Occurrence and Toxicology of Selenium in Halogeton and Associated Species[1]

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Seleniferous soils occur throughout extensive areas in the western United States, Canada, and Mexico. In the semiarid regions of the Great Plains and Rocky Mountains where annual precipitation is 20 inches or less, many soils contain sufficient quantities of selenium to produce plants high in selenium.

Halogeton glomeratus (M. Bieb.) C. A. Mey., first identified in the United States during the mid-thirties, did not invade the seleniferous areas of Wyoming and eastern Utah until after most of the mapping of seleniferous soils and analyses of flora indigenous to these regions had been completed. Therefore, this species had not been studied for its ability to accumulate selenium or the effect such accumulation might have on its toxicity to sheep. In addition, no studies had been conducted to determine whether sheep would be more adversely affected by consuming both halogeton and selenium-bearing species. Ingesting both seleniferous species and halogeton would involve intake of two separate and very toxic compounds.

Halogeton is poisonous to livestock, especially sheep, because of its high soluble oxalate content. The leaves of this annual may contain over 20 per cent soluble oxalates (dry weight) after June 15, and more than 30 to 35 percent soluble oxalates by mid-October. The soluble oxalate content of the whole plant varies during the season but may reach 16 to 18 percent when the plant is fully mature.

Highly seleniferous soils in Utah occur principally in the east central area of the state. Soils derived from the Morrison formation of the Jurassic system and the Mancos formation of the Cretaceous system are high in selenium and usually support a variety of seleniferous plants wherever outcrops occur. Many of these plants are utilized as forage by livestock. The Morrison is widely exposed in an area southeast of Thompson, Utah, and immediately east and northeast of the Arches National Monument. Here the Salt Wash Sandstone member of the formation forms the so-called "poison strips" which contain such high levels of selenium that nearly all vegetation rooted there is seleniferous. Trelease and Beath (1949) reported numerous losses of cattle and sheep from selenium poisoning in this area. The Mancos Formation is exposed in the vicinity of Cisco, Utah, and highly seleniferous indicator species are prevalent on derived soil. Indicator plants are species of certain genera which absorb large quantities of selenium and thrive only on seleniferous soils (Trelease and Beath, 1949).

The invasion of halogeton east of the Wasatch Mountains in Utah has to date been limited principally to the Uinta Basin and an area adjacent to U. S. Highway 50 from Price, Utah, to Grand Junction, Colorado. The spread of halogeton over the seleniferous areas from Green River to Cisco, Utah, and south of Thompson was accelerated in part by the extensive uranium boom during the 1940's and early 1950's. Construction of new roads and disturbance of the soil by prospectors and miners created an ecological situation favorable for invasion by halogeton. Today, halogeton is widespread throughout the mining area and may be found in dense stands on deposits of ore waste, at mine entrances, along the borders of roads, and in washes. It is still generally confined to disturbed sites in the Yellow Cat Mining District and has not yet invaded the surrounding desert.

The research reported was initiated to study the ability of halogeton to absorb selenium and to determine the effect of the combination of selenium and soluble oxalates on sheep.

Methods

Greenhouse

Halogeton seeds were germinated in sand on a greenhouse bench. After germination, the sand was kept moist with a 1:4 dilution of Hoagland's nutrient solution. A 0.001 M solution of NaCl was added to the sand to stimulate vigorous growth (Williams, 1960). When the first leaves appeared, two plants were transferred to one-gallon polyethylene containers with full-strength Hoagland's solution, 0.01 M NaCl, and 0, 2, 4, and 8 ppm of selenium as Na_2SeO_4. There were six replications per treatment. Each container was aerated continuously via a capillary tube from a 1/6-HP pump.

The solutions were replaced every two weeks. The plants were harvested at the end of six weeks and divided into leaf and stem fractions. These fractions were weighed fresh, and then divided into two samples of equal weight. One sample was digested immediately in HNO_3 and H_2SO_4 with HgO fixative; the other was dried at 60 degrees

[1] *Cooperative investigation of the Crops Research Division and Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Utah Agricultural Experiment Station.*

C for 24 hours. Both samples were analyzed for selenium (Lepper *et al*, 1950). A fraction of the leaf sample was analyzed for soluble oxalates (Dye, 1956).

Plant Collections

Five sites were chosen for study: 6.5 miles west of Green River, ½ mile north of Crescent Junction, 1 mile east of Thompson, 1 mile west of Cisco, and 11 miles southeast of Thompson near the Yellow Cat mine. The first four sites were adjacent to Highway U.S. 50.

Halogeton was collected at these sites between the 14th and 16th of the month beginning in June and ending in November. In addition, samples of fourwing saltbush, *Atriplex canescens* (Pursh) Nutt., milk vetch, *Astragalus preussii* A. Gray, and dropseed, *Sporobolus flexuosus* (Thurb.) Rydb. were collected occasionally.

Two 50-gram fresh samples of halogeton leaves (and later flowers, sepals, and seeds) and two 25-gram samples of stems were collected. One sample was placed in a cardboard container and oven-dried at 60 degrees C upon return to the laboratory. The other sample was immediately placed in a pint polyethylene bottle containing 100 ml HNO_3, 50 ml H_2SO_4, and 10 ml of a fixative solution containing 1 gram of HgO. These methods were used to determine the volatility of selenium in halogeton. In many plants, particularly those which absorb large quantities of selenium, a considerable portion of the element is lost through volatilization by air and oven drying. This error is minimized when fresh material is digested upon harvest. The Hg in the digestion solution prevents loss of selenium by combining with this element to form a nonvolatile compound. The digestion mixture was prepared in the laboratory. The fixative was prepared separately and added to the acid in the field just before the addition of the plant material.

Table 1. Dry weight and selenium and soluble oxalate contents of halogeton grown in nutrient solution containing 0, 2, 4, and 8 ppm selenium.

Sample	Selenium added	Dry weight per plant	Selenium in		Soluble oxalates
			Digested sample	Oven-dried sample	
	ppm	mg	ppm	ppm	Percent
Leaves	0	210	0.0	0.0	14.9
Leaves	2	190	70.7	55.4	13.5
Stems	2	90	27.8	14.5	.....
Leaves	4	190	128.2	126.7	12.4
Stems	4	70	129.8	79.2	.....
Leaves	8	140	500.2	229.7	9.0
Stems	8	60	264.8	207.8	.....

The same procedures were used for collection and analysis of the other species except that the terminal 4 inches of branches and leaves of milk vetch and fourwing saltbush was analyzed and whole plant samples were taken of the dropseed.

The leaf or the leaf-seed-sepal fraction of halogeton was analyzed for soluble oxalates to determine whether uptake of selenium affected the amount of soluble oxalates which would be formed. Soil samples 1 to 12 and 12 to 24 inches in depth were taken for selenium analysis at Yellow Cat and Cisco at the site of maximum halogeton infestation.

Precipitation from January 1 to October 15, 1959, measured only 2.83 inches at the weather station at Green River. Of this, only 0.3 inch fell between April 19 and August 18.

Sheep Feeding Experiments

Halogeton containing 15.3 percent soluble oxalates and 11 ppm selenium was harvested 1 mile west of Cisco, Utah, in September, 1959. *Astragalus preussii* and *Atriplex canescens* containing 2044 ppm and 295 ppm selenium, respectively, were collected in August near the Yellow Cat mine. Nonseleniferous halogeton was collected west of Snowville, Utah. The plant material was dried at 60 degrees C, ground to a 20-mesh powder, and stored in airtight polyethylene containers and bags.

Nine rumen-fistulated ewes 3 to 5 years old were divided into 3 groups of 3 each. Halogeton was fed to all animals at a level to provide a daily dose of 847 mg of soluble oxalate per kg of body weight.

Group one was fed seleniferous halogeton which provided 0.061 mg selenium per kg of body weight daily. Group two received nonseleniferous halogeton and sufficient seleniferous milk vetch to provide a daily dose of 0.167 mg of selenium per kg of body weight. Group three was fed nonseleniferous halogeton and enough seleniferous fourwing saltbush to provide a daily dose of 0.344 mg of selenium per kg of body weight.

Selenium occurs in the organic form in species of *Astragalus* but in species of *Atriplex* it is primarily of an inorganic type (Beath and Eppson, 1947).

The ingestion of organic selenium by laboratory animals caused a higher accumulation of selenium in the tissue with less excreted in the urine than did ingestion of the inorganic form (Smith, Westfall, and Stohlman, 1938).

All animals were fed ½ pound of rolled barley each morning and given free access to good quality alfalfa hay until they had a normal fill. They were then fed the respective plants through a plastic rumen fistula apparatus daily for 30 days. Blood calcium and blood urea nitrogen levels were determined on each animal just before the

Table 2. Selenium content of dry tissue of *Halogeton glomeratus*, *Atriplex canescens*, *Sporobolus flexuosus*, and *Astragalus preussii* collected in eastern Utah, June to November, 1959.

Sample	Site	June	July	Aug.	Sept.	Oct.	Nov.
		— — — — — — (ppm) — — — — — —					
Halogeton							
Leaves	Cisco	0.5	35.0	3.3	11.1	6.6	0.5
Stems	Cisco	0.3	6.1	0.6	0.9	6.1	0.9
Leaves	Yellow Cat	8.9	2.4	0.3	1.9	5.4	0.0
Stems	Yellow Cat	3.0	1.8	0.3	2.2	9.0	0.9
Leaves	Thompson	6.4	1.2	2.2	1.8	2.1	0.5
Stems	Thompson	1.2	1.8	1.0	0.5	1.8	0.0
Leaves	Green River	0.9	0.8	0.9	2.9	3.4	0.5
Stems	Green River	3.1	1.3	0.0	0.3	0.0	0.0
Leaves	Crescent Junction	0.3	0.0	2.0	0.0	0.0	0.0
Stems	Crescent Junction	0.2	0.2	0.5	0.0	0.0	0.0
Sporobolus	Yellow Cat	2.4	....	4.0	....	....	....
Atriplex	Yellow Cat	102.4	....	1014.2	382.5	....	....
Astragalus	Yellow Cat	1332.0	....	564.7	1853.9	....	....

beginning of the feeding trial and once each week throughout the feeding period, with the final test before death. Data by Binns and James (1960) indicated that neither clinical symptoms nor fatalities occurred during a 90-day feeding period at sublethal levels of either selenium or halogeton fed separately to different animals maintained on a medium protein diet.

Results

Symptoms of selenium toxicity have never been reported in plants growing under natural conditions regardless of the availability or content of the selenium in the soil. Symptoms of toxicity are common, however, when sodium selenate or sodium selenite is added to greenhouse soils or nutrient cultures. The most obvious symptoms are reduced vigor and size and varying degrees of chlorosis. The addition of sodium selenate to nutrient solution in which halogeton was growing resulted in these symptoms. As shown in Table 1, the dry weight of the stems decreased with each increase in selenium. Leaf dry weight was not seriously affected until the concentration of selenium reached 8 ppm. Soluble oxalate content became lower with each additional level of selenium. Slight chlorosis was noted in halogeton grown in the solution containing 8 ppm selenium.

The plant tissue was carefully examined after harvest for any selenium odor but none was detected in the fresh material. After drying, the characteristic garlicky odor of selenium was faintly detectable. The odor was intensified and easily detected when the leaf tissue was ground to a powder preliminary to analyzing for oxalates. It would therefore appear to be very difficult to detect seleniferous halogeton in the field through odor. Halogeton is not likely to have 500 ppm in the field and the characteristic sour odor in the leaves tends to mask what odor of selenium might be present.

The analyses for selenium (Table 1) show that a considerable portion of the selenium present may be lost through drying and indicates the merit of immediate digestion of fresh samples.

The selenium content of halogeton collected in eastern Utah was low (Table 2). The highest selenium content was found at Cisco. All analyses except 2 yielded less than 10 ppm selenium in the leaves and stems. Samples of leaves taken in August, 1958, however, yielded 90 ppm at Cisco, 95 ppm at Yellow Cat, 20 ppm at Crescent Junction and Green River, and 10 ppm at Thompson. That year was favorable for good growth because of near normal precipitation in the area. Selenium concentration decreased rapidly in November after the death of the plants.

The low selenium accumulating power of halogeton is further shown in that milk vetch and fourwing saltbush growing near halogeton accumulated much higher concentrations of this element. The digestions of samples in the field was decidedly advantageous for these species but little or no difference was apparent in halogeton because of the very low selenium concentration. Data in Table 2 are from samples digested in the field.

The selenium content of the soil from the Salt Wash near Yellow Cat ranged from 0.6 to 0.8 ppm at the 10 to 12-inch level, and from 1.1 ppm to 1.5 ppm at the 12 to 24-inch level. At Cisco, selenium content averaged 4.2 ppm in the surface 12 inches and 2.9 in the 12- to 24-inch sample. The findings at Cisco compare favorably with soil analyses reported by Beath (1943) for this general area. In the Morrison alluvium, however, he reported a selenium content of 35 ppm in the 1- to 12-inch layer and 82 ppm in the 12- to 24-inch layer. The difference apparently results from the soil types and sites studied. Our samples were taken from very sandy deposits at the bottom of the wash. This area is subject to periodic flooding, and one would therefore expect much of the selenium to be leached. Since halogeton infestations were confined to these sandy deposits, soil samples were taken only from these particular sites.

The soluble oxalate content of the leaf-seed-sepal fraction of halogeton collected at sites in eastern Utah was similar to that found in halogeton collected on nonseleniferous soils by Williams (1960). The almost pure leaf sample from Cisco collected in

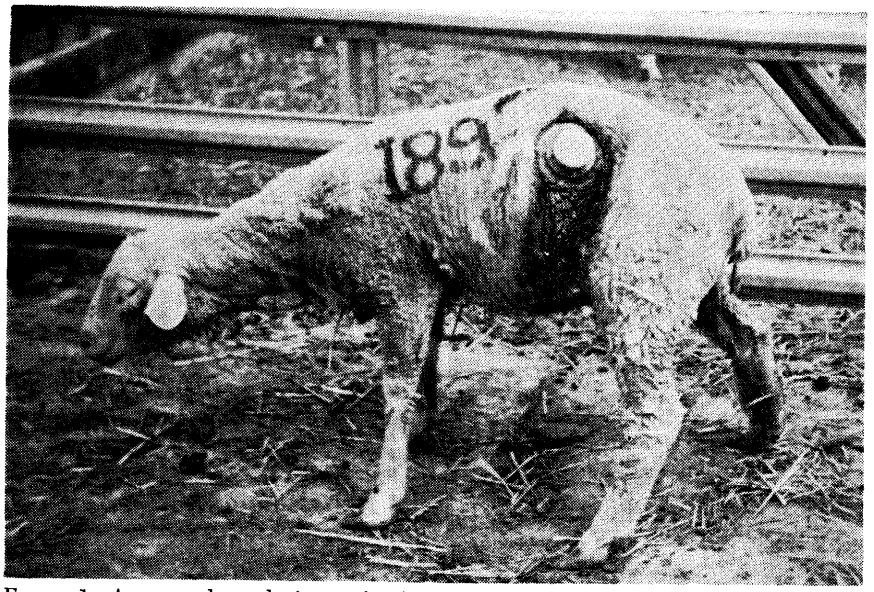

FIGURE 1. Acute oxalate-selenium poisoning in a ewe fed nonseleniferous halogeton and seleniferous fourwing saltbush. The head dropped, the hindquarters were stiff, and respiration was 130 to 140 vs a normal of 70 to 80.

November contained 38.3 percent soluble oxalates. This was among the highest concentrations of this compound ever found in Utah by the authors.

Sheep Feeding Trials

The blood calcium and urea nitrogen concentrations shown in Table 3 are those taken May 23, one day preceding the beginning of the feeding experiment, and June 22, the day the feeding was terminated. The final samples from the 4 animals which died during the 30-day feeding trial were obtained at the time of death.

One ewe fed seleniferous halogeton died 7 hours after feeding on the 5th day. Total selenium administered up to time of death had been only 20.15 mg. The pathology was unusual in that necropsy revealed symptoms typical of oxalate poisoning; but blood calcium concentration was 9.4 mg per 100 ml of serum which, while below normal, was much higher than the level associated with acute hypocalcemia. It would be difficult to assess the role of the small quantity of selenium present. Blood urea nitrogen was normal.

Two of the 3 ewes fed seleniferous fourwing saltbush and nonseleniferous halogeton died (Figure 1). The first ewe died on the 15th day of the feeding trial after receiving 237 mg of selenium and the second died on the 27th day after ingesting 470 mg of selenium. Pathological examination revealed discoloration, swelling, and congestion of the kidneys and liver. The lungs were marked by large areas of hepatization, passive congestion, and atelectasis. Heart muscles were flaccid, but no hemorrhages were present. The abdominal cavity contained about a quart of clear fluid. Blood calcium was 8.0 and 8.6 mg per 100 ml of serum at the time of death. Again, the blood calcium was not at a level which should result in hypocalcemia. It seemed that an interraction occurred which produced acute toxicity without typical hypocalcemia. Moderate to severe injury to the liver and kidneys was indicated. Blood urea nitrogen concentration was 22.8 and 35.5 mg per 100 ml of blood plasma. Normal range for sheep is 8 to 20 mg per 100 ml of blood plasma.

One ewe died after receiving 187 mg of selenium in milk vetch plus nonseleniferous halogeton over a 22-day period. Petechial hemorrhages occurred in the lungs, the heart was flaccid, with a slight swelling and mottling of the liver. No excess fluid was found in the peritoneal cavity, but hypocalcemia was marked with 4.9 mg calcium per 100 ml of serum. Blood urea nitrogen increased to 43.3 mg per 100 ml of plasma which indicated an increase in blood urea nitrogen from disfunction of the kidneys.

The remaining animals were sacrificed at 33 days. Swelling and mottling of the liver were present in each case. Kidney abnormalities were found in one ewe fed seleniferous milk vetch and halogeton and one which had been fed seleniferous fourwing saltbush and halogeton. Blood

Table 3. Blood calcium and urea nitrogen levels in 100 ml of blood plasma of sheep fed sublethal concentrations of soluble oxalates and selenium for 30 days.

Group and sheep no.	First sample Ca	First sample Urea N	Final sample Ca	Final sample Urea N	Remarks
	— — — (mg)		— — —		
Seleniferous Halogeton					
176	13.0	6.1	9.4	11.2	Died May 28
146	13.8	14.0	12.3	17.3	Killed June 25
180	14.2	10.3	12.3	14.9	Killed June 25
Atriplex and *Halogeton*					
175	13.6	10.3	11.7	10.3	Killed June 25
185	13.7	14.9	8.6	22.8	Died June 19
189	13.5	11.2	8.0	35.5	Died June 7
Astragalus and *Halogeton*					
160	14.3	12.1	11.7	9.3	Killed June 25
142	13.5	11.7	11.5	8.4	Killed June 25
153	13.1	6.5	4.9	43.3	Died June 15

calcium and urea nitrogen levels were normal.

Death of the experimental animals during the first 30 days was considered to be primarily due to respiratory and heart failure induced partly by extensive impairment of the liver and disfunction of the kidneys. Necropsy revealed pathological changes in the liver, lungs, heart, and kidneys characteristic of oxalate and selenium poisoning (Cook and Stoddart, 1953; Dudley, 1936; Rosenfeld and Beath, 1945). The kidneys, liver and lungs are primarily the target organs in selenium poisoning, whereas the kidneys are most severely affected in halogeton poisoning. When the normal pathways for detoxification and elimination are reduced, a sustained sublethal dosage will result in steadily increasing concentration of oxalates and selenium within the tissues.

Discussion

Halogeton is one of the many plant species which have a very low accumulating power for selenium. The inability of halogeton to absorb sizable quantities of this element is further stressed by the analyses of milk vetch and halogeton which grew adjacent to each other in the poison strips near the Yellow Cat mine. In one instance, a robust halogeton plant rooted in alluvium near the bottom of the wash contained 1.8 ppm selenium while milk vetch growing 20 inches away contained 385 ppm. Milk vetch rooted on ore waste near the entrance of an abandoned mine yielded 2378 ppm selenium, while an exceptionally large plant of halogeton 3 feet away on the same ore deposit contained only 2 ppm.

Sheep feeding experiments by Cook and Stoddart (1953) have indicated that symptoms of oxalate poisoning did not become apparent until blood calcium level dropped to about 7.4 mg per 100 ml of blood. Fatalities are seldom encountered until the calcium drops below 7, but no recoveries can be expected when the calcium concentration reaches 6.2 mg per 100 ml or below.

Rosenfeld and Beath (1945) reported that sheep excreted more selenium in the urine when they were fed a medium and high protein diet as compared with a low protein diet. The elimination of selenium decreased as kidney damage increased. They also found that selenosis did not become apparent in the medium and high protein group until 860 mg of selenium had been administered, whereas symptoms appeared after the ingestion of only 360 mg in sheep on the low protein diet. Since our experiments utilized a medium protein diet, the quantity of selenium ingested by the 4 sheep which died was far below that reported as necessary to produce symptoms of selenosis.

The work reported herein indicated, with the exception of one death from acute hypocalcemia, that the majority of the animals died from the combined effects of oxalate-selenium poisoning. The liver and kidneys appeared to be more severely affected when selenium and oxalates were fed in combination rather than when they were fed separately. As the ability of these organs to eliminate the poisonous substance decreases, the lethal dose of either selenium or soluble oxalates required also decreases. Thus, when soluble oxalates and selenium were fed in combination, the acute stage of intoxification was achieved in at least some of the experimental animals at levels which, if administered separately, would have resulted in no external symptoms.

The occurrence of small quantities of selenium in halogeton is not considered of great importance on the range. The very rapid decrease in the selenium following the death of the plant would remove any danger of this element from practical consideration. Oxalate content, however, usually remains high well into the winter. If conditions prevailed under which the selenium content in halogeton exceeded 60 to 70 ppm, the selenium-oxalate concentration would then equal that of the fourwing saltbush and nonseleniferous halogeton used in the experiment.

The ingestion of moderate amounts of halogeton and selenium-bearing plants could result in increased losses of sheep on ranges where both types of plants occurred. The extent to which this problem would exist would depend upon range condition and the protein content of the diet. Since both halogeton and selenium-bearing plants are unpalatable, little or no poisoning would likely be anticipated under good range conditions. As range condition deteriorates and these plants constitute a greater part of the available forage and general diet, one might expect greater losses where both plants occurred than if only one type were present. There is no evidence to indicate that any losses of sheep poisoned by both halogeton and seleniferous plants have occurred. Because of the similarity of symptoms characteristic of each type of poisoning, it is doubtful that field evaluation would be possible without a complete necropsy and laboratory examination of tissues.

Summary

The selenium content of halogeton collected from seleniferous soils in eastern Utah ranged from 0 to 95 ppm. Maximum selenium concentration was usually less than 10 ppm.

Halogeton grown in nutrient cultures containing 8 ppm selenium as sodium selenate accumulated 500 ppm selenium in the leaves. Both dry weight and soluble oxalate content were reduced at this concentration.

Growth and oxalate content were not affected by selenium accumulation in the field.

Sublethal doses of selenium and soluble oxalates administered daily to sheep were more toxic than when each was fed separately. The increased toxicity resulted from a more rapid and severe injury of the liver, lungs, and kidneys. Necropsy of sheep which died during the feeding trials indicated pathology characteristic of both selenium and oxalate poisoning. Death was attributed directly to acute hypocalcemia in only one case. No symptoms of poisoning occurred in sheep fed only soluble oxalates or only selenium for 90 days at comparable dosages.

Except under unusual circumstances, halogeton is unlikely to contain enough selenium to increase its toxicity. Where range condition is so poor that halogeton and seleniferous species constitute an unusually high proportion of the diet, losses among sheep may be more severe when both types of plants are eaten together.

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