Minor Element Content of Forage Plants and Soils

N. O. PRICE, W. N. LINKOUS, and R. W. ENGEL

Virginia Agricultural Experiment Station, Blacksburg, Va.

The extensive use of minor element supplements in the livestock feed industry emphasizes the need for establishing more precisely the contribution of natural feed ingredients to the diet of farm animals with respect to minerals. One hundred and fifty-three forage samples and 88 soil samples from Piedmont Virginia were analyzed for the minor elements—boron, copper, cobalt, manganese, molybdenum, and zinc. Except for a few isolated sampling areas, where plants were deficient in copper and cobalt, this study indicates that forages grown in this area generally contain sufficient amounts of the minor elements to meet the requirements of grazing animals.

RECENT WORK in Virginia relative to the minor element content of forage plants dealt with the study of mixed pasture plants and legumes grown on five soil types under different fertilizer treatments (*16*). This was the first of a series of studies that was undertaken in this state to determine the minor element content of forage plants. In 1949 Orange and Culpeper counties, a representative agricultural and livestock-producing section of north-central Virginia, were selected for a survey of the minor element content of the forage plants and soils.

Orange and Culpeper counties are in the north-central part of Virginia, situated entirely within the middle Piedmont plateau. The land is rolling or hilly, elevations range from 200 to 1200 feet above sea level, and drainage is excellent. The principal crops of these two counties are corn, wheat, oats, soybeans, cowpeas, clover, timothy hay, alfalfa, lespedeza, and bluegrass pasture. A considerable acreage is in orchards. Dairying is an important animal industry, and beef cattle, hogs, and poultry are raised, in greater or smaller numbers, on most farms. The four major soil belts within these two counties are the Penn-Bucks, Tatum-Nason, Davidson, and Cecil series (*21, 22*).

The purpose of this study was to determine the boron, cobalt, copper, manganese, molybdenum, and zinc contents of the forage plants and soils from four of the major soil belts in the Piedmont region; and to determine if the forages contained sufficient amounts of these elements, as now recommended, to meet the needs of grazing animals. The extensive use of minor element supplements in the livestock feed industry emphasizes the need for establishing more precisely the contribution of minerals in natural feed ingredients to the diet of farm animals.

Procedures

As a sampling procedure, the two counties were divided into 33 areas, ranging in size from 280 to 2566 acres. Every 6th acre of alfalfa; every 23rd acre of red clover, timothy, and lespedeza; and every 15th acre of orchard grass and ladino clover were sampled for minor element analysis of the forages. Soil samples, for analysis, were taken at the same location as the plants. Because of the limited acreages of a number of forages, the sampling procedure did not allow uniform representation of forages and soil types. The total soil and plant samples analyzed were 88 and 153, respectively. As was anticipated, alfalfa and lespedeza were most frequently represented in the plant samples. All samples were taken between mid-July and mid-August, 1949.

The determinations of boron were made by the quinalizarin reaction method of Berger and Truog (*6*); cobalt was determined on the plant tissue by the colorimetric procedure involving the use of nitroso R salt with dithizone extraction. The cobalt method used in this laboratory is that published by Beeson (*3*) with one exception: The wet-digestion method (nitric and perchloric acids) was used in the preparation of the samples instead of ashing. Cobalt in the soils was determined by first extracting with hydrochloric acid and then following the procedure for plant tissues. Copper, manganese, and zinc were determined according to the official methods of the Association of Official Agricultural Chemists (*2*). Molybdenum was determined in the plants by the thiocyanate–stannous chloride method of Evans (*14*) and in the soils by the method of Robinson (*20*). The minor element analyses for the soils represent total content.

A summary of the minor element composition of the forage plants and soils is given in Tables I and II.

Results

All of the legumes studied contain considerably more boron than timothy or orchard grass (Table I). The legumes, in general, require larger amounts of boron for development than do nonlegume forage plants. Alfalfa, in particular, is very sensitive to a lack of boron and this may explain the great concentration of boron by this plant. The boron analyses of the soils reveal rather marked differences between and within soil belts. The average boron content of Cecil soils is consistently lower than the Davidson and Tatum-Nason, while Penn-Bucks is the highest in boron.

The cobalt content of the forages (Table I) ranges from a low of 0.05 p.p.m. for a lespedeza sample from the Tatum-Nason soil to a high of 1.10 p.p.m. for a red clover sample from the Davidson soil belt. Most of the samples are in the range of 0.10 to 0.5 p.p.m. of cobalt. The legumes show a higher concentration of cobalt than the nonlegumes. As is the case with boron, the soils vary greatly in cobalt content. The Davidson soils are consistently higher in this element than the other soil belts. Cecil soils and all but a few of the Tatum-Nason soils are consistently lower in cobalt than Penn-Bucks or Davidson.

Copper in the forages (Table I) ranges from a low of 1.5 p.p.m. in a timothy sample to a high of 29.0 p.p.m. in a red clover sample. In general, the concentration of copper is higher in red clover than in any of the other legumes studied. The limited data on orchard grass indicate that this grass concentrates copper as effectively as alfalfa, lespedeza, or ladino clover. Timothy is consistently low in copper. Copper is relatively abundant in all of the soil belts represented in this study and there is no indication of differences in copper content between soil types. The soil type yielding the lowest copper (12.6 p.p.m. in Cecil) also yields the highest copper value (191.0 p.p.m.).

The manganese content of the grasses and legumes (Table I) ranges from 12.0 to 368.0 p.p.m. The relatively greater capacity to accumulate manganese by orchard grass than by the legumes confirms the observation of Bolin (*7*) that grasses are usually higher in this element than legumes. The soil belts vary greatly in manganese concentration. The Tatum-Nason and Cecil soils generally do not exceed 600 p.p.m., whereas the Penn-Bucks and Davidson exceed 1000 p.p.m. in the majority of samples.

The majority of forage samples contain less than 1.0 p.p.m. of molybdenum (Table I), and there appear to be no forage differences in the ability to concentrate this element, and no real differences in the soil concentration of this element that can be attributed to soil type.

Although the limited number of nonlegume samples average somewhat lower in zinc concentration than the legume forages, there are no great differences in the concentration of this element among the plant species sampled. Soils of the Davidson belt are somewhat higher in zinc than those of the Penn-Bucks or Cecil belt, but the differences are not great. On the average, soils of the Tatum-Nason belt contain only about one half as much zinc as those of the Davidson belt.

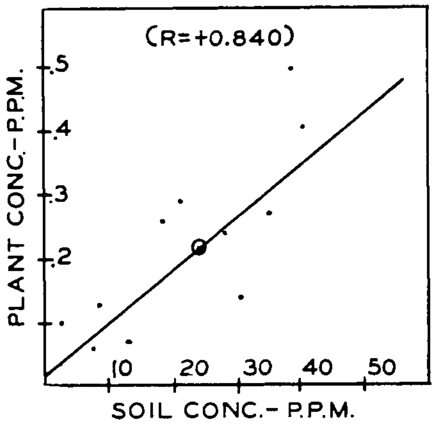

Figure 1. Relation of soil (Davidson) concentration of cobalt to plant (alfalfa) concentration

⊙ Mean

The data on alfalfa and lespedeza afforded an opportunity to determine whether or not there was any relationship between soil and plant concentration of the minor elements. The correlation coefficients between minor element soil and plant concentration for these species were calculated. In nearly all instances the trend was for increased soil concentrations to be reflected in increased plant concentration, yielding positive r values. However, in only one case—alfalfa cobalt concentration and Davidson soil cobalt concentration—was the correlation highly significant (Figure 1, $r = +0.840$, 11 degrees of freedom, $P = <0.01$).

Discussion

The minimum requirements of the minor elements necessary to meet the need of grazing animals has been fairly well established for some of the minor elements. Beeson (*4*), in summarizing a great deal of data on cobalt content of good and deficient pastures, stated that an average value throughout the year, greater or less than 0.07 p.p.m. of cobalt, might represent the minimum level for health in ruminants. Allman (*1*) stated that cobalt-deficient pastures contain 0.01 to 0.07 p.p.m., whereas healthy pastures generally afford 0.07 p.p.m. to 0.30 p.p.m. Cobalt has not been shown to be essential for forage plants, but is essential for animal health. Copper, on the other hand, is essential to both plants and animals. Cunningham (*11*) stated that dried grass from pastures in

Table I. Minor Element Content of Forage Plants of Culpeper and Orange Counties

(P.p.m., dry basis)

Plant	*Soil Belt*	*No. of Samples*		*Boron Range*	*Cobalt Range*	*Copper Range*	*Manganese Range*	*Molybdenum Range*	*Zinc Range*
Alfalfa	Penn-Bucks	21		25.0–58.4	0.19–0.71	6.5–19.7	25.0–113.0	0.18–1.11	21.0–41.0
			Mean	38.4	0.39	11.2	70.0	0.46	28.7
	Tatum-Nason	4		35.0–40.6	0.12–0.74	9.8–16.2	22.0–35.0	0.20–1.44	18.5–28.0
			Mean	37.5	0.36	13.5	26.8	0.85	24.6
	Davidson	22		13.5–59.3	0.06–0.90	10.2–18.5	12.0–81.0	0.06–1.44	17.0–48.0
			Mean	35.6	0.30	12.6	47.0	0.49	26.5
Lespedeza	Penn-Bucks	12		12.6–31.6	0.09–0.41	6.0–14.2	45.0–154.0	0.14–6.00	23.0–52.0
			Mean	23.0	0.20	9.8	86.1	1.14	34.8
	Tatum-Nason	29		10.2–38.6	0.05–0.35	6.4–12.8	27.0–120.0	0.01–2.25	21.0–42.0
			Mean	15.9	0.15	8.6	67.7	0.37	28.7
	Davidson	15		12.3–23.7	0.06–0.46	6.4–13.7	22.0–138.0	0.05–1.86	20.0–37.0
			Mean	17.5	0.23	9.4	63.5	0.52	27.3
	Cecil	7		10.2–23.2	0.09–0.31	7.3–11.8	24.0–102.0	0.01–2.33	23.5–34.0
			Mean	14.2	0.18	10.2	61.3	0.83	29.4
Red clover	Penn-Bucks	3		15.2–20.8	0.33–0.68	19.5–21.0	59.0–85.0	0.24–1.58	31.0–41.0
			Mean	17.1	0.48	20.0	69.0	0.69	35.6
	Tatum-Nason	4		16.4–22.0	0.10–0.49	11.5–20.5	59.0–75.0	0.11–0.98	27.0–60.0
			Mean	19.6	0.26	15.0	63.0	0.52	41.8
	Davidson	10		11.7–25.6	0.29–1.10	13.7–29.0	55.0–112.0	0.01–1.89	28.0–47.0
			Mean	17.7	0.67	18.2	74.9	0.35	35.7
	Cecil	7		10.8–17.7	0.13–0.97	10.5–29.0	38.0–81.0	0.09–0.46	24.0–41.0
			Mean	14.4	0.41	18.7	62.3	0.25	35.0
Ladino clover	Penn-Bucks	5		11.4–23.7	0.13–0.48	10.2–15.2	70.0–120.0	0.06–0.45	25.0–30.0
			Mean	18.0	0.27	13.0	82.6	0.25	27.6
	Davidson	2		17.7–18.5	0.26–0.80	12.0–12.5	85.0–130.0	0.01–0.35	24.0–24.0
			Mean	18.1	0.51	12.2	107.5	0.18	24.0
Timothy	Penn-Bucks	5		2.5–4.1	0.07–0.28	1.5–9.7	40.0–160.0	0.31–1.83	14.0–35.0
			Mean	3.3	0.16	5.1	89.4	0.69	21.4
	Cecil	1		3.8	0.17	6.7	59.0	0.90	7.5
			Mean	3.8	0.17	6.7	59.0	0.90	7.5
Orchard grass	Penn-Bucks	2		1.7–3.0	0.16–0.16	13.7–16.0	298.0–368.0	0.10–0.40	17.0–24.0
			Mean	2.3	0.16	14.8	333.0	0.25	20.5
	Tatum-Nason	4		1.7–5.2	0.06–0.60	8.0–18.5	73.0–168.0	0.34–1.61	20.0–28.0
			Mean	3.3	0.20	12.0	122.0	0.67	23.6

Table II. Minor Element Content and pH of Soils of Culpeper and Orange Counties

(P.p.m., dry basis)

Soil Belt	Soil Cover	No. of Samples		Boron Range	Cobalt Range	Copper Range	Manganese Range	Molybdenum Range	Zinc Range	pH Range
Penn-Bucks	Alfalfa	12		19.2–77.1	2.88–34.00	19.4–105.0	276.0–2128.0	0.80–1.90	43.8–108.7	5.8–7.1
			Mean	48.1	18.54	61.8	1419.0	1.27	66.3	
	Lespedeza	2		20.2–36.2	3.48–20.40	56.0–62.0	448.0–1916.0	0.95–1.10	46.3–70.0	6.2–6.4
			Mean	28.2	11.94	59.0	1182.0	1.02	58.1	
	Red clover	1		88.3	22.96	62.0	1100.0	1.00	65.0	6.3
			Mean	88.3	22.96	62.0	1100.0	1.00	65.0	
	Ladino clover	1		28.3	19.60	24.4	648.0	0.90	73.7	6.0
			Mean	28.3	19.60	24.4	648.0	0.90	73.7	
	Orchard grass	1		49.3	22.96	105.0	2180.0	1.40	80.0	5.7
			Mean	49.3	22.96	105.0	2180.0	1.40	80.0	5.7
Tatum-Nason	Alfalfa	3		17.8–21.1	3.52–47.64	27.0–104.0	392.0–612.0	0.90–2.85	17.5–58.7	6.1–6.5
			Mean	19.8	20.48	60.4	489.0	1.58	40.8	
	Lespedeza	22		5.9–69.3	1.48–10.24	13.4–92.0	132.0–1580.0	0.40–1.40	17.5–79.0	4.9–5.9
			Mean	22.5	4.03	40.5	412.0	0.82	39.4	
	Red clover	1		50.6	4.80	62.0	380.0	0.60	32.5	6.7
			Mean	50.6	4.80	62.0	380.0	0.60	32.5	
	Orchard grass	2		34.2–96.5	1.92–3.88	26.4–67.0	184.0–208.0	0.60–0.90	22.5–41.3	5.6–6.7
			Mean	65.3	2.90	46.7	196.0	0.75	31.3	
Davidson	Alfalfa	11		3.5–39.7	2.88–56.64	24.0–125.0	408.0–2580.0	0.30–2.10	45.0–141.3	5.8–7.1
			Mean	15.8	24.27	70.6	1225.0	1.50	88.3	
	Lespedeza	13		4.8–46.9	5.72–60.60	25.5–77.5	364.0–3200.0	0.70–2.20	27.5–118.7	5.4–7.2
			Mean	22.3	23.11	49.9	1284.0	1.40	68.0	
	Red clover	8		9.6–98.1	14.00–57.84	35.0–128.0	1000.0–2176.0	0.50–2.00	42.5–150.0	5.9–7.4
			Mean	25.6	38.25	59.9	1467.0	1.29	90.0	
Cecil	Lespedeza	5		3.8–16.3	3.80–11.88	43.0–191.0	324.0–472.0	0.45–0.70	45.0–66.5	5.2–6.4
			Mean	9.4	8.00	78.5	384.0	0.59	61.0	
	Red clover	5		2.2–18.7	3.08–21.28	12.6–81.5	184.0–468.0	0.25–1.35	22.5–91.7	5.9–6.4
			Mean	9.3	7.73	48.6	303.0	0.68	49.1	
	Timothy	1		12.3	4.20	63.4	124.0	1.55	57.5	6.1
			Mean	12.3	4.20	63.4	124.0	1.55	57.5	

deficient areas contain between 2.8 and 7.5 p.p.m. of copper (normal, 10 to 15 p.p.m.). Allman (*1*) also found that 7.5 p.p.m. of copper is generally sufficient for healthy pastures and below this amount a deficiency may exist.

The wide distribution of manganese in the plants and soils establishes this element as one of importance for the normal development of plants and animals. The recent report of Bentley and Phillips (*5*) indicates that cattle are affected by a deficiency of manganese in the ration. They state that a satisfactory level of manganese, which provides a margin of safety to meet the requirements of dairy cattle, is 20 p.p.m.; 10 p.p.m. is distinctly in the marginal or deficient zone. Coppenent (*9*) concluded from his studies that a manganese content of less than 20 p.p.m. is diagnostic of deficiency.

It has been known for some time that molybdenum performs an essential function in the higher plants and certain microorganisms (*15*). The recent observation that molybdenum is an essential dietary factor for the deposition and maintenance of normal levels of intestinal xanthine oxidase in the rat suggests that this element may be important in the nutrition of animals (*13*, *17*). Prior to this, extensive evidence was accumulated establishing the toxicity of forages high in molybdenum for animals and the antagonism of this element to copper (*15*). Neither the minimum requirement nor the tolerance level for molybdenum in animals has been established definitely. Cunningham (*10*) concluded that toxic herbage contained up to 25.6 p.p.m. of molybdenum, while normal pasture grasses contained less than 2.00 p.p.m. Davis (*12*), in summarizing his work, pointed out that in the herbivorous animal the presence of small amounts of molybdenum, ranging from 2 to 25 p.p.m. in young growing forage, has been associated with an increased requirement for copper.

Zinc is essential to the growth of plants and also necessary for animal health. Underwood (*23*) concluded that a zinc deficiency is unlikely to be observed in ruminants, since the zinc content of pasture plants and forages usually lies within the range of 30 to 100 p.p.m. on the dry basis. Pastures in Western Australia respond to applications of 10 to 15 p.p.m. of zinc on a dry basis, which is more than sufficient for animal requirements. Brown (*8*) found that deficient alfalfa plants averaged 8.00 p.p.m. of zinc, while normally growing plants averaged 13.8 p.p.m.

If judged by the minimum amounts of the minor elements that are deemed necessary for animal health, it is evident that only a very limited number of the forage plants analyzed in the present study are deficient in cobalt and copper. The molybdenum, zinc, manganese, and boron concentrations found in the forage are within the normal ranges reported by others and should be satisfactory for grazing animals.

The limited data establishing a positive correlation between plant (legume) and soil cobalt concentration are of interest and deserve further study. In extensive studies reported from New Zealand (*18*) no direct correlation was found between soil and pasture cobalt contents, although low cobalt status of the soil was usually associated with low cobalt content of pastures (*19*).

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CHELATE FUNGICIDES

Fungitoxicity of the 8-Quinolinols

S. S. BLOCK

Engineering and Industrial Experiment Station, University of Florida, Gainesville, Fla.

The 8-quinolinols (oxines) and their chelates have been of commercial importance as fungicides in industry and medicine and highly effective in agricultural applications. Their fungitoxic activity was studied, employing different derivatives under different conditions of acidity. Ability to chelate and lipoid solubility were found requisite for the activity of this group. The copper chelates were, in most cases, many times more fungitoxic than the unchelated compounds. It is suggested that both the chelator and the metal function in producing the unusually high antifungal activity of these chelates.

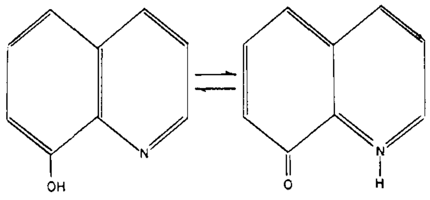
THE COPPER CHELATE of 8-quinolinol (oxine, 8-hydroxyquinoline, *22*) is one of the most highly rated fungicides developed in recent years. Its effectiveness in the preservation of textiles, paint, and miscellaneous industrial products has been the subject of numerous papers (*10–12, 14, 17, 18, 24, 28, 30, 38–40*). A method of rendering the compound soluble in many common organic solvents (*25–27*) has extended its usefulness. Its merit as an agricultural fungicide has been recognized (*8, 15, 16, 35, 45, 48, 49*), but economic considerations have limited this application. Recent results, however, indicate that the cost factor may be minimized by combining this copper chelate with low-cost fungicides, without losing its protective properties against certain plant diseases (*36*).

Unlike the copper chelate, the parent compound, oxine, is no newcomer to the field of antimicrobial chemicals. As the active agent of Chinosol, it has been in use as an antiseptic and disinfectant since about 1895 (*5*), but only in recent years have its antibacterial and antifungal properties been re-examined in some detail.

Analytical chemists have shown great interest in oxine and its relatives because of the ability of these compounds to chelate with trace quantities of metals and form precipitates and colored solutions. Indeed, the ability to chelate with and precipitate metals essential for cell metabolism has been proposed as the mechanism for the antimicrobial activity of oxine (*1, 50, 51*). Zentmyer (*51*) demonstrated that its toxicity to fungi could be overcome by the addition of excess zinc to the medium. Albert and coworkers (*6*) found that structural modifications preventing chelation resulted in markedly decreased toxicity to bacteria. Other workers (*31, 43, 46, 47*) found that an excess of certain metals in the medium could eliminate the toxicity of oxine and copper oxinate.

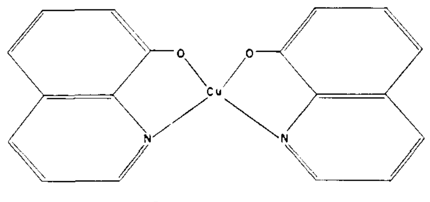
On the other hand, the fact that the copper, nickel, cadmium, and silver salts of oxine, which are saturated with respect to metal, had high fungistatic activity indicated to Sexton (*44*) that chelation was not the basis for the toxicity. He suggested, as had Hata (*23*), that oxine owes its toxicity to its phenolic properties. Mason (*32*) and Manten and coworkers (*31*) shared this view. The examination of many chelating compounds for toxicity to fungi and bacteria (*6, 7, 42*) has clearly demonstrated that ability to chelate is not necessarily synonymous with toxicity.

In more recent work, Rubbo and coworkers (*41*) and Albert and coworkers (*3*) demonstrated the startling fact that oxine owes its toxic effect on bacteria to the metal chelate alone, and if a medium is depleted of iron and copper, oxine is no longer inhibitory. Their theory of the mechanism of action (*3*) is that the chelate enters the cell as the 2 to 1 (oxine to divalent metal) complex and that



Phenolic tautomer of oxine

Quinoidal tautomer of oxine



Copper oxinate