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A STUDY OF SOME FACTORS INFLUENCING THE
NITRATE CONTENT OF OAT SEEDLINGS

By
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THIS BOOK DOES
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A STUDY OF SOME FACTORS INFLUENCING THE
NITRATE CONTENT OF OAT SEEDLINGS

Analysis of samples of oat hay frequently reveals concentrations of nitrates sufficient to be toxic and cause occasional livestock losses.* Pigweeds (Amaranthus retroflexus L.) and sorghum having high concentrations of nitrate nitrogen have also been reported as being toxic to cattle.^{1,2,3} In view of this fact this study was begun to determine what factors might play a role in nitrate metabolism and the extent of their influence.

⁴ Steinberg, working with Aspergillus niger, found that when nitrate is the source of nitrogen, molybdenum is required to a greater degree than when ammonia or organic nitrogen is the source. Interpreting his results he concluded that molybdenum is necessary for the activation of nitrate reductase.⁵ Working with maize seedlings in water and sand cultures, Scharrer and Schropp found that seedlings grown in cultures with 10^{-4} to 10^{-1} mg. molybdenum per 2 liter flask grew better than the control plants. Above this concentration molybdenum was definitely toxic. Numerous other workers likewise assign catalytic roles to manganese and arsenic in nitrogen metabolism.

This paper is a preliminary study of the affects of molybdenum, arsenic, manganese, pH, and soil moisture on the nitrate nitrogen content of Richland oat plants grown in water culture and in soil.

* The symptoms of nitrate poisoning are similar to asphyxia. Whereas normally hemoglobin (the red pigment in the blood) forms a loose combination with oxygen, in cases of nitrate poisoning methemoglobin (a darker and browner pigment) is formed. In this perverted form of hemoglobin the oxygen is so firmly bound that it is not released to the tissues.

Procedure for Analysis:

The following method was used in the analysis of the plant samples for nitrates:⁵

Five grams of the finely-ground, air-dry sample was placed in a 250cc. volumetric flask. About 100cc. of boiling distilled water was added to the flask and it was placed on a steam bath for two hours, during which time it was shaken occasionally. Then about 125cc. of distilled water and 5cc. of a solution of saturated neutral lead acetate were added to the flask and its contents were mixed and allowed to cool to room temperature. After making up to volume the mixture was shaken well and filtered. 150cc. of the filtrate, 100cc. of distilled water, 25cc. of 5% NaOH solution, a small amount of paraffin and a few glass beads were placed in a Kjeldahl flask and the mixture was boiled for 30 minutes. (A blank containing 150cc. of distilled water in place of the extract was run simultaneously.) After cooling to almost room temperature, 200cc. of distilled water and three grams of Devarda's alloy were added to the flask and the ammonia resulting from the reduction of the nitrate nitrogen was distilled over into standard HCL solution. After back-titrating with standard NaOH solution the amount of nitrate nitrogen was calculated. The nitrate nitrogen is reported in this study as the percent of KNO_3 in the air-dry sample.

Experimental

Molybdenum:

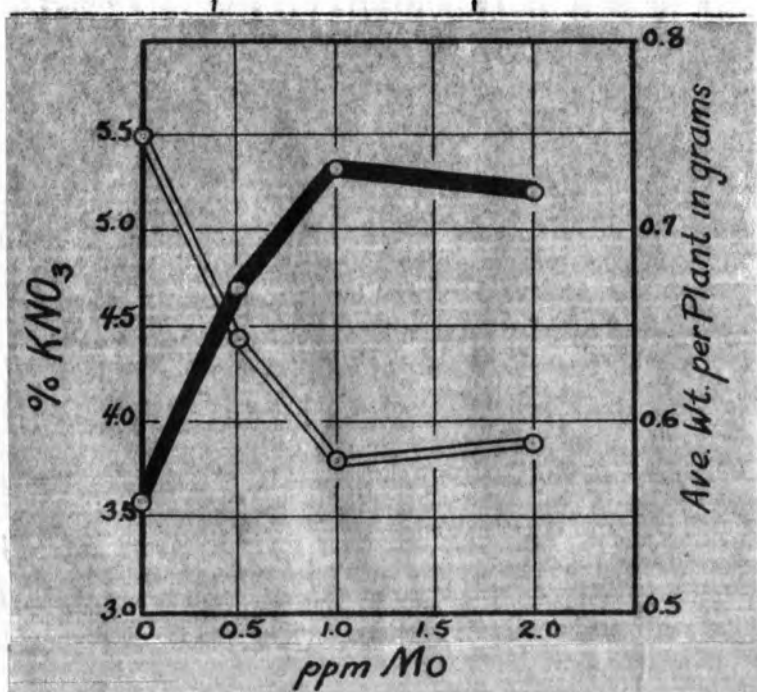
The oat seedlings were grown in water culture solutions and were supplied nutrients as recommended by Trelease and Trelease,⁶ the culture solutions being changed weekly. As four plants were grown in each quart jar and the varying concentrations were run in duplicate, the percent KNO_3 as reported in the following tables is an average for the eight plants.

Concentrations of molybdenum at 0.5 ppm, 1.0 ppm, and 2.0 ppm were maintained by appropriate additions of a standard ammonium hepta molybdate

solution. The control series supplied the 0.0 ppm Mo level. At the end of a six weeks growing period, the plants were cut, air dried, and analyzed for nitrates. The values obtained are given in Table I.

Table I

Treatment	% KNO_3	Ave. Wt. Per plant.
0.0 ppm Mo	3.59	0.75 g.
0.5 ppm Mo	4.71	0.64 g.
1.0 ppm Mo	5.33	0.58 g.
2.0 ppm Mo	5.20	0.59 g.



The results clearly show the toxicity of Molybdenum to the plants and also, that the percent KNO_3 rises as the average plant growth diminishes. (In view of Scharrer and Schropp's work the lower Mo levels deserve further study.)

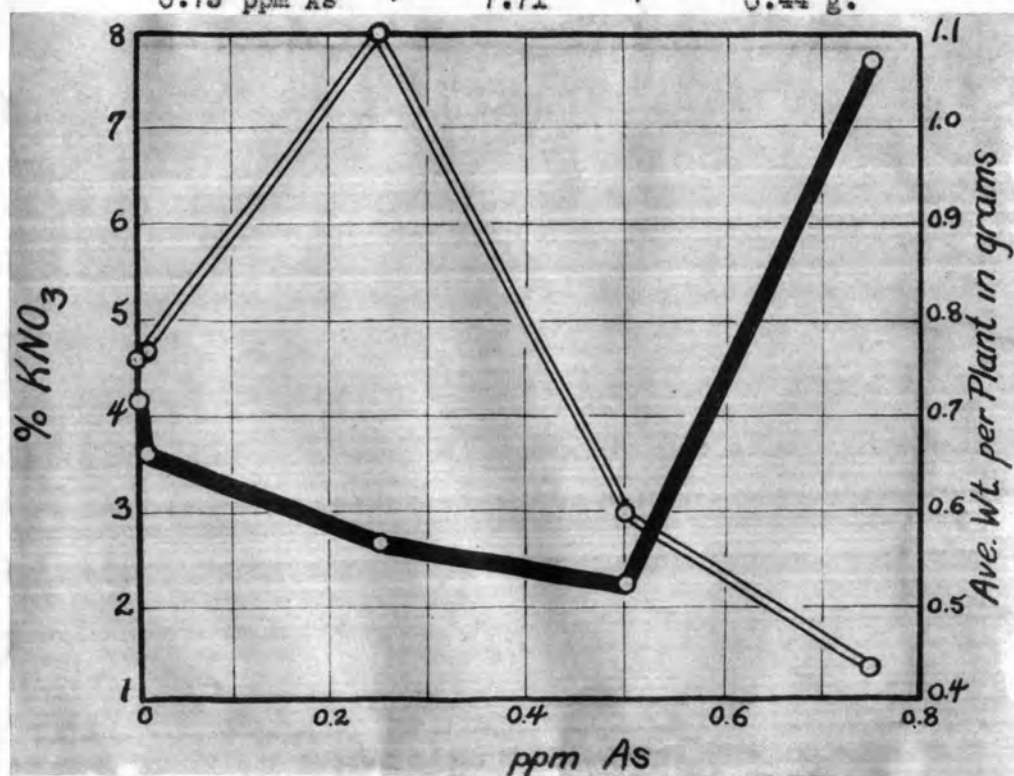
Arsenic:

In this series nutrient solutions were supplied with 0.0 ppm, 0.01 ppm,

0.25 ppm, 0.5 ppm, 0.75 ppm, and 1.0 ppm As by the appropriate addition of a standard solution of sodium arsenite. At the end of a six weeks period of growth, the plants were cut, air-dried, and the percent KNO_3 determined.

Table II

Treatment	% KNO_3	Ave. Wt. Per Plant.
0.0 ppm As	4.14	0.74 g.
0.01 ppm As	3.59	0.76 g.
0.25 ppm As	2.70	1.10 g.
0.50 ppm As	2.25	0.60 g.
0.75 ppm As	7.71	0.44 g.



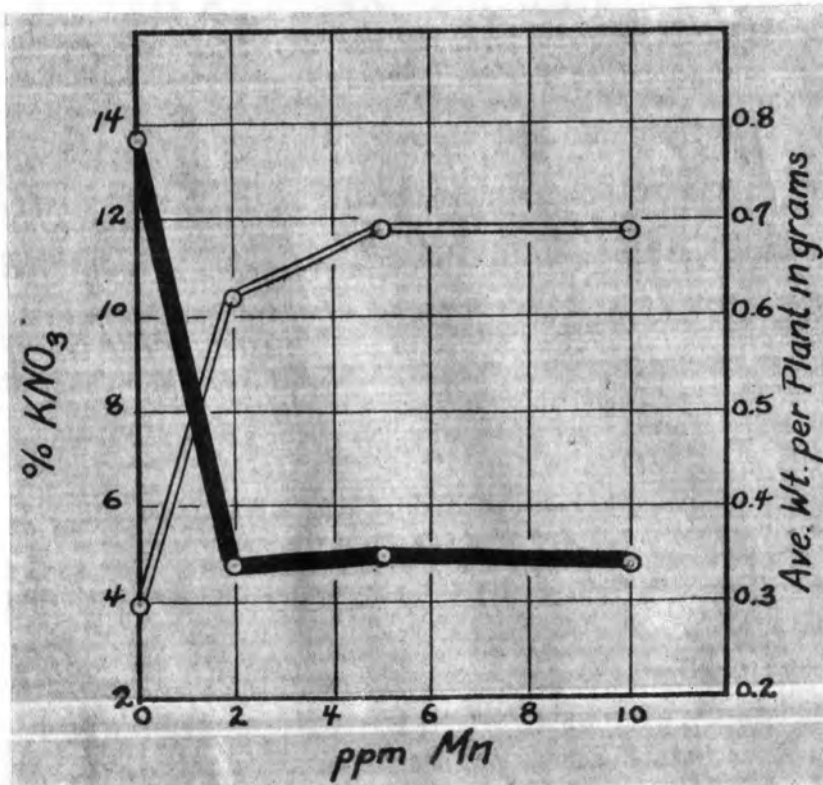
A study of the graph would indicate that concentrations of arsenic up to the 0.25 ppm level stimulated plant growth and that there is a corresponding decrease in the KNO_3 content. Beyond the 0.25 ppm level arsenic is toxic to plants grown in water culture and at the 1.0 ppm level, the plants grew for only a short period of time.

Manganese:

Nutrient solutions with concentrations of 0.0 ppm, 2.0 ppm, 5.0 ppm, and 10.0 ppm Mn were maintained by appropriate additions of a standard solution of manganese sulfate to a complete nutrient solution. The plants were cut at the end of a six weeks growth period, air-dried, and analyzed for the percent KNO_3 content.

Table III

Treatment	% KNO_3	Ave. Wt. Per Plant.
0.0 ppm Mn	13.8	0.29 g.
2.0 ppm Mn	4.83	0.62 g.
5.0 ppm Mn	5.09	0.69 g.
10.0 ppm Mn	4.80	0.69 g.



The manganese deficient solution (0.0 ppm) yielded plants with an

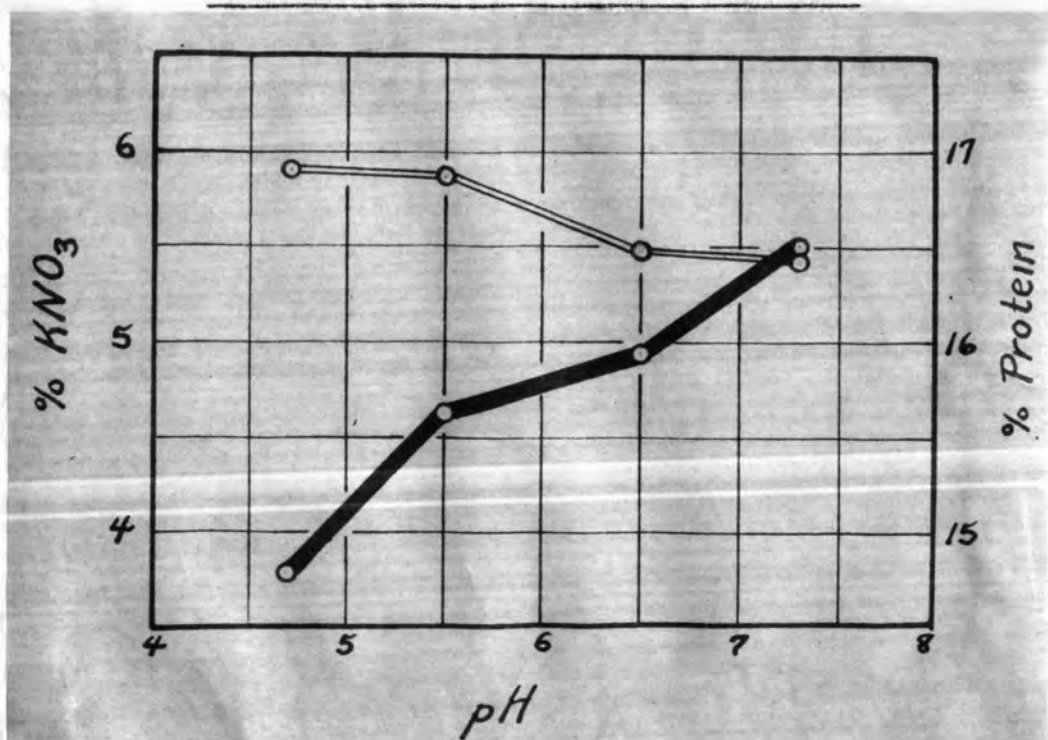
extremely high nitrate content. These plants displayed all of the symptoms of manganese deficiency. The graph shows definite growth stimulation by manganese and that toxic levels were not within the range of concentrations studied.

pH:

In this experiment a Trelease and Trelease nutrient solution was used, except that the KH_2PO_4 and K_2HPO_4 , and the $(\text{NH}_4)_2\text{SO}_4$ and KNO_3 ratios were adjusted as necessary to give nutrient solutions of different pH. Solutions with pH above 6.5 were maintained by appropriate addition of 0.2M NaOH. At the end of five weeks the plants were cut, air-dried, and the percent KNO_3 content determined. The values recorded are the average for sixteen plants.

Table IV

pH	% KNO_3	% Protein
4.7	5.79	16.90
5.5	4.63	16.87
6.5	4.94	16.48
7.3	5.51	16.43



A great many difficulties were encountered in maintaining the pH of the solutions, when in contact with the plant roots, the pH tending to fall fairly rapidly at the higher levels and remaining more constant at the two lower levels. In-as-much as time did not permit a more careful adjustment of the nutrient solutions, the pH was determined at the beginning and end of each week, the solutions being prepared fresh weekly. The average of the two pH values is recorded in Table IV.

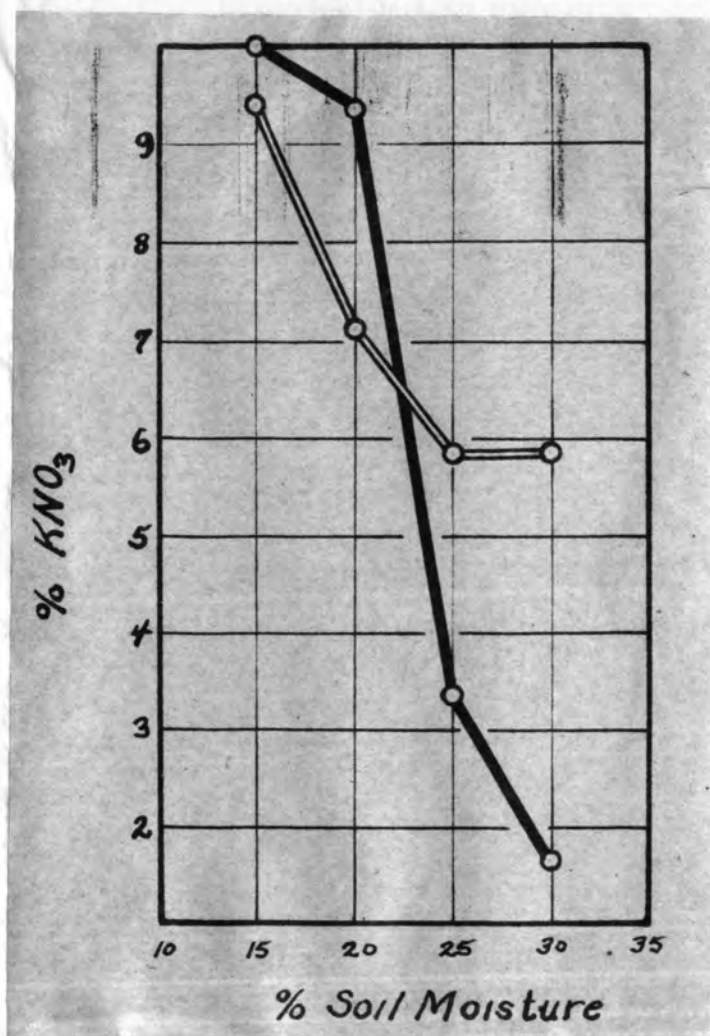
As can be seen from the graph a wider pH range should be studied. However, the increase in nitrate nitrogen with increase in pH is significant.

Soil Moisture:

By maintaining soil moisture of pot grown plants at levels of 15%, 20%, 25%, and 30% another factor was found to affect the concentration of nitrates in oat plants. The experiment has been carried out twice in duplicate and the average percent KNO_3 content at the different moisture levels is recorded in Table V.

Table V

Treatment	1st Trial % KNO_3	2nd Trial % KNO_3
15 % H_2O	9.40	9.97
20 % H_2O	7.10	9.38
25 % H_2O	5.85	3.35
30 % H_2O	5.86	1.63



Droughty soil conditions markedly affect the nitrate concentration, as the graph denotes. While the average weight per plant was not recorded in this experiment, it is obvious that plant growth would be poorer at the lower moisture levels.

In order to further ascertain the influence of drought on the nitrate content of the oats, two large tubs of soil were planted, No. I being kept as dry as possible without causing death, No. II being watered copiously. After four weeks, some of the plants from each tub were cut, air-dried, and analyzed for nitrate nitrogen and crude protein nitrogen. The procedure was then reversed, tub No. I now being well-watered, while tub No. II was kept on the dry side. After an interval of a week, samples were again cut, air-dried, and analyzed. The results are recorded in Table VI.

Table VI

Tub No.	I		II	
	Dry_____	Moist	Dry_____	Moist
% KNO ₃	11.33	7.51	6.62	9.39
% Crude Protein	23.85	22.93	22.24	20.58

These results seem to substantiate a belief that the accumulation of nitrates in field grown plants is in part, at least, a drought problem. Had the time interval been greater, no doubt there would have been a greater decrease in the nitrate content in the first case, and a greater increase in the second.

Affect of Continuous Light

By supplying one set of plants with continuous light (daylight plus the continuous light from a 100 watt light) and comparing the nitrate concentration in plants grown under these conditions with that in plants receiving only daylight, it is apparent that the percent KNO₃ is lower in the plants receiving continuous light.

Table VII

Treatment	Pot grown		Water Culture	
	% KNO ₃	% Crude Protein	% KNO ₃	Average Wt. per plant
Continuous light	4.94	14.98	2.04	0.73 g.
Daylight (only)	7.06	18.43	3.59	0.75 g.

Protein analyses were also made on the plants thus treated, and the protein content of the plants subjected to continuous light was found to be lower than that in the plants receiving daylight only. Likewise, with plants grown in water cultures, but subjected to the same treatment, there is no significant increase in the growth of plants receiving continuous light.

Inasmuch as protein synthesis takes place more rapidly when ample light is received, the above data would seem to indicate that the plants were receiving too much light and as a result there is cell elongation rather than cell division. This was much in evidence, for the plants grown with continuous light had short, narrow leaves and extremely long inter-nodes, while plants receiving daylight only had long, wide leaves and comparatively short inter-nodes. The fact that this decrease in plant growth did not result in a corresponding rise in nitrates is perhaps best explained by Strowd⁷, who found that nitrates accumulate most rapidly in plants in the dark.

Conclusions

The data obtained from the water culture experiments with arsenic, molybdenum, manganese, and continuous light provides evidence that an increase in nitrates is accompanied by a corresponding decrease in plant growth. The soil moisture experiments, also, lead to this observation. It would seem evident that the nitrate nitrogen content of a plant rises, whenever conditions prove unfavorable for plant growth. Therefore, it is reasonable to conclude that for some reason, the nitrate nitrogen is not being reduced to a form available for amino acid synthesis, consequently plant growth is retarded.

Future Work

It should be kept in mind that this thesis is a preliminary study and that only a few of the experiments have been repeated. Therefore, before elaborating a theory for the accumulation of nitrates in plants, this work should be rechecked. Also, work should be done on the nitrogen cycle of the growing plant with particular attention paid to nitrate reduction.

Summary

1. Nitrates accumulate in increasing amounts as molybdenum toxicity increases.
2. Arsenic stimulates plant growth up to 0.25 ppm, becoming increasingly toxic above this level.
3. Manganese stimulated plant growth at all of the levels studied. Manganese deficiency affects nitrate content in a manner similar to toxic conditions in 1 and 2.
4. A rise in pH of the culture solution is accompanied by an increase in the nitrate content of the oat seedlings.
5. Whenever a lack of moisture is introduced as a factor limiting growth, there is a corresponding nitrate nitrogen increase.
6. Continuous light depresses the nitrate content of oat seedlings.

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