with three replications; control, compost, (alum+NPK) and (compost+NPK). No fertilizer was applied to the experimenting plot (Control). Alum sludge from Daedeog water treatment plant, 133 kg/ha, was applied to the plots with nitrogen+ phosphorus+ potassium fertilizers (Alum+NPK). On a volume basis, at first, sawdust and another sludge (1:2) were mixed. Then, alum sludge, on dry matter (DM) basis, 25% of this mixture, was added and composted during 55 days. Finally, 67 kg/ha of the compost (fermented alum sludge) prepared on this procedure was applied to the plot (compost). The 67 kg/ha of the compost (fermented alum sludge) were applied with (N+P+K) fertilizers to the plot (Compost+NPK). The fertilizers were added on a ratio of 200:200:200 kg/ha as a component of N: P₂O₅: K₂O, respectively. The alum sludge and fertilizers were applied on June 7 and June 17, 1993, respectively. The seeds of sorghum hybrid and

Pioneer 931 (*Sorghum bicolor* (L.) Moench) were sown on June 23 and the forage was harvested on November 4, 1993 (Kim et al., 1997).

The analysis of manganese (Mn) was carried out from October 2, 2007 through March 26, 2008 in a laboratory at the Department of Companion Animal and Animal Resources Science in Joongbu University. Samples were air-dried and milled for analysis of Mn. Then, a ¹/₂ g each of milled sample was extracted for 18 h by 25 ml of 1 M hydrochloric acid (HCI), and adequate volume of extracts was diluted with distilled water filling up to 50 ml or 100 ml, and filtered (Norinsuisan-sho, 1979). While this method had been used for K or Mg determination, it was used for Mn element analysis on the present experiment. The Mn analysis was made with an atomic absorption/flame emission spectrophotometer (AA 680, Shimadzu Co. Ltd., Kyoto), with the method of atomic absorption spectrophotometry (Pinta et al., 1979). The condition was as follows: wave length, 279.5 nm; mode, back ground correction (BGC); slit, 0.40 nm; lamp burner height, 6 mm; acetylene flow, 2.0 I/min. The Mn content was measured at three different lamp currents of the apparatus. In order to draw calibration curve of Mn standard solutions, the solutions were measured on the electric currents of the AA-680 lamp as follows: 4, 5 and 6 mA of the hollow cathode lamp of the spectrophotometer. The two values of absorbance and of background were observed. The t-test was carried out to determine the significant difference of absorbance and of background values among the three electric currents.

Experiment 2

The analysis of manganese (Mn) in sorohum hybrid was carried out from October 2, 2007 through March 26, 2008 in a laboratory at Department of Companion Animal and Animal Resources Sciencezin Joongbu University. The methods of Kim et al. (1997) was utilized. The Mn content was measured at the three different electric currents of lamp of the apparatus. As shown in Table 4, the solutions were calculated three times in order to draw calibration curve of standard solutions for Mn, with the following lamp currents of AA-680 as follows: 4 mA of the hollow cathode lamp of the AA-680, 5 mA of the hollow cathode lamp and 6 mA of the hollow cathode lamp. The Mn contents, shown on a unit of ppm in Figure 4, were directly calculated without regard to its filling up to volume with the absorbance value on the calibration curve (Table 4). The subsequent calculation for Mn content was as follows (Table 5): (calculated Mn content from the absorbance value) / 10 x 2,000 in the case of 100 ml, and (calculated Mn from absorbance) / 20 x 2,000 in the case 50 ml filling up, respectively.

The analysis of variance (ANOVA) table was prepared (Table 6). The mean Mn contents of each treatment were shown in order to compare statistically the difference among them (Table 7 and Figure 5), and it was done through the least significance difference (LSD) method (Snedecor and Cochran 1980, Son and Park 1999).

RESULTS

Experiment 1

Figure 1 shows the emission value on different currents in the emission mode of the atomic absorption/flame emission spectrophotometer (AA-680). The emission value linearly increased with the advance of lamp current. Table 1 shows the absorbance of standard solutions for the Mn analysis of sorghum hybrid (*Sorghum bicolor* (L.) Moench) upon the three different electric currents of lamp on the atomic absorption spectrophotometer, AA-680.

Table 1. Absorbances of standard solutions for the Mn analysis of sorghum hybrid(*Sorghum bicolor* (L.) Moench) upon the three different electric currents of lamp on the atomic absorption spectrophotometer, AA-680.

Lamp current (m	A) Absorbance (/10.000)	Mn content of standard solutions (ppm)
	000	0.00
4	610	0.50
	1220	1.00
	000	0.00
5	600	0.50
	1250	1.00
	000	0.00
6	630	0.50
	1270	1.00



Figure 1. Effect of electric current on emission value in emission mode of an atomic absorption spectrophotometer (AA-680).

From Table 1, the absorbance values did not differ with the advance of lamp current. Figure 2 shows the absorbance values on electric current of 4, 5 and 6 mA on AA-680 spectrophotometer. From Figure 2, the absorbance value decreased (4 > 6 mA), and again increased with the advance of the lamp current (5 < 6 mA).

Table 2 shows background value on the three different electric current of hollow cathode lamp on the atomic

absorption spectrophotometer. As was shown in Figure 3 and will be shown in Table 3, Table 2 shows the mean value of the background was the least, 32.2/10,000, on 5 mA condition while the greatest, 50.9 on 6 mA. On the other hand, the standard deviation of the background was the least, 9.7 on 5 mA and the greatest, 18, on 6 mA current.

Figure 3 shows the relation between absorbance and background during analysis of manganese in sorghum

Deremeter	Electric current of hollow cathode lamp				
Parameter	4 mA	5 mA	6 mA		
AlumNPK3-1†	20	20	20		
AlumNPK3-2 †	10	30	30		
AlumNPK2-1	20	20	30		
AlumNPK2-2	20	20	20		
Control2-1 †	30	20	40		
Control2-2 †	40	30	40		
Compost3-1	30	20	30		
Compost3-2	30	30	30		
CompostNPK1-1	40	30	50		
CompostNPK1-2	40	20	50		
CompostNPK2-1	40	30	50		
CompostNPK2-2	50	40	50		
Compost2-1 †	50	40	60		
Compost2-2†	50	30	60		
Compost1-1	50	40	60		
Compost1-2	50	40	60		
CompostNPK3-1	60	30	70		
CompostNPK3-2	60	40	70		
Control1-1	60	40	70		
Control1-2	60	40	70		
AlumNPK1-1 †	70	50	80		
AlumNPK1-2 †	70	50	80		
Mean ‡	43.1 ^b	32.2 ^a	50.9 ^c		

Table 2. Background value on the three different electric current

 of hollow cathode lamp on the atomic absorption pectrophotometer.

†, Filled up to 50 ml, the others are up to 100 ml; ‡, characters horizontally show difference significantly at 1% level.



Figure 2. Absorbance values on electric current of 4, 5 and 6 mA on AA-680 spectrophotometer. **, 50 ml filling instead of 100 ml.

Table 3. Effects of electric current of hollow cathode lamp on the ratio among background(/10,000) and absorbances(/10,000) values on the Mn analyses of sorghum hybrid with AA-680 spectrophotometer.

Decemeter	Electric current of hollow cathode lamp		
Parameter	4 mA 5 mA 6 m		6 mA
Mean of 22 absorbance values(AM)†	565.3 ^c	497.3 ^a	526.5 ^b
Standard deviation of 22 absorbance values(AS)	197.0	197.5	195.3
Mean of 22 background values(BM) †	43.1 ^b	32.2 ^a	50.9 ^c
Standard deviation of 22 background values(BS)	17.0	9.7	18.7
(BM)/(AS) (%)	21.8	16.3	26.0
(BS)/(AS) (%)	8.6	4.9	9.5
(2BS)/(AM) (%)	6.0	3.9	7.1

†, Characters horizontally show difference significantly at 1% level.



Figure 3. Relation between absorbance and background during analysis of manganese in sorghum hybrid.

hybrid, and indicates the absorbance and background values on the measuring order on electric lamp current of 4, 5 and 6 mA during Mn analysis of sorghum hybrid with an atomic absorption spectrophotometer (AA-680). The absorbance value differed on the four treatments and three replicates. While the changing pattern of absorbance values was similar on all the three lamp currents, absorbance values on 4 mA was the highest and those on 5 mA lamp current the lowest. And the background

values increased with the lapse of measuring time, being apparently high on 6 and low on 5 mA electric lamp current, respectively. It was indicated from the figure that 5 mA lamp current condition shows the lowest values of absorbance and of background.

Table 3 shows the effects of electric current of hollow cathode lamp on the ratio among background (/10,000) and absorbance (/10,000) values on the Mn analyses of sorghum hybrid with AA-680 spectrophotometer. The

Table 4. Calibration curve for the Mn analysis of sorghum hybrid (*Sorghum bicolor* (L.) Moench) upon the three different electric currents of lamp on the atomic absorption spectrophotometer, AA-680.

Lamp current	Absorbance(/10.000)(X) and Mn con	tent of Standard solutions(Y)
4 mA	Y=8.1967 x 10 -4 X,	n=3, r=1.0000 (p<0.01)
5 mA	Y=7.9957 x 10 -4 X +6.9296 x 10 -3,	n=3, r=0.9997 (p<0.05)
6 mA	Y=7.8738 x 10 -4 X +1.3226 x 10 -3,	n=3, r=0.9999 (p<0.05)

Table 5. The real manganese(Mn) concentration of sorghum hybrid(*Sorghum bicolor* (L.) Moench) at different lamp currents with an AA-680 spectrophotometer (mg Mn / kg DW) \$.

Treetment number	Electric current of hollow cathode lamp			
Treatment number	4 mA	5 mA	6 mA	
Control 1-1	75.6	59.4	61.8	
Control 1-2	75.8	59.4	61.4	
Control 2-1 #	67.5	63.8	64.5	
Control 2-2 #	70.7	65.7	65.7	
Compost 1-1	91.8	78.4	80.2	
Compost 1-2	91.4	78.4	80.2	
Compost 2-1 #	81.7	74.0	74.3	
Compost 2-2 #	82.3	75.1	75.2	
Compost 3-1	62.8	54.6	57.4	
Compost 3-2	64.6	55.4	59.0	
Alum+NPK 1-1 #	53.3	44.2	45.0	
Alum+NPK 1-2 #	51.7	42.8	43.4	
Alum+NPK 2-1	54.0	48.0	50.8	
Alum+NPK 2-2	50.6	45.2	48.4	
Alum+NPK 3-1 #	49.0	46.5	47.9	
Alum+NPK 3-2 #	49.7	46.2	48.1	
Compost+NPK 1-1	75.4	63.8	67.4	
Compost+NPK 1-2	77.2	65.2	69.2	
Compost+NPK 2-1	81.2	69.6	73.4	
Compost+NPK 2-2	80.8	69.4	71.8	
Compost+NPK 3-1	73.8	59.4	61.2	
Compost+NPK 3-2	70.0	55.0	57.6	

&, Real Mn concentration = (Mn concentration in Figure 1) / 10 x 2,000 (filling up to 100 ml), or calculated from (Mn concentration in Figure 1) / 20 x 2,000 (filling up to 50 ml); \$, on a dry matter basis; #, filling up to 50 ml instead of 100 ml.

standard deviations of absorbance values on the three lamp currents were nearly same, from 195.3 to 197.5/10,000. On Table 3, the ratio of the (Mean of backgrounds) / (Standard deviation of absorbances), (Standard deviation of backgrounds) / (Standard deviation of absorbances), and (2 x Standard deviation of backgrounds) / (Mean of absorbances) was 16.3, 4.9 and 3.9%, respectively; the lowest values at 5 mA electric current of the hollow cathode lamp among the three measuring conditions.

Experiment 2

Table 4 shows the calibration curve for the Mn analysis of

sorghum hybrid (*S. bicolor* (L.) Moench) upon the three different electric currents of lamp on the atomic absorption spectrophotometer, AA-680. In the table, absorbance values were similar on the same Mn concentration level among the three currents. Figure 4 shows the Mn content in the extracted solution from the sorghum hybrid (*S. bicolor* (L.) Moench) samples. These data were obtained with an AA-680 spectrophotometer at different lamp currents, but the values were without regard to filling up to volume. The order of Mn content was on the three lamp currents as follows: 4 mA> 6 mA> 5 mA.

Table 5 shows the real Mn concentration of sorghum hybrid (*S.bicolor* (L.) Moench) at different lamp currents



Figure 4. The manganese content of sorghum hybrid (*Sorghum bicolor* (L.) Moench) at different lamp currents. Directly calculated from its absorbance. *, Filling up to 50 ml instead of 100 ml.

Table 6. The analysis of variance(ANOVA) table of the Mn content of sorghum hybrid(Sorghum bicolor (L.) Moench).

Parameter	SS	d.f. ^{&}	MS	F- value
Total	1,412.22	11		
Replication	278.615	2	139.3075	4.1901NS [#]
Treatment	934.126	3	311.3753	9.3656 *
Error	199.479	6	33.2465	

⁸, degree of freedom; # , not significant statistically at 5 % level; *, significant difference at 5% level (p<0.05).

Table 7. The mean manganese concentration of sorghum hybrid (*Sorghum bicolor* (L.) Moench) on the condition of 5 mA of hollows cathode lamp of AA 680 spectrophotometer (mg Mn / kg DW)\$.

Treatment -	Replication			
	1	2	3	wean
Control	59.4	64.7	52.0*	(58.7 ^a)#
Compost	78.4	74.5	55.0	(69.3 ^a)
Alum+NPK ^{&}	43.5	46.6	46.3	(45.4 ^b)
Compost+NPK ^{&}	64.5	69.5	57.2	(63.7 ^a)

Treatments were statistically significant at 5% level. Here, the value of LSD (least significant difference) was 11.5, it was obtained from (t value x standard error; $2.447 \times 4.7079 = 11.520$). *, an estimated value; [&], nitrogen (N) + phosphorus (P) + potassium (K). ^{\$}DW, on a dry matter basis; #, the same letter vertically indicates that there was not a significant difference.

with the spectrophotometer. The value of Mn were in order of 4 > 6 > 5 mA. The Mn values on 5 mA were smaller than those on 4 and 6 mA lamp currents. Table 6

shows the analysis of variance (ANOVA) table of the Mn content of sorghum hybrid (*S. bicolor* (L.) Moench). There was significance among treatments at 5% level. Table 7

shows the mean Mn concentration (mg Mn/kg DW) of sorghum hybrid (*S. bicolor* (L.) Moench) on the condition of 5 mA of hollows cathode lamp of the spectrophotometer. The result was in the order of Alum+NPK< Control, Compost, Compost+NPK. The Mn contents of the sorghum for all the four treatments were higher than 40 mg/kg DM.

DISCUSSION

Experiment 1

The present extracting method for Mn analysis is much weaker compared to the method of sample preparation by Pal and Sethunathan (1982). The emission value linearly increased with the advance of lamp current (Figure 1), but the change of absorbance on atomic absorption was not similar to that of emission value (Table 1, Figure 2). Pinta et al. (1979) described the change of atomic absorption with the advance of electric current as follows: though theoretically the sensibility increased with the advance of lamp current, there were three patterns of absorbance change: The first pattern was the decreased absorbance on Cd analysis, the second was the increased absorbance on lead (Pb), the third was the absorbance increased but decreased again on aluminum (AI) analysis with the advance of lamp current while the variation of absorbance was feeble on the Cd analysis with the advance of electric current up to 5 mA.

In the present experiment, we wanted to know the changing pattern of absorbance values with the advance of lamp current on Mn analysis: "How much would we control the lamp current to obtain appropriate absorbance values on Mn analysis?" It is necessary to know that the lamp current is not fixed, but to lessen or to increase the current of the spectrophotometer. However, there is an another not-advantageous fact, the intensity of the hollow cathode lamp on an (atomic absorption / flame emission spectrophotometer) varies with the advance of utilizing time. In other words, the function of the lamp current itself does not have an important meaning, and accordingly, stabilization is not easy to analyze Mn in sorghum hybrid.

In another words, it can be said like this: On the traditional *Kimzang* (*Kimchi* making) in late autumn or beginning of winter season in Korea, it is very important to lessen water content of Chinese cabbages with salt in order to prepare the important *Kimzang* travail for a Korean housewife; decrease water content to what degree? It is necessary to find another index. Therefore, we considered if there is any meaningful index for the Mn analysis. This is our intention of the present experiment. From Figure 2, the changing pattern of Mn analysis was opposite to Al analysis shown by Pinta et al. (1979); de-

creased and again increased with the advance of the lamp current on the present experiment. Here, we considered that 5 mA current was the best condition with the least value of standard deviation (SD) of backgrounds among the three lamp currents. Until now, we considered that three ratios, (Mean of background values)/(Standard deviation of absorbance values), (Standard deviation of background values)/(Standard deviation of background values), (2 x standard deviation of background values), (mean of absorbance values), are good tools of Mn analysis on BGC (background correction) mode of the atomic absorption spectrophotometer.

Experiment 2

Two manganese values, both Mn calculated (Figure 4) and real Mn content (Table 5), were continually the least on 5 mA current among the three lamp currents, because both the Mn content came from the same sources of absorbances. The least values in Figure 4 and in Table 5 were the best. The result on Table 5 was based and obtained on Mn absorbance. The Mn content on 5 mA electric current, is taken as the actual value on Table 6, Table 7, and Figure 5. From Table 6 on the analysis of variance (ANOVA), it was possible to calculate the mean value of Mn as in Table 7. The data in Figure 5 were taken from Table 7. Figure 5 made it clear the difference among mean Mn content of sorghum hybrid (Sorghum bicolor (L.) Moench) among the 4 treatments as follows on a dry matter basis. Except the difference between (Compost) and (Compost+NPK), the Mn contents differed significantly among the treatments (Figure 5). The tendency was shown, in more detail on Figure 5 than on Table 7, as follows; Compost or Compost+NPK > Control > Alum+NPK, or Compost > Compost+NPK > Control> Alum+NPK.

The fresh yield of top plant parts on Control plots were much less than other plots (Kim et al., 1997). And the higher content on control than on compost or (compost+NPK) might result from the less yield on control plot. The Mn content of the sorghum seemed overoptimum for ruminants, because 40 mg/kg DW was advised (McDonald et al., 1985). As a result, alum sludge application might be desirable where Mn toxicity can be expected, for example, near wasted mine.

There were three blocks, and it can be said that there is an interval of time elapsed. Though repeated Mn data of three blocks on 4 treatments were not studied (Table 7 and Figure 5), there is a tendency of Mn decrease on Control, Compost, and Compost+NPK. The mean Mn content was the smallest on (Alum+NPK) treatment (p<0.05) (Table 7). As a kind of suggestion for the environment as written before, alum sludge may be used at the wasted mineral caves in order to prevent excessive absorption of Mn by forage crops or human consuming crops such as barley, rice among others.



Time after cultivation

Figure 5. The mean manganese content of Sorghum hybrid (*Sorghum bicolor* (L.) Moench on the condition of 5 mA of hollows cathode lamp of AA-680 spectrophotometer. *DW, on a dry weight basis; **NPK, nitrogen, phosphorus and potassium fertilizers.

A soil scientist wrote that "If a plant grows well and is healthy, will it be a satisfactory source of food for man or animals? " (Foth, 1978). It's a very important question. Sorohum hybrid was famous for its tall height up to 5 m and some sorghum cultivars may produce more DM per hectare than does corn (Fribourg, 1985). The Mn contents of the sorghum for all the four treatments were higher than 40 mg/kg DM, and they were considered over-optimum level for the ruminants' feed, because the Mn content of sorghum was 16 mg/kg DM (McDonald, 1985). In an ecological viewpoint, even though the Mn content was not the toxicant level for sorghum itself or not for ruminants, the over-optimum level of Mn content in plants or in animals could be delivered to human when man eats the meat of the animal. Park et al. (2009) investigated that the range of cadmium (Cd) content in this sorghum hybrid was 1.3 to 5.0 ppm on Control plot. This heavy metal, Cd, is one of the carcinogens. Even though this range of Cd is not suggested dangerous to plant or to ruminants, the effect of higher Cd in plant and potentially higher Cd in animal which eats the plant can be, therefore, a dangerous source of Cd for cancer to human-beings who take the animal's meat as food.

Conclusion

The experimental field was situated at a mountainous site

with an altitude of 260 m. The objective of the present report was measuring manganese (Mn) content in sorghum hybrid (*S. bicolor* (L.) Moench) on an appropriate lamp current with an atomic absorption spectrophotometer. As a result, 5 mA current was favorable for the analysis, therefore, the Mn content was calculated on 5 mA lamp current. There were significant differences of Mn contents among the four treatments, and the Mn content on (Alum+NPK) was higher than that of Control (no fertilizer was applied to the experimental plot). The Mn contents of the sorghum for all the four treatments were higher than 40 mg/kg DM (dry matter), and they were considered over-optimum level for the ruminants' feed.

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