Northern NY Agricultural Development Program
2005-2006 Project Report

The effect of $CuSO_4$ from dairy manure on the growth, and composition of cool season forage grasses and corn

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Introduction

The use of copper sulfate ($CuSO_4$) in footbaths as a preventative maintenance for foot health has been a common practice on dairy farms for the last 10 years. In general the waste material from footbaths ends up in the manure storage system and then applied to fields. At Miner Institute, before $CuSO_4$ footbaths were employed, the manure slurry concentration of Cu was 4.8 g /1000 L. After $CuSO_4$ footbath use, the concentration of Cu reached a high of 88.6 g/1000 L in 2000. Records for manure analysis and application rates for Miner Institute over the last 10 years show single year application rates of Cu as high as 31.7 kg/ha, with 18% of annual applications of liquid manure above 4.54 kg Cu /ha. Spreading of manure slurry with high Cu concentrations may adversely impact crop growth and quality. In 2001 on average 1.93 ± 1.28 kg Cu/ha was imported on to farms surveyed in Vermont and New York (Flis *et al*., 2006). This decreased to 1.35 ± 0.75 kg Cu/ha in 2004 (Flis *et al*., 2006). For these farms the concentration of Cu (ppm) was higher in 2001 than in 2004 for both corn silage ($P = 0.04$) and haylage ($P = 0.009$, Table 1, Flis *et al*., 2006). Copper is not highly mobile in plants and toxicity affects root growth first, resulting in decreased root growth. Finally, when applied to the soil the Cu cation binds tightly to negatively charged soil particles, resulting in accumulation of Cu in the soil.

Therefore, the objectives of this study were to determine:

1. the effects of rate of application of copper sulfate from dairy manure on the establishment, growth, and quality of cool season forage grasses and corn
2. the fate of Cu applied from dairy manure containing copper sulfate in the soil.

Table 1. Copper tissue concentration from surveyed farms for 2001 and 2004.

	Cu concentration (ppm)			
Forage	**2001**	**Standard Deviation**	**2004**	**Standard Deviation**
Corn Silage	11.97	6.94	6.56	2.24
Haylage	21.17	9.48	9.17	7.62

Materials and Methods – Greenhouse Grass

Soil

Bulk soils were collected by removing the top 15 cm of soil was collected from the edges of fields for Trout River gravelly loamy sand (**sand**) and Roundabout silt loam (**silt**). The Trout River series is very deep, somewhat excessively well-drained soil formed in water-sorted deposits (USDA-NRCS). The Roundabout series is very deep, and somewhat poorly drained soils that formed in glaciolacustrine and glaciomarine deposits on lake or marine plains (USDA-NRCS). The total volume of soil collected was 0.42 m^3 for each type. After collection soil was dried at 105°C for 24 h and sifted to remove large rocks and debris. Pre-trial soil analysis was done at the UVM Agriculture and Environmental Testing Labs (Burlington, VT) on a dried soil sample. Soil was stored dry until potted.

Species

Three cool season forage grasses were used; orchardgrass (*Dactylis glomerata* L.), timothy (*Phleum pretense* L.), and reed canarygrass (*Phalaris arundinacea* L.). The seeding rate used was 9, 6, and 10 lbs/acre (10, 6.7, and 11.2 kg/ha) for the orchardgrass, timothy, and reed canarygrass, respectively. After adjusting the seeding rates for the purity and germination, the seeding rate per pot was determined to be 0.037, 0.022, and 0.039 g/pot for the orchardgrass, timothy, and reed canarygrass, respectively. These seeding rates result in 35, 54, and 33 seeds per pot for the orchardgrass, timothy, and reed canarygrass, respectively.

Treatments

For the 2 soil types there were 3 treatment levels with 6 replications for each of the 3 cultivars for a total of 108 pots. Treatments were 0 (**Control**), 2.27 (**Medium**), and 4.54 (**High**) kg of Cu/acre from $CuSO_4$. Soil was mixed by cultivar by treatment. Manure was added to treatments based on P requirement for establishment of a grass based on the soil test available P.

Manure

Manure was colleted 1-week prior to mixing with soil and the treatment level of $CuSO_4$ was added. Copper sulfate was put into solution and mixed with the manure for each treatment level. After 1-week manure mixes were sampled and analyzed at the UVM Agriculture and Environmental Testing Labs (Burlington, VT).

Pots

Soil and manure were mixed and transported to the greenhouse at Plattsburgh State University (Plattsburgh, NY). Pots were filled with 0.005 m^3 of the correct mixture. Seeding was done in the greenhouse and seeds were covered with 0.64 cm of the soil mixture and watered.

Watering, Temperature and Lighting

Pots received water twice daily as needed. Temperature was maintained above 13°C. Lighting was provided for a 12 h photoperiod. Due to differences in light intensity and temperature in the greenhouse, pots were rotated once a week.

Monitoring and Harvesting

Percent germination of the pots was measured for two consecutive weeks after seedlings emerged. Growth was measured once weekly for each pot and recorded. Shoots were harvested when one treatment for that species and soil type reached 40.6 cm of growth or at least 7 weeks of growth after planting. Grasses were cut to leave 5.1 cm of stubble for re-growth and were allowed to re-grow until one treatment reached 40.6 cm at which time the entire plant was harvested and the number of days after planting and height of each treatment was recorded. Roots were washed to remove soil, weighed, and dried at 55°C for 24 h to determine dry matter (**DM**) and mineral concentration. Shoots were harvested from each pot and dried at 55°C for 18-24 h to determine DM. Samples were ground to 2 mm and used to determine ash, crude protein (**CP**), neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), lignin (Miner Institute Forage Lab, Chazy, NY) and mineral analysis (UVM Agriculture and Environmental Testing labs, Burlington, VT). Soil tests were repeated at the end of the trial.

Statistical Analysis

Analysis was performed for a treatment effect, species effect, the treatment by species interaction, and the linear effect of Cu treatment level. Differences were significant at $P \leq 0.05$ and tendencies at $P \leq 0.15$.

Materials and Methods – Corn Plots

Location, Plot size, Treatments, and Corn Hybrids

Plots were located at The William H. Miner Agricultural Research Institute (Chazy, NY). Plots were 3.68 m (12', 4 rows of corn) wide and 7.62 m (25') long. Treatments were control, medium (8.1 lbs/acre or 9.12 kg/ha), and high (16.3 lbs/acre or 18.23 kg/ha). Additionally, the 3 treatment levels were tested on two corn hybrids, a short day corn (39D81 – 84 DRM, Pioneer Seeds) and a long day corn (36M28 – 103 DRM, Pioneer Seeds). Each corn hybrid at each Cu level were replicated 4 times for a total of 24 plots.

Manure Collection, Application, and Planting

Approximately 105.9 L (28 gal) of manure was applied to each plot. Manure was hand applied and incorporated with a roto-tiller immediately following application. Corn was planted on May 9, 2006 in 72.6 cm (30") rows with plants within rows spaced at 17.15 cm (6.75"). At planting, 302.7 kg/ha (270 lbs/acre) of 14 – 21 – 21 + Zn was applied. Additionally, Force 3G (Syngenta) was applied for protection of early-season insect pests, including: corn rootworms, cutworms, wireworms, white grubs and seedcorn maggots. Approximately 45.7 cm 100 lbs/acre of N was also applied.

Soil Sampling

A soil sample was taken on the harvest day for analysis of total P, K, Mg, Al, Ca, Zn, Na, Fe, B, Mn, Cu, and S.

Corn Measurements and Harvest
Number of plants per plot was counted at the V1 stage to determine the plant population per plot. Corn height was measured at 5 different stages (V3: 9- 12 days after emergence, V5: 14 to 21 days after emergence, V6: 21 to 25 days after emergence, V13: 42 to 49 days after emergence, and R1 or silking: 63 to 68 days after emergence). At these times, days after planting, height, number of leaves, and stalk diameter were measured. Additionally, at R1, 4 plants were harvested from each plot in the center 2 rows. These plants were used for counts and measurements. At 1/3 milk line, 4 plants were harvested from each plot for dry matter determination. Corn was harvested at approximately 70 % moisture. At harvest, 10' (3.1 m) of length from the 2 center rows were hand harvested and weighed. From the plants harvested, 6 were chopped with a wood chipper and samples taken for chemical analysis of DM, NDF, ADF, lignin and CP (Miner Institute Forage Lab, Chazy, NY) and mineral analysis (UVM Agriculture and Environmental Testing labs, Burlington, VT).

Statistical Analysis
Analysis was performed for a treatment effect, hybrid effect, the treatment by hybrid interaction, and the linear effect of Cu treatment level. Differences were significant at $P \leq 0.05$ and tendencies at $P \leq 0.15$.

Results and Discussion

<u>Greenhouse Grass</u>
The method for seeding the pots resulted in poor germination and growth in the silt loam soil regardless of Cu treatment level. Based on this result, the silt loam treatments will be repeated beginning in November of 2006. Additionally, the reed canarygrass grown in the sand soil was cut too short at the first harvest and did not re-grow for the second harvest. Therefore, results are the comparison of the orchard grass and the timothy grown in the sandy loam soil only.

Tillering rate and re-growth rate both decreased as copper application level increased regardless of grass species (Table 2). A linear treatment effect was observed for harvest 2 number of shoots and the tillering rate from harvest 1 to harvest 2 (Table 2, Figure 1a). Additionally, there was a decreased shoot yield and dry root weight with increased copper application level (Table 2). A linear effect of Cu treatment on root dry weight suggests that there is a decrease root dry weight as the Cu treatment level increases (Figure 1b).These effects may result in a decrease in the longevity of the stand and an overall decrease in yield. There may be concern for a larger decrease in re-growth rate and yield in drought conditions due to the decrease in root dry matter with increased Cu application rate.

The Cu concentration of the shoots tended to increase in copper concentration with increased Cu application (Table 3). Additionally, there was a significant linear effect of Cu treatment level on Cu concentration in the shoots, with Cu concentration in the shoots increasing as Cu treatment level increases (Table 3, $P = 0.06$). However, the Cu concentrations observed are with in the normal range for crop plants (0 – 50 ppm, Epstein and Bloom, 2005).

There was only a numerical increase in the Cu concentration in the roots with increased Cu application (Table 4). Overall, the Cu concentration in the shoots was lower than in the roots, 33.2 vs. 66.6 ppm, respectively. This is expected because Cu is a mineral that is generally sequestered in the roots (Epstein and Bloom, 2005).

Corn Plot Study

There was no effect of copper treatment level on plant number per plot, total harvest weight, number of plants harvested, or the weight per plant harvested (Table 5). Long-term studies with the application of high Cu swine manure have consistently reported no effect of the level of Cu application on corn grain or silage yields (Mullins et al., 1982; Sutton et al., 1983; Payne et al., 1988a; and Payne et al., 1988b).

There was no effect of Cu treatment level on harvest DM or the Cu concentration of the plants (Table 6). Copper concentration in the corn was within the normal range for crop plants andmuch lower than the grass shoot Cu concentrations. Studies with long-term application of high Cu swine manure have consistently reported leaf or grain Cu concentrations that were not outside expected values (Mullins et al., 1982; Sutton et al., 1983; Payne et al., 1988a; and Payne et al., 1988b).

There was a significant effect of copper treatment on total soil Cu concentration (Table 7). This effect is further explained by a significant linear effect of treatment on soil Cu concentration (Figure 2). As expected the total soil Cu concentration increased as the copper treatment level increased. The difference in the Cu applied per acre and the difference of the soil test lbs/acre is approximately the removal of Cu in the shoots of the corn (3.7 and 1.9 lbs/acre removal from the soil for the medium and high Cu treatments, respectively, Table 8). This indicates that the only loss of Cu from the soil is from plant removal and with one application at these rates there is no leaching of Cu.

Conclusions and Further Research

A single high application of Cu appears to have greater effect on grass growth and yield than corn growth and yield. From the results reported here it appears that one year of application of at least 16 lbs/acre of Cu from dairy manure is not a concern for growth, yield, or plant Cu concentration. However, continued research is needed on the effect of multiple high application rates on corn since a single application of dairy manure to corn land is not a common practice.

Additionally, the method of soil analysis for Cu needs further examination. Differences in soil Cu concentration were observed in the corn soil samples when total mineral digestion was performed, but the typical test for soil is an extraction on available minerals which may not be showing actual increases in soil Cu loading.

Education and Outreach Plan. Results of this research will be published in the monthly Miner Institute Farm Report, with readership of over 10,000. This newsletter is not copyrighted, and articles are often used by farm newspapers and county Cooperative Extension publications. Sally Flis presented these results on December 20, 2006 at a producer meeting for Carovail, Inc. (Salem, NY).

References

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Table 2. Copper treatment effects on growth measurements.

Item	Copper Treatment Level			SE	Treatment
	Control	Medium	High		*P*-value
Number of seedlings	34.5	35.8	32.8	0.87	0.08
Harvest 1 number of shoots	34.9	35.7	32.5	1.03	0.06
Harvest 2 number of shoots	87.6	90.3	71.3	4.55	0.01
Tillering rate from Harvest 1 to Harvest 2	52.7	54.7	39.1	4.63	0.05
Harvest 2 shoot dry weight (g)	6.57	7.04	5.45	0.50	0.09
Shoot weight per plant (g)	0.076	0.079	0.075	0.004	0.81
Re-growth rate (g/d)	0.18	0.19	0.15	0.013	0.08
Root dry weight (g)	3.25	3.44	2.19	0.37	0.05

Table 3. Shoot chemical and mineral analysis by copper treatment level for Timothy and Orchard Grass grown in Trout River Gravelly Sandy Loam.

Item	Copper Treatment Level			SE	*P*-value
	0	5	10		Trt
NDF, % DM	47.7	46.2	47.3	0.58	0.21
ADF, % DM	38.2	37.5	39.4	0.59	0.14
Lignin, % DM	13.1	13.5	14.8	0.67	0.29
CP, % DM	20.3	21.7	21.9	0.95	0.44
Ca, % DM	0.72	0.75	0.72	0.03	0.76
P, % DM	0.34	0.36	0.40	0.03	0.35
K, % DM	4.64	5.39	5.25	0.26	0.11
Mg, % DM	0.37	0.38	0.35	0.01	0.26
Na, % DM	0.02	0.02	0.03	0.003	0.55
Al, ppm	1146	914	1329	165	0.22
Fe, ppm	1298	1039	1477	172	0.21
Mn, ppm	554	548	542	30.5	0.96
B, ppm	12.9	14.0	12.2	1.84	0.77
Cu, ppm	29.8	30.9	39.0	3.32	0.12
Zn, ppm	41.9	46.1	46.8	2.89	0.44
S, % DM	0.28	0.29	0.29	0.009	0.51

Table 4. Root mineral concentration by copper treatment level for Timothy and Orchard Grass grown in Trout River Gravelly Sandy Loam.

Item	Copper Treatment Level			SE	*P*-value
	0	5	10		Trt
Ash, % DM	22.7	16.9	14.4	1.92	0.01
Ca, % DM	0.46	0.46	0.47	0.02	0.97
P, % DM	0.20	0.21	0.22	0.01	0.31
K, % DM	1.94	2.05	2.28	0.11	0.10
Mg, % DM	0.21	0.22	0.21	0.008	0.92
Na, % DM	0.12	0.12	0.13	0.01	0.77
Al, ppm	2878	2905	2640	281	0.77
Fe, ppm	4568	4181	4550	548	0.85
Mn, ppm	1243	1321	1377	105	0.67
B, ppm	24.6	21.4	24.7	2.37	0.56
Cu, ppm	63.3	65.6	70.9	5.32	0.59
Zn, ppm	82.6	92.9	96.7	10.9	0.64
S, % DM	0.19	0.20	0.21	0.01	0.29

Table 5. Plant population and harvest data for corn grown with three different levels of Cu application in dairy manure.

Item	Cu Treatment Level			SE	*P*-Value
	Control	Medium	High		
Plant Number	160.25	159.38	161.0	5.96	0.98
Total Harvest Weight (kg)	45.5	47.63	46.75	1.93	0.74
Number of Plants Harvested	33.5	32.88	32.88	1.50	0.94
Weight per Plant at Harvest (kg)	1.37	1.45	1.41	0.05	0.48

Table 6. Chemical and mineral analysis of corn grown with three different levels of Cu application from dairy manure.

	Cu Treatment Level				
Item	**Control**	**Medium**	**High**	**SE**	***P*-Value**
DM	36.1	36.6	36.5	0.35	0.58
NDF, % DM	44.27	43.38	41.81	1.46	0.49
ADF, % DM	24.71	24.44	24.29	0.41	0.77
Lignin, % DM	3.77	3.88	3.79	0.07	0.52
CP, % DM	7.10	7.19	7.04	0.14	0.73
Ca, % DM	0.17	0.16	0.17	0.003	0.45
P, % DM	0.21	0.21	0.21	0.007	0.95
K, % DM	0.61	0.59	0.61	0.02	0.71
Mg, % DM	0.15	0.15	0.15	0.004	0.72
Na, % DM	0.008	0.006	0.007	0.002	0.47
Al, ppm	56.90	46.30	61.46	5.08	0.12
Fe, ppm	74.98	72.73	78.79	5.46	0.73
Mn, ppm	12.09	11.16	11.95	0.95	0.76
B, ppm	5.96	4.94	8.10	0.91	0.07
Cu, ppm	3.22	2.76	3.37	0.36	0.49
Zn, ppm	12.68	12.10	12.84	0.96	0.85
S, % DM	0.08	0.08	0.08	0.003	0.97

Table 7. Soil mineral analysis for corn plots (ppm).

	Cu Treatment Level				
Item	**Control**	**Medium**	**High**	**SE**	***P*-Value**
Ca, % DM	3422	3292	3462	52.37	0.08
P, % DM	583	547	578	12.7	0.13
K, % DM	891.7	765.6	829.9	46.6	0.18
Mg, % DM	224.9	2016.4	2191.2	58.04	0.13
Na, % DM	140.9	121.1	130.9	9.78	0.38
Al, ppm	11454	10408	11074	397.3	0.19
Fe, ppm	14797	13694	14582	472.8	0.24
Mn, ppm	158.5	150.9	158.8	5.06	0.47
B, ppm	35.01	34.69	30.48	2.53	0.38
Cu, ppm	10.98	13.18	18.19	0.78	<0.0001
Zn, ppm	31.81	30.01	32.03	0.76	0.14
S, % DM	189.5	184.5	192.6	6.44	0.67

Table 8. Changes in soil Cu concentration compared to Cu application rate.

Treatment	**Lbs/acre applied**	**Soil Test lbs/acre**	**Difference from Control**
Control	0	21.96	-
Medium	8.1	26.36	4.4
High	16.3	36.38	14.42

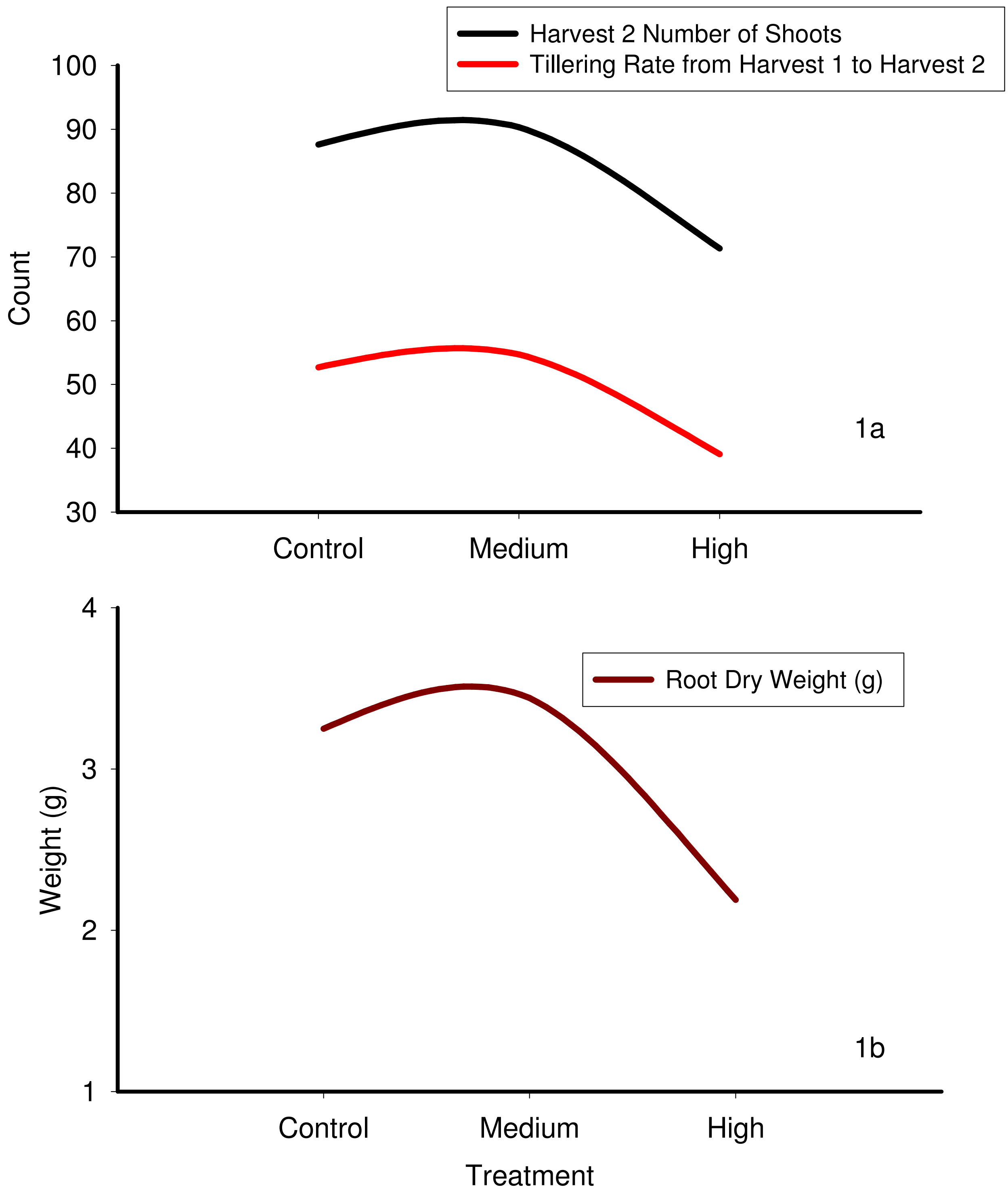

Figure 1a,b. Linear effect of Cu treatment level on harvest 2 number of shoots, tillering rate from harvest 1 to harvst 2, and root dry weight (g).

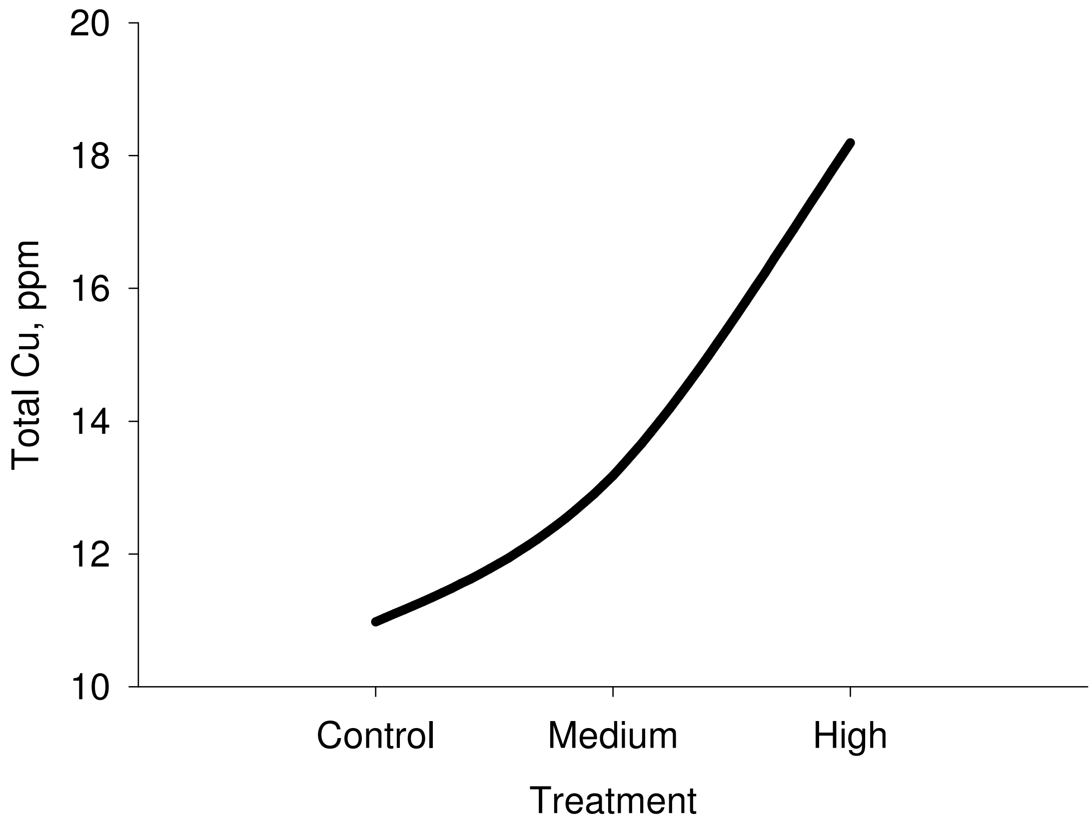

Figure 2. Linear effect of Cu treatment level on total soil test Cu (ppm).