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INTRODUCTION

The presence of trace minerals in plant material has been shown in recent years to be of nutritional importance in the feeding of farm animals. Current investigations of the distribution of these elements and their role in animal nutrition have offered a possible explanation for the unthrifty nature of beef cattle in the eastern and southeastern areas of Oklahoma. The unthriftiness of these cattle is characterized by poor weight gains, poor calf crops, and physical conditions not desirable in finished beef cattle. Nervous manifestations which occur in cattle in some areas, especially in the eastern area near Sallisaw, may possibly be related to the mineral composition of the forage in this area.

Previous studies have shown that the soil and the forage grown in the eastern and southeastern areas are frequently deficient in phosphorus. Although calcium and phosphorus supplementation has some effect in relieving the condition of cattle in these areas, results have not been sufficiently conclusive to be considered satisfactory. A study of the occurrence of some of the trace elements was therefore desirable, so as to characterize more completely the conditions existing in these two areas. The trace elements chosen for study were manganese, molybdenum, and cobalt.

A study of the occurrence of trace elements requires analytical procedures that are capable of determining these elements at very low concentration. The present investigation, therefore, had two objectives: first, the evaluation of procedures sufficiently accurate and simple to permit a large number of forage samples to be analyzed, and

second, the determination of the concentration of these trace elements in native forage from the two areas in question, and in that from one area considered normal with respect to beef cattle health.

Specific areas chosen for forage analysis were the Sallisaw area in eastern Oklahoma, the Range Cattle Minerals Station near Wilburton in southeastern Oklahoma, and the experimental beef cattle range at Lake C. P. Blackwell in north central Oklahoma. The latter area was considered a normal one while the Wilburton and Sallisaw areas were considered as typical afflicted areas.

REVIEW OF LITERATURE

Nutritional Importance of Manganese, Molybdenum, and Cobalt

Manganese: The complete role of manganese in animal nutrition is not understood. It is essential for normal development and growth of plants and animals, yet it is toxic to both at high levels of intake. Manganese has been shown by several workers to be required for reproduction, lactation, and proper bone formation of a number of animals. Among those animals known to require manganese are the rat (1), the rabbit (2), swine (3, 4), cattle (5), and chickens (6). Bulls require in excess of 28 parts per million for production of good quality semen (5). Chickens require 35 to 50 parts per million for the prevention of perosis (6).

Manganese is known to function in at least one enzyme system--liver arginase (7)--and it has been suggested that it functions in several other ones.

Soluble manganese salts, at high levels of intake, are toxic. According to Carratala and Carbonischi (8), rabbits fed manganous salts at levels of 0.7 to 0.9 mg of manganous sulfate or 0.1 to 0.94 mg of manganous chloride (equivalent to about 0.043 to 0.41 mg of elemental manganese) per day over a period of three weeks show liver degeneration and changes in the bones. Becker and McCollum (9) added up to 9,980 parts per million (ca. 1 percent of manganese) to the ration of rats and observed that reproduction was satisfactory although growth was somewhat retarded. Monier-Williams (10) states that for animals in general, the toxic level is 0.1 gram of manganese per pound of body weight per day.

Blakemore et al. (11) have expressed the opinion that grasses particularly rich in manganese (650 to 1320 parts per million) cause a lowering

of blood magnesium, leading to lactation tetany of cows and sheep. Their results have not been verified (12).

Chornock et al. (13), in studying the effect of manganese on calcium and phosphorus metabolism, fed large amounts of manganese to rats on a rachitic diet and obtained some very interesting results. When manganese was added to a ration having a high calcium-phosphorus ratio, it caused increased fecal excretion of both calcium and phosphorus. Calcium excretion increased as dietary manganese was increased, while phosphorus excretion was greatly accelerated. Large amounts of bone calcium and phosphorus were mobilized and excreted through the feces. Addition of potassium citrate or vitamin D brought about an abrupt reversal of this process. Addition of extra phosphorus brought about a remission of the rachitic condition.

Reid et al. (14), in experiments with cows in early stages of lactation, obtained results similar in some respects to those of Chornock. They found that manganese in amounts from 0.18 to 0.47 gram per day fed to cows receiving calcium and phosphorus in ratios which varied from 2.2 to 1.6 caused negative calcium balances, but had no effect on phosphorus balances. However, it should be pointed out that negative calcium and phosphorus balances are often observed in cows during the early part of lactation, even with liberal quantities of calcium and phosphorus in the ration.

Reid and Ward (15) observed that regardless of the quantity of manganese fed to cows (622.4 to 1325.6 mg per day) only 154.4 ± 9.8 mg per day are retained.

It is interesting to note that there is an apparent interrelationship between manganese and thiamin. In Sweden, military horses fed

roughage containing appreciable quantities of manganese developed a "forage anemia" which was corrected by thiamin (16). Perla and Sandberg (17) claim to have found an interrelationship of manganese and thiamin in rats; high levels of manganese accelerated thiamin depletion in rats fed a thiamin deficient ration.

Molybdenum: While plants require molybdenum for proper growth, it has never been shown to be an essential component of the diet of animals. It is toxic to ruminants when present at levels of 20 parts per million, causing scouring, loss of weight and condition, and sometimes death of animals.

Beath et al. (18) in 1935 reported that in Wyoming ruminants eating green forage containing 89 parts per million of molybdenum suffered pathological changes similar to selenium poisoning.

Near Somerset, in England, a condition known as "teart pastures" has existed for years. Ferguson et al. (19), in studying this condition, determined the mineral composition of the forage from teart and healthy pastures, and found molybdenum to be present to the extent of 20 to 100 parts per million in teart pastures and up to 5 parts per million in healthy pastures. They reported that cows were cured of molybdenum poisoning when fed from 1 to 2 grams of $CuSO_4 \cdot 5H_2O$ per day.

Comar et al. (20) and Monier-Williams (10) have noted the similarity of molybdenum toxicity to that of copper deficiency. Their observations are in accord with the report by Danish workers (21) of a condition in cattle on reclaimed land in Denmark. These cattle showed symptoms very similar to those of molybdenum poisoning, although no molybdenum was found in the forage. The condition was corrected by copper administration.

Comar et al. (20) have studied the interrelationships of copper, molybdenum and phosphorus in rats and cattle. In studying the effects of molybdenum on phosphorus metabolism they found that molybdenum does not affect the assimilation of phosphorus. Molybdenum and copper, administered together, reduced phosphorus accumulation in the tissues while phosphorus and molybdenum, administered together, reduced copper retention in rat and calf livers 2.5 to 5 fold. Copper and phosphorus apparently had little effect on the accumulation of molybdenum by the tissues.

Shirley et al. (22) studied the deposition and alimentary excretion of radioactive phosphorus in steers on high molybdenum and copper diets. They found that molybdenum changed the pathway of excretion of phosphorus to one where the majority of the phosphorus appeared in the feces. Molybdenum and copper administered together in large amounts produced the same effect, the magnitude, however, being somewhat less. Copper administered alone had the least effect on the path of phosphorus excretion.

Fragile bones have been found in animals fed high molybdenum diets (22). Comar et al. (20) have suggested that the effect of molybdenum may be

> ".....interference, due to a lowered liver copper, in enzyme systems necessary for skeletal metabolism; (b) inhibition of these enzyme systems by molybdenum; and (c) competition between phosphorus and molybdenum for deposition in the bone."

Cobalt: The knowledge of cobalt in animal nutrition has expanded quite rapidly since 1932. Orten et al. (23) in 1932 discovered that as little as 0.5 mg of cobalt per day in the diet of rats caused a decided polycythemia. This phenomena, which was confirmed by Stare and Elvehjem (24) the following year, has since been observed in a number of animals.

A disease of long standing known as enzoötic marasmus has been noted in West Australia. Animals affected are anemic and the disease is further characterized by lethargy, loss of weight, quick pulse, rapid respiration, loss of milk production, absence of estrus, and abortion. Filmer (25) in writing of this disease in 1933 suggested that it was due to a deficiency of a mineral necessary for the metabolism of iron. Filmer and Underwood (26) in 1934, as quoted by Neal and Ahmann (27), reported that 50 grams of limonite, an iron bearing ore, would cure the disease. They also found that a weak acid extract of the ore, free of iron, would cure the disease while the residue would not. They announced in 1935 (28) that of the minerals in the acid extract, they had isolated the active portion. This was found to be cobalt.

Analysis of the soil of pastures in New Zealand was made by Kidson (29) in 1937. He found that the cobalt content varied from 0.3 to 40 parts per million, with occasional samples containing even more. Kidson (30) in 1938 found that soils containing less than 4 parts per million were likely to cause cobalt deficiencies, while healthy soils contained 11 to 30 parts per million. Other workers, however, have shown a rather wide variation in the cobalt content of soil and the occurrence of cobalt deficiency disease (29, 31, 32).

Forages found to precipitate a cobalt deficiency in sheep in general contain less than 0.07 parts per million of this element while deficiencies in cattle occur if cobalt is present in quantities less than 0.04 parts per million (32). Lee (33) has reported the requirement for sheep and cattle to be about 0.1 mg per 100 pound of body weight per day. While this much cobalt may be required to prevent deficiency symptoms, very little is absorbed. Comar, Davis and Taylor (34), using radioactive

cobalt found that only 0.25 percent of a 1.33 mg dose administered orally to a steer was present in the liver 10 days later. Very little was found in the other tissues. Comar and Davis (35), studying the alimentary absorption of radioactive cobalt by young calves, found about 2.49 percent of a .06 mg dose in the liver 5 days later as compared to the maximum of 0.42 percent in the liver of mature animals (36) given the same size dose and killed 5 days later.

Cobalt is effective in relieving deficiencies only by oral administration (37).

The use of vitamin B_{12} to relieve cobalt deficiency has proved unsuccessful. Hale et al. (38) have shown that B_{12} synthesis in the rumen of cobalt deficient sheep is very poor, as shown by chick assay of the rumen contents. Later they showed that B_{12} was of no value in relieving the symptoms of cobalt deficiency (39).

Another interesting experiment by Gall et al. (40) has shown that the bacteria content of the rumen of cobalt deficient sheep is only approximately 55 percent as great as that in sheep receiving sufficient cobalt.

Analytical Methods for Manganese, Molybdenum, and Cobalt

Trace elements may be quantitatively determined in a number of ways. Procedures described in the chemical literature include gravimetric, colorimetric, polarographic, spectrographic (emission by arc and flame excitation) and microbiological methods. Of these procedures the colorimetric methods were chosen because of convenience and available equipment.

Manganese: Willard and Greathouse (41) in 1917 described a colorimetric method for the determination of manganese as permanganate with

periodate as the oxidizing agent that has proved to be accurate and the most versatile of any method yet described.

Although the above method leaves little to be desired, there are a number of other procedures worthy of mention. Sandell (42) has reviewed the literature quite completely and lists the following reagents as suitable, but not specific, for colorimetric determination of manganese following its oxidation to permanganate: ortho-tolidine, tetramethyldiaminodiphenylmethane, tetramethyldiaminotriphenylmethane, 4,4'- tetramethyldiaminodiphenylmethane, and benzidine. The reaction of manganese with formaldoxime in a basic solution has also been recommended.

Marshall in 1901, as quoted by Monier-Williams (10), described a method for the determination of manganese by oxidation with persulfate in the presence of a small amount of silver. This method as modified by Nydahl (43) in 1949 has the advantage over periodate when manganese is present at very low concentration. Collins and Foster (44) in 1924 used bismuthate ion in the presence of nitric acid as the oxidizing agent. Solarino (45) in 1928 described a method in which lead peroxide was used with nitric acid as the oxidizing agent. These two procedures have the disadvantage that the oxidizing agent is not sufficiently soluble to insure stability of the color formed.

Molybdenum: A number of reagents have been recommended for the colorimetric determination of molybdenum. Sandell (42) in reviewing the literature lists phenylhydrazine, potassium ethyl xanthate, sodium thiosulfate, and dithiol (4-methyl-1,2-dimercaptobenzene) as reagents for molybdenum. These reagents, however, are either not sufficiently sensitive to be of great importance or are subject to interference by other heavy metals.

Stanfield (46) in 1935 proposed a method for the determination of molybdenum by its reduction with stannous chloride in the presence of potassium thiocyanate. This method has undergone considerable modification (47, 48, 49), but has proved to be accurate and the most sensitive of any method described.

Cobalt: The determination of cobalt by colorimetric procedures has been accomplished with several reagents, with varying degrees of success. A number of reagents which form colored compounds with cobalt are listed by Sandell (42); however, only two are of sufficient sensitivity to receive further consideration.

Van Klooster (50) in 1921 observed that the nitroso derivative of the sodium salt of R-acid reacted with cobalt to give a water soluble compound having an intense red color. Stare and Elvehjem (24) in 1933 first used this reagent for the determination of cobalt in biological tissues. Kidson, Askew and Dixon (51) in 1936 and a number of workers after them have modified the procedure and increased its sensitivity (52, 53).

Ellis and Thompson (54) in 1945 presented a method for cobalt that is even more sensitive than the nitroso-R-salt procedure. In this procedure the dithizonate salt is formed and separated from interfering ions by extraction with carbon tetrachloride. A cobalt·ortho-nitroso-cresol complex is then formed, and its absorption in ligroin determined at 360 mu, the near ultraviolet region of the spectrum.

Sandell (42), Monier-Williams (10), and C. S. Piper (55) have discussed procedures in which metal complexes of dithizone (diphenylthiocarbazone) are formed and separated by extraction with the proper organic solvent. Scott and Mitchell (56) employed 8-hydroxyquinoline in the separation of trace elements for spectrographic analysis. Bayliss and

Pickering (57) have found that ammonium thiocyanate and 35 percent amyl alcohol in ether is very useful in extracting cobalt from aqueous solutions.

In the present experiments, the procedure of Beeson and Gregory (58) was followed. This procedure was used because of its applicability to plant material.

EXPERIMENTAL PROCEDURES

Samples: Grass and other green forages secured for chemical analysis were cut by hand from pastures located near Wilburton and Lake C. P. Blackwell. While part of these samples were representative of the entire pasture at each location, a few samples were representative of areas in a pasture where the cattle were observed to be grazing heavily. Other samples were of specific genus and species of grasses either taken in the field or separated from baled native-grass (prairie) hay produced in the particular area. Small samples of hay taken at random from a large number of bales were combined to form composite samples of native-grass hay from each location. Hay samples from the Sallisaw area were secured in this manner.

All samples were clipped into short lengths with shears and stored in half-gallon glass jars. Mineral analysis was made on these samples. A portion of each sample was ground in a Wiley mill for proximate analysis.

Ashing: While ashing in a muffle furnace was used exclusively in the analysis of these samples, it is well to mention the advantages and disadvantages of other ashing procedures. A rather complete discussion of ashing procedures has been given by Bailey and McHargue (59) as applied to the determination of copper, and by C. S. Piper (55) as applied to the determination of all the common trace elements. While it is generally recognized that wet ashing with combinations of nitric, sulfuric, and perchloric acids may in some instances be superior to dry ashing, lack of available hood space made it impossible to carry out this type of preparation. Wet ashing is less time consuming than dry ashing and reduces the possibility of formation of insoluble silicates or volatilization of the

elements being determined. However, it usually presents the problem of disposing of a large amount of strong acid before proceeding with the analysis.

Dry ashing is most successfully accomplished in a muffle furnace equipped with controls to prevent over heating. This has the advantage of affording careful temperature control, while flame procedures do not. Sandell (42) shows that in samples ashed in a muffle furnace, the quantity of manganese forming insoluble silicates is considerably less than that formed by ashing over a burner. The quantity forming insoluble silicates at low muffle furnace temperatures (500° C.) is about 1.0 percent of the total manganese present in the sample. The quantity of molybdenum forming insoluble silicates under these conditions is negligible as shown by spectrographic analysis of the silica residue by Barshad (49). Both wet and dry ashing have been used by other investigators in the determination of cobalt with equal success (59).

Manganese: The method used in the determination of manganese was a modification of the method of Willard and Greathouse (41). Because of the relatively high manganese content of the forages, the same sample was used for the determination of both manganese and molybdenum.

A ten-gram sample of forage was weighed into a 150 ml pyrex beaker and ashed in a muffle furnace overnight at a temperature of 500° to 550° C.. The ash was taken up with 25 ml of 1:1 HCl (1 part of concentrated HCl to 1 part of water), the solution heated to boiling and digested for four hours on an electric hot plate. A steam plate, however, is to be preferred for this digestion and subsequent heating. The solution was then evaporated to dryness and the silica dehydrated by further heating of the dry

residue for one hour at 100° C.. The residue was again taken up in 1:1 HCl and heated near boiling for another four hours to assure complete solution of all the minerals present.

The entire contents of the beaker was transferred to a 200 ml volumetric flask and made to volume. From this solution a 10 or 25 ml aliquot was transferred to a 50 ml beaker. To this solution 5 ml of 1:1 nitric acid was added and the solution evaporated to dryness. This evaporation is necessary to remove chlorides and any oxidizable material (60) that would use excess periodate or cause fading of the permanganate color by reduction of the manganese to a lower state of oxidation. This step was repeated twice to assure complete removal of chlorides and oxidation of any extraneous material.

The chloride-free residue was then taken up in 10 ml of 1 N nitric acid and 1.0 ml of ortho-phosphoric acid added to deionize and decolorize the ferric ion, thus preventing its possible interference (61). Phosphoric acid, in addition to preventing the precipitation of ferric periodate and decolorizing iron, also prevents the precipitation of manganese periodates, iodates, and hydrated manganese oxides.

The solution was heated to near boiling and about 50 mg of potassium periodate were added in small amounts to convert the manganous ion to the colored permanganate ion. The beaker was covered with a watch glass and the solution kept near boiling for an hour--or until the color had reached its maximum intensity. The solution was then made to volume, transferred to a colorimeter tube and transmittance measured at 515 mu in an Evelyn photoelectric colorimeter. The concentration of manganese was found by use of a standard curve prepared from known quantities of manganese.

The concentration of acid in the solution in which the color development is being carried out is of some importance. Unless the concentration of manganese is very small, the acid concentration may be increased considerably over the quantity required to prevent precipitation of periodates. The speed of oxidation is increased if the concentration of acid is above 3.5 N (60).

The reaction of periodate with manganese in hot acid solution is as follows: $2Mn^{++} + 5IO_4^- + 3H_2O \text{ ----- } 2MnO_4^- + 5IO_3^- + 6H^+$

Molybdenum: In the determination of molybdenum, the method of Barshad (49) was followed with slight modifications. In this method the thiocyanate complex of quinquevalent molybdenum is formed, the color of which is proportional to the concentration of molybdenum present and dependent to some extent on the acidity of the solution.

The sample was prepared as described under the determination of manganese. An aliquot, usually 150 ml of the hydrochloric acid solution, was transferred to a 150 ml beaker and evaporated to dryness. The residue was taken up in 4 ml of hot 14 percent HCl (14 ml of concentrated HCl and 86 ml of water) and transferred to a 50 ml centrifuge tube. The beaker was rinsed with four 4-ml portions of the HCl solution and the rinsings added to the centrifuge tube.

To this solution there were added in the following order: 0.25 ml of 5 N sodium nitrate, 2 drops of 0.01 N ferric chloride, and 1.5 ml of 10 percent (w/v) potassium thiocyanate. The solution was then diluted to 23.5 ml with 14 percent HCl. The colored molybdenum complex was then formed by the addition of 1.5 ml of 10 percent (w/v) stannous chloride in 1:9 HCl. Each addition of reagent was followed by shaking to insure complete mixing.

After the addition of stannous chloride the analysis may be handled by either of two procedures. The percent transmission may be determined on the aqueous solution, or the color may be extracted by a suitable organic solvent and transmission determined on the non-aqueous extract. In the present study, 15 ml of diethyl ether, saturated by shaking with 10 percent stannous chloride and 10 percent potassium thiocyanate, was used to extract the colored molybdenum complex. After the ether was added, the solution was shaken vigorously for 30 seconds and percent transmission of the ether extract determined at 470 mu.

The presence and order of addition of each reagent is of extreme importance. Iron in amounts no less than the concentration of molybdenum is necessary for the full development and stability of color (48). Nitrate ion is added to oxidize any molybdenum present in a low state of oxidation to hexavalent molybdenum. It also prevents the reduction of molybdenum to less than quinquevalency (49).

The thiocyanate ion should be present in excess, as the complex formed by quinquevalent molybdenum and thiocyanate is an equilibrium reaction (62). Thus the thiocyanate ion concentration should be considerably higher than required for direct combination. This was noted by Sandell (42) and others who state that the concentration of potassium thiocyanate should be from 0.4 to 0.8 percent, while Perrin (63) states that the concentration should not be less than 1.0 percent.

For maximum intensity of color, the solution should have an acidity equivalent to about 1.5 to 2.5 N HCl (64). For that reason, the color was developed in a solution of 14 percent (ca. 1.7 N) HCl.

Contrary to the finding of Barshad (49), there is a definite intensification of the color of the molybdenum-thiocyanate complex in the ether

solution. The aqueous solution does not obey Beer's law above a certain concentration. The ether solution closely obeys Beer's law at the low concentrations used.

It has also been noted that a number of authors, when performing an extraction, repeat the extraction until there is no more color present in the extracting solvent. It should be pointed out that in the case where the solubility of the solute in the extracting solvent is not exceeded, one extraction only need be made where the volumes of the two solutions are held constant. The quantity extracted is a function of the distribution coefficient and even though the extraction may not be complete, the percent extracted remains constant. The use of the standard curve prepared in like manner makes this type of extraction as accurate as where complete extraction is carried out.

Results of recovery experiments in which known amounts of molybdenum were added to grass samples are shown in table 3.

Cobalt: The determination of cobalt was made by the method of Beeson and Gregory (58). A 10-gram sample was ashed and taken up in 25 ml of 1:1 HCl as for manganese. The contents of the beaker were transferred to a 100 ml volumetric flask and an aliquot, usually 75 ml, transferred to a 100 ml beaker. To bring this solution to a small volume it was evaporated to dryness and the residue dissolved in 5 ml of 0.5 N HCl and 5 ml of distilled water. To this solution 2 ml of a 1 percent solution of nitroso-R-salt was added, followed by approximately 2 grams of sodium acetate. The solution was then heated to about 70° C., and neutralized with 20 percent sodium hydroxide, phenolphthalein being used as the indicator. The red phenolphthalein color was just discharged with 0.5 N HCl. The solution was then brought to boiling to hasten the development of the cobalt•nitroso-R-salt

color. Five ml of 1:1 nitric acid were then added and boiling continued for 2 minutes to destroy colored complexes of ions other than cobalt (50). The solution was cooled, made to volume and transmittance measured at 490 mu.

A number of items should be carefully controlled in the development of color in this procedure. The colored nitroso-R-salt should be added in exact amounts.

The length of time the solution is boiled after the addition of nitric acid is also important. Excessive boiling causes a bleaching of the color. Solutions of known amounts of cobalt were subjected to boiling, after the addition of nitric acid, for 1, 2, 3, 4, and 5 minutes. It was found that boiling for more than 2 minutes decreased the intensity of the color. These results are shown in table 7.

The presence of other ions has received considerable attention by a number of workers. In a series of recovery experiments it was found that 12.8 mg percent of manganese did not interfere greatly while 19.2 mg percent of manganese caused severe interference. These results are shown in table 5.

The presence of iron, regardless of the quantity present, caused an error of about 10 percent. Results of recovery experiments in which iron was added to cobalt solutions are shown in table 6.

Standard Solutions of Manganese, Molybdenum, and Cobalt

Manganese: Standard solutions of manganese were prepared by dilution of a 0.0496 N solution of $KMnO_4$ which had been standardized against sodium oxalate. 45.97 ml of this stock solution, containing 0.5448 mg of manganese per ml, were diluted to 1 liter to make a solution in which 1 ml contained 25 mcgms of manganese. Aliquots of this solution, containing from 25 to 275

mcgms of manganese, were oxidized with periodate as described under procedure and made to a volume of 50 ml. Transmittance was determined on the solutions at 515 mu and the galvanometer readings plotted against concentrations on semi-logarithmic graph paper. The resulting points formed a straight line. The K_1 value for each galvanometer reading was calculated by the formula:

$$\text{Log}\frac{I_o}{I} = klC \qquad k1 = K_1 \qquad \text{or} \quad \frac{\text{Log 100 - Log Galv defl}}{\text{conc in mg per ml soln}} = K_1$$

The average K_1 value was 0.636.

Molybdenum: For the preparation of a standard solution of molybdenum 0.9198 gram of C.P. $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ was dissolved in one liter of distilled water. This solution contained 0.5 mg of molybdenum per ml. Aliquots of this solution were diluted to make solutions containing 1, 5, 10, and 25 mcgms of molybdenum per ml. The standard curves were prepared from these solutions, as described under procedures. The galvanometer readings were plotted against concentrations on semi-logarithmic graph paper. Since the color in aqueous solution does not follow Beer's law, the K_1 values were not constant. The K_1 value for the color in ether solution was found to be 0.0042, when molybdenum is expressed in micrograms per ml of solution.

Cobalt: The standard solution for cobalt was prepared by dissolving 4.0372 grams of $CoCl_2\cdot 6H_2O$ in one liter of water. From this solution, which contained 1 mg of cobalt per ml, solutions containing 2 and 5 mcgms of cobalt per ml were prepared. On aliquots of these solutions, containing from 5 to 25 mcgms of cobalt, the color was developed as described under procedure, and the curve prepared by plotting galvanometer deflections against concentrations on semi-logarithmic graph paper. The resulting curve was found to be a straight line. The value of K_1 was found to be 0.00436 when cobalt was expressed in micrograms per ml.

RESULTS AND DISCUSSION

Table 1 gives the date of collection and description of grass and forage samples taken from the eastern, southeastern and north central parts of Oklahoma for the determination of manganese, molybdenum and cobalt. The results of the determinations are presented in table 2. The calcium, phosphorus and magnesium content of a large number of these samples is also presented in table 2 in order to bring out any relationship between these more common elements and the trace minerals under investigation.

The average manganese content of three samples of prairie hay produced in the Sallisaw area in 1949 and one sample produced in 1950 was 417 parts per million. The manganese content of similar native hays produced near Wilburton in 1948, 1949, and 1950 ranged from 150 to 270 parts per million while hay produced during corresponding years in the Lake C. P. Blackwell area only contained from 25 to 75 parts per million. The results clearly show that despite considerable variation in the manganese content of different samples of hay produced in the same location, hay produced at the Sallisaw and Wilburton areas in eastern and southeastern Oklahoma, respectively, contains far greater quantities of manganese than that produced at the Lake C. P. Blackwell area in north central Oklahoma. If the average manganese content of the Blackwell hay samples is given a rating of 1, the average values for the Wilburton and Sallisaw areas would be rated 4.6 and 9.0, respectively.

The amount of manganese in these different hay samples was unrelated to their content of calcium or phosphorus. The Blackwell hay was lower than the Wilburton hay in magnesium; the magnesium content of the Sallisaw hay was not determined.

Climax grasses separated from the Wilburton and Blackwell hays collected

TABLE 1

Samples

Sample number	Laboratory number	Description
Hay samples from Sallisaw area.		
1	49-576-18	Baled prairie hay cut early in June, 1949
2	49-576-19	Baled prairie hay cut in the summer of 1949
3	49-576-20	Baled prairie hay cut in the summer of 1949
4	51-576-15	Baled prairie hay cut in the summer of 1950
Samples from the Range Cattle Minerals Station near Wilburton.		
5	48-526-68	Baled prairie hay cut in the summer of 1948
6	49-647-1	Baled prairie hay cut in the fall of 1949
7	49-526-84	Baled prairie hay cut in the summer of 1949
8	50-526-97	Baled prairie hay purchased in the winter of 1949-50
9	50-526-118	Baled prairie hay cut in the summer of 1950
10	49-526-81	Climax grasses separated from Sample 7
11	50-526-112	Big Bluestem grass sampled in October, 1950
12	50-526-113	Little Bluestem grass sampled in October, 1950
13	50-526-114	Indian grass sampled in October, 1950
14	50-526-102	Big Bluestem grass sampled in June, 1950
15	50-526-103	Little Bluestem grass sampled in June, 1950
16	49-526-82	Weedy grasses separated from Sample 7
17	49-526-83	Foreign matter (weeds) separated from Sample 7
18	50-526-117	False dandelion (weed) sampled in October, 1950
19	50-526-116	Broom weed or many-flowered aster sampled in October, 1950
20	50-526-115	Dog-hair grass sampled in October, 1950
21	51-526-138	Spring grass sampled in April, 1951
22	51-526-139	Spring grass sampled in April, 1951
23	51-526-140	Dog-hair grass sampled in April, 1951
Samples from the Lake C. P. Blackwell experimental range.		
24	48-526-73	Baled prairie hay cut in the summer of 1948
25*	49-647-2	Baled prairie hay cut in the summer of 1949
26	49-647-3	Baled prairie hay cut in the summer of 1949
27	49-526-80	Baled prairie hay cut in the summer of 1949
28	50-526-119	Baled prairie hay cut in the summer of 1950
29	49-526-78	Climax grasses separated from Sample 27
30	50-526-120	Big Bluestem grass sampled in October, 1950
31	50-526-121	Little Bluestem grass sampled in October, 1950
32	50-526-122	Indian grass sampled in October, 1950

*This sample was from a meadow that had been fertilized with superphosphate.

TABLE 2

Results of Analyses

Sample number	Description	Mineral composition on a dry matter basis					
		Mn	Mo	Co	Ca	P	Mg
Hay samples from Sallisaw area.		ppm	ppm	ppm	%	%	%
1	Baled prairie hay	380	.11	.16	.438	.076	N.D.
2	Baled prairie hay	567	.11	.14	.429	.083	N.D.
3	Baled prairie hay	321	.08	.24	.489	.074	N.D.
4	Baled prairie hay	400	.05	—	N.D.	N.D.	N.D.
Samples from the Range Cattle Minerals Station near Wilburton.							
5	Baled prairie hay	253	—	.25	.524	.053	.516
6	Baled prairie hay	150	.03	.13	.65	.056	.339
7	Baled prairie hay	270	.02	.07	.75	.047	.406
8	Baled prairie hay	170	.07	.11	.53	.086	.334
9	Baled prairie hay	215	.05	.03	.44	.052	N.D.
10	Climax grasses	290	.02	.07	.57	.045	.330
11	Big Bluestem grass	205	.15	.17	.324	.035	.113
12	Little Bluestem grass	224	.14	.12	.363	.035	.083
13	Indian grass	210	.14	.12	.285	.031	.099
14	Big Bluestem grass	168	.06	.09	.332	.070	.221
15	Little Bluestem grass	219	.13	.08	.322	.069	.176
16	Weedy grasses	342	.25	N.S.	.41	.056	.301
17	Foreign material (weeds)	455	.19	N.S.	1.22	.052	.509
18	False dandelion	231	—	.03	1.111	.043	.342
19	Broom weed	881	.03	—	.748	.115	.213
20	Dog-hair grass	730	3.39	—	.372	.087	.150
21*	Spring grass	267	N.S.	.11	N.D.	N.D.	N.D.
22*	Spring grass	269	.11	N.S.	N.D.	N.D.	N.D.
23*	Dog-hair grass	480	.45	—	N.D.	N.D.	N.D.
Samples from the Lake C. P. Blackwell experimental range.							
24	Baled prairie hay	75	.14	.26	.442	.061	.159
25	Baled prairie hay	25	.18	.26	.54	.078	.221
26	Baled prairie hay	32	.13	.19	.38	.049	.115
27	Baled prairie hay	30	.14	.29	.46	.064	.117
28	Baled prairie hay	69	.13	.03	.32	.079	N.D.
29	Climax grasses	45	.30	.29	.40	.067	.153
30	Big Bluestem grass	47	.11	.03	.22	.032	N.D.
31	Little Bluestem grass	47	.06	.07	.25	.018	N.D.
32	Indian grass	47	.11	.12	.25	.018	N.D.

*Mineral composition of these samples expressed on the air-dry basis
N.D. — Not determined
N.S. — Insufficient sample for this determination

in 1949 showed differences in manganese content similar to the hays from these two areas, the grasses from the Wilburton hay containing 290 parts per million and those from the Blackwell hay containing only 45 parts per million. The high manganese content of the Wilburton hay, therefore, can not be attributed entirely to the presence of a large proportion of weeds or foreign grasses.

A comparison of hand-cut samples of dry grass of the same genus and species taken from the Wilburton and Lake C. P. Blackwell areas in October, 1950, also shows the relatively higher manganese content of the Wilburton grass. Big Bluestem grass from the Wilburton station contained 205 parts per million of manganese as compared to 47 parts per million in the sample from the Lake C. P. Blackwell range. Little Bluestem grass from these two areas contained 224 and 47 parts per million of manganese, respectively. Indian grass from these two areas showed similar differences in manganese content. Individual species of grass were not available from the Sallisaw area. Further comparisons of grass species from the Wilburton and Lake C. P. Blackwell areas were not possible, since collection of grass from the latter area was discontinued during the grazing season of 1950 and early spring of 1951.

Weedy grasses (genus Panicum) and foreign material consisting mostly of weeds and stemmy unclassified material separated from the 1949 Wilburton hay sample contained 342 and 455 parts per million of manganese, respectively. Other samples of weeds and one weedy grass, "dog-hair grass", were collected from the pastures in October of 1950 for manganese determination. One weed, commonly known as "broom weed" or "many-flowered aster", contained 881 parts per million of manganese. Another weed from the same area, "false dandelion", contained only 231 parts per million.

The "dog-hair grass", which was found growing near one of the range ponds contained 730 parts per million of manganese, while a sample of this grass taken in April, 1951, contained 480 parts per million.

Two samples of early spring grass taken in April, 1951, from the south and southeast pastures at the station near Wilburton contained 267 and 269 parts per million of manganese, a surprisingly high manganese content for early spring grasses. Soil samples were taken at the same time for manganese determination by the Agronomy Department. One sample, representative of an area near the pond in the south pasture where the cattle grazed heavily, contained 1440 parts per million of available (water soluble) manganese and a total of 3340 parts per million. The second sample, representative of the entire southeast pasture, contained 1350 parts per million of available manganese and a total of 2930 parts per million.

Comparisons of the concentration of manganese in the plants from these three areas with concentrations considered normal show that only the hays from the Lake C. P. Blackwell area are within the normal range of 50 to 100 parts per million as given by Monier-Williams (10).

Molybdenum content of the various forage and grass samples showed considerable variation. Of the prairie hay samples, those from the Wilburton station were the lowest in molybdenum, 0.02 to 0.07 parts per million, while those from the Blackwell area contained from 0.13 to 0.18 parts per million. Dry fall grass collected in October from the Wilburton area, however, contained about 0.14 parts per million of molybdenum, which is about the same amount as was found in similar dry grass from Lake C. P. Blackwell.

These values are much lower than those reported by Ferguson et al. (19), who found that most pasture grasses in England contained 5 parts per million or less of molybdenum.

"Broom weed", which was exceptionally high in manganese, contained only 0.03 parts per million of molybdenum, while one sample of "dog-hair grass", also exceptionally high in manganese, contained over 3 parts per million of molybdenum. No molybdenum could be detected in the sample of "false dandelion".

From these comparisons and other values presented in table 2, it does not appear that the molybdenum content of the plants is related to their manganese content, or for that matter, to their content of such common elements as calcium, phosphorus, and magnesium.

Recovery experiments presented in table 3 show that molybdenum added to plant material, either before or after ashing, can be recovered to the extent of 97 to 100 percent.

TABLE 3

Recovery of molybdenum

Molybdenum in sample	Molybdenum added	Molybdenum found	Molybdenum recovered	Percent recovered
mcgm/gm	mcgm/gm	mcgm/gm	mcgm/gm	
0.0	3.5*	3.5	3.5	100
0.0	3.5**	3.5	3.5	100
0.0	6.6*	6.6	6.6	100
0.0	6.6**	6.4	6.4	97

*Molybdenum added before sample was ashed
**Molybdenum added after sample was ashed but before subsequent treatment

The values for the cobalt content of the different grass and forage samples shown in table 2 must be considered as only relative. Recovery experiments presented in table 4 show inconsistent recoveries of cobalt added in increasing amounts to plant material previous to ashing.

Comparison of results of experiments in which cobalt was added to the plant material before and after ashing indicate that losses occured after the samples were ashed. The nature of the results do not preclude the possibility, however, that some cobalt was lost by volatilization or by formation of insoluble silicates.

TABLE 4

Recovery of cobalt

Sample number	Cobalt in sample	Cobalt added	Cobalt found	Added cobalt recovered	Percent of added cobalt recovered
	mcgm/gm	mcgm/gm	mcgm/gm	mcgm/gm	
Cobalt added before ashing.					
35	0.083	0.5	0.516	0.433	86.6
	0.083	1.0	0.866	0.783	78.3
	0.083	1.5	1.349	1.266	84.3
36	0.219	0.3	0.406	0.187	62.3
	0.219	0.3	0.438	0.219	73.0
	0.219	0.3	0.406	0.187	62.3
	0.219	0.5	0.549	0.385	77.0
	0.219	0.5	0.500	0.281	56.2
	0.219	0.5	0.672	0.453	90.6
37	0.12	2.0	1.65	1.53	76.5
	0.12	2.0	1.38	1.26	63.0
	0.12	3.0	2.88	2.76	92.0
	0.12	3.0	2.45	2.33	77.7
	0.12	4.0	3.40	3.28	80.2
	0.12	4.0	4.00	3.88	97.0
38	—*	5.0	4.40	4.40	88.0
	—*	5.0	4.25	4.25	85.3
	—*	5.0	4.60	4.60	92.2
	—*	5.0	4.45	4.45	89.1

TABLE 4 (continued)

Cobalt added after ashing but before subsequent treatment.					
35	0.083	0.5	0.533	0.450	90.0
	0.083	1.0	0.882	0.799	79.9
	0.083	1.5	1.232	1.149	76.5
38	—*	5.0	4.95	4.95	99.0
	—*	5.0	4.90	4.90	98.0
	—*	5.0	4.40	4.40	88.0
	—*	5.0	4.85	4.85	97.0
Cobalt added to one of duplicate samples just previous to color development.					
30	0.30	0.0	0.30	—	—
	0.30	2.0	1.90	1.60	80.0
31	0.15	0.0	0.15	—	—
	0.15	2.0	1.50	1.35	67.5
32	0.20	0.0	0.20	—	—
	0.20	2.0	1.90	1.70	85.0

*Because of the large quantity of cobalt added, the cobalt present was considered to be negligible.

The possibility of interfering ions causing failure of color development or bleaching of the final color is clearly shown in the results of recovery experiments in the last part of table 4. In these experiments, equal amounts of cobalt were added to samples 30, 31, and 32 just previous to color development and their color compared to duplicates, without added cobalt, handled in exactly the same manner. Colorimetric measurement gave recovery values of 67.5, 80.0, and 85.0 percent of the theoretical amount added. The possibility that maximum color development was prevented by the presence of another ion is in agreement with the data shown in table 5, concerned with the interference of manganese in the determination of cobalt. The presence of 12.8 mg percent of manganese in the final solution used for color development can be tolerated, however, the presence of over 19.0

mg percent of manganese caused severe interference. The final solution of samples 30, 31, and 32 contained 2 mg percent of manganese. It appears, therefore, that some other ions were causing interference.

TABLE 5

Effect of added manganese on cobalt recovery

Cobalt in standard solution	Manganese added	Cobalt found	Percent recovered
mcgms	mg percent	mcgms	
0.00	6.4	.20	—
3.80	6.4	4.15	109.2
3.80	12.8	4.10	108.0
3.80	19.2	2.25	59.2
3.80	25.6	0.75	19.7
3.80	32.0	0.15	4.1
3.80	38.4	0.0	0.0
3.80	44.8	0.0	0.0
3.80	51.2	0.0	0.0
3.80	0.0	3.80	100.0

TABLE 6

Effect of added iron on cobalt recovery

Cobalt in standard solution	Iron added	Cobalt found	Percent recovered
mcgms	mg percent	mcgms	
3.80	0.0	3.80	100.0
3.80	11.6	4.20	110.5
3.80	23.2	4.20	110.5
3.80	34.8	4.25	111.8
3.80	46.4	4.15	109.2
3.80	58.1	4.10	108.0
3.80	69.7	3.80	100.0
3.80	81.3	4.20	110.5
3.80	92.9	4.15	109.2

The effect of iron, as shown in the recovery data in table 6, is relatively slight and opposed to that of manganese. It increases color intensity slightly and gives high results.

Excessive boiling of the solution of the cobalt-nitroso-R-salt after color development causes bleaching of the color. Data in table 7 show that boiling in excess of 2 minutes causes a definite reduction in the intensity of the color.

TABLE 7

Effect of boiling time on recovery of cobalt

Cobalt in standard solution	Length of boiling in minutes	Cobalt found
mcgms		mcgms
3.70	1	3.70
3.70	2	3.70
3.70	3	3.65
3.70	4	3.40
3.70	5	3.45

From these recovery studies it is suggested that all the forage samples shown in table 2 apparently contained cobalt in concentrations too small for accurate determination by present colorimetric procedures. That these forages are not cobalt deficient is indicated in the results of feeding trials conducted by the Department of Animal Husbandry.

SUMMARY

Methods for the determination of manganese, molybdenum, and cobalt were investigated for the purpose of selecting those methods most applicable to the determination of these elements in native forages.

Satisfactory methods were found for the determination of manganese and molybdenum which could be adapted to general plant analysis procedures. The method for cobalt, however, did not prove to be satisfactory due largely to the minute amounts of cobalt in plant material and unsatisfactory procedures for its separation from other interfering elements. Manganese in relatively low concentrations caused serious interference in the determination of cobalt.

Determinations of manganese, molybdenum, and cobalt were made on numerous samples of hay, grass, and other plant material from the Sallisaw area in eastern Oklahoma, the Range Cattle Minerals Station near Wilburton in southeastern Oklahoma, and the Lake C. P. Blackwell experimental range in the north central part of Oklahoma.

Prairie hays from the Sallisaw area were found to contain an average of 417 parts per million of manganese and 0.09 parts per million of molybdenum. Similar hay from the Wilburton area contained an average of 212 parts per million of manganese and 0.04 parts per million of molybdenum, while hay from the Lake C. P. Blackwell range contained an average of 46 parts per million of manganese and 0.14 parts per million of molybdenum.

Mature Big Bluestem, Little Bluestem, and Indian grasses, which make up the climax grasses in native pastures, from the Wilburton area contained 205 parts per million of manganese and .14 parts per million of molybdenum, while grasses of the same species from the Blackwell area contained 47 and 0.09 parts per million of manganese and molybdenum, respectively.

Weeds and weedy grasses from the Wilburton area contained manganese in excess of 230 parts per million, one sample of "broom weed" containing 881 parts per million. A sample of "dog-hair grass" from this area containing 730 parts per million of manganese had a molybdenum content of over 3 parts per million. Other samples of weeds from Wilburton contained an average of 0.1 parts per million of molybdenum.

Less than 0.3 parts per million of cobalt was found in the various grasses and weeds from the different areas. The accuracy of these cobalt determinations is in doubt.

The concentration of manganese, molybdenum, and cobalt in the forage samples appeared to be unrelated to the concentration of calcium, phosphorus and magnesium.

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