The Effects of Chloramine on Alfalfa

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Introduction

Chloramine (NH_2Cl) is an alternative bactericide that is used in place of chlorine in the treatment of water supplies. Currently, 20 % of the nation's water agencies have converted to chloramine disinfection. This prevents the taste of chlorine and the formation of carcinogenic chlorine byproducts such as trihalomethides (Behrman, 1968). Surprisingly little research has been done on the effect of chloramine on animals and even less on plants. This experiment focuses on determining the effects it has on plants. Will chloramine concentrations of 4 (the maximum tap water concentration), 20, or 40 ppm affect the growth rate, mass development or germination rate of *Medicago sativa*?

Materials and Methods

Four groups were created of 25 alfalfa sprouts each. These were all approximately 2 inches high. Each plant had been germinated and watered with deionized water until they reached this height. Each plant was grown in a separate compartment of plastic "six pack" containers available at any nursery. They were grown in "Unigro" organic soil which I sterilized by heating to 120 degrees Fahrenheit. Each group was arranged identically, 8" beneath two 4' fluorescent grow lights. This sole source of light was turned on and off by an electronic timer every twelve hours.

Starting with day zero, each plant received 2.0 ml of the chloramine concentration which corresponds to their group as shown by Table 1. Table 1 also shows the method of chloramine synthesis created with the assistance of Dr Rusay.

Table 1: Concentrations and synthesis of chloramine solutions.

Group	Concentration	Synthesis
A	0 ppm	Deionized water
B	4 ppm	Add 500 ml 2.81×10^{-4} M Nh_4OH to 500 ml 2.81×10^{-4} M NaOCl
C	20 ppm	Add 500 ml 7.8×10^{-4} M NH_4OH to 500 ml 1.5×10^{-3} M NaOCl
D	40 ppm	Add 500 ml 1.56×10^{-3} M NH_4OH to 500 ml 3.00×10^{-3} M NaOCl

Plants were watered every day in this fashion for the duration of the experiment, and waterings were periodically skipped (for all plants at a given time) to prevent over-saturation of the soil.

Measurements were made of the height of each plant every other day starting with day one. This was done from the top of the soil to the highest point of the plant to a tenth of a centimeter. On a few occasions, the act of measuring damaged or broke the plants. The heights of those plants were not included in the averages for their group from that point on. The plants were cut at the soil on the 29th day, and their masses were recorded.

For the second part of the experiment, four groups of one hundred seeds each were placed on folded cheesecloth soaked in their corresponding concentration of chloramine solution as described by table 1. Organic alfalfa seeds which can be found at any health food store were used. Each group was stored with their soaked cloth in separate glass containers sealed with plastic wrap. The number of sprouted seeds was recorded every twelve hours for a total of 60 hours. Germination was defined as the emergence of the root tip from the seed shell. As with the other measurements, data were not analyzed statistically.

Results

As Figure 1 shows, the average growth rate did not vary significantly over the 29 days between the groups watered with 0 , 4 , 20 , and 40 ppm chloramine solutions. In fact, the cumulative data for each group follows a nearly identical curve. At the end of the experiment, the average height of each group was nearly identical. The difference between the shortest and tallest group after 29 days, Group C and Group A respectively, was only 0.4 cm. The greatest variance between groups measured over the entire study was 0.7 cm between Group A (highest) and C

(lowest) on day 15.

As figure 2 shows, there was significant variation in the average masses of the groups after 29 days. Group A was .0864 grams, Group B was .0840 grams, Group C was .0634 grams, and Group D was .0723 grams. The average mass of the control group was slightly higher than that of Group B and significantly higher than that of groups C and D. Group B was 3% lighter on average, Group C was 27% lighter on average, and Group D was 16 % lighter on average than the control group. Interestingly, Group D was 14 % more massive than Group C even though it had twice the concentration of chloramine.

As Figure 3 shows, there was significant variation in the germination rates of the groups. The germination rate of the control group was higher than any of the chloraminated groups. The seeds began to sprout during the second twelve hour period during which 47 % of the control group sprouted. This is 27% more sprouts than produced by the most productive of the chloraminated groups during this period, Group C. In the first 36 hours, the control group germinated dramatically faster than all other groups. From this point until the end of the study, the groups watered with 20 and 40 ppm began to catch up with the control group. However, the 4 ppm group germinated consistently slower than the other groups.

Discussion

The average growth rate of the plants watered with 0, 4, 20, and 40 ppm chloramine solutions did not vary significantly during the 29 days of the study. This data suggests that these concentrations of chloramine do not inhibit or promote the vertical growth of alfalfa over this length of time. A possible source of error in these measurements was the fact that each plant did not grow perfectly straight. The stems of the plants often appeared to be curved. Since this occurred in all of the groups, the error should not have affected the relative differences in average height between the

groups. It is possible that a longer study may have revealed an inhibition of the growth rate. Further research is needed to determine conclusively if chloramine affects the growth rate of alfalfa. At the conclusion of this study, the average masses recorded of the four groups varied significantly. The fact that the chloraminated groups were 4% to 27% less massive than the control group suggests that chloramine inhibits the development of mass in alfalfa. In the early sixteenth century, Paracelsus discovered that the degree of harm which toxic substances have on organisms is usually directly proportionality to their concentrations (Rusay, 1998). This relationship was not observed between the average masses of the four groups in this experiment. If an error was made in the preparation of one or more of the chloramine solutions (I am sure that this is very unlikely), all of the data recorded would have been affected and distorted.

In the germination portion of this experiment, the variation in rates suggest that chloramine inhibits the germination rate of alfalfa. Again, the rate was not determined to be inversely proportional to the concentration of chloramine as may be expected. Since the anomalous group in this experiment (B), is not the same as those of the average mass comparison (C and D), this suggests that error in solution preparation is not the source of the anomalies. Further research is needed to determine the effect chloramine concentration has on germination rate and mass development of plants conclusively.

Chloramine is known to have adverse effects on some living organisms. In a study initiated by the Environmental Protection Agency, rats receiving chloraminated water in 50, 100, and 200 ppm concentrations weighed less after two years than the rats of the control groups (Exon, 1987). Though plants and animals differ enormously, this data is similar to that of the mass comparison in this experiment. This suggests that chloramine may inhibit some of the developmental functions of a broad range of organisms. Unfortunately, very little research has been done on the effects of

chloramine on plants. In fact, a fairly extensive search yielded no information whatsoever in this area.

There are a huge variety of mechanisms which control the developmental functions of plants. It would be extremely difficult to determine the exact way in which environmental pollutants negatively affect the biological functions of plants. On the other hand, certain general principles are understood about how toxins react with organisms on a cellular level. Many complex series of enzymes exist which catalyze the reactions that sustain the development and life of all organisms. Hormones and their interactions with enzymes also play a crucial role in plant growth and germination. It is known that many toxins, such as DDT, function by inhibiting crucial enzymes.

One possible explanation for the fact that chloramine inhibited the germination rate and mass development of alfalfa but not the growth rate in this experiment is that these functions each utilize different enzymes. The chloramine may have inhibited the enzymes which are used in mass development and germination but not those used in vertical growth (mitosis and elongation of cells). Obviously, more data is needed to determine exactly why and how chloramine inhibits some of the developmental functions of alfalfa and not others .

The fact remains that evidence is accumulating which suggests that organisms are adversely affected to a certain degree by chloramine in water. This may have important implications to the health of plants, animals, and perhaps the entire ecosystem. It is important to remember that in this experiment, the only significant effect which chloramine of the maximum concentration allowed in tap water had on alfalfa was a reduction in the germination rate. On the other hand, we don't know how the wide spread use of chloraminated or even chlorinated water will affect any aspect of the ecosystem over long periods of time. This is true of any man-made chemical introduced into the environment. As human civilization continues to grow in size and

complexity, its byproducts may pose a substantial threat to the natural world. For now, no safer alternative to chloraminating water is known.

Research cited

Behrnam, A. S., 1968. Water is Everybody's business; the Chemistry of Water Purification. Anchor Books, New York. pp. 132-134.

Exon , J. H., 1987. Toxicology and Carcinogenesis Studies of Chlorinated Water and Chloraminated Water. Toxicology, 44 (3): 267-269

Rusay, Ph.D., R. J., Chemistry instructor, Diablo Valley College, personal communication., March-April 1998.

Figure 1.

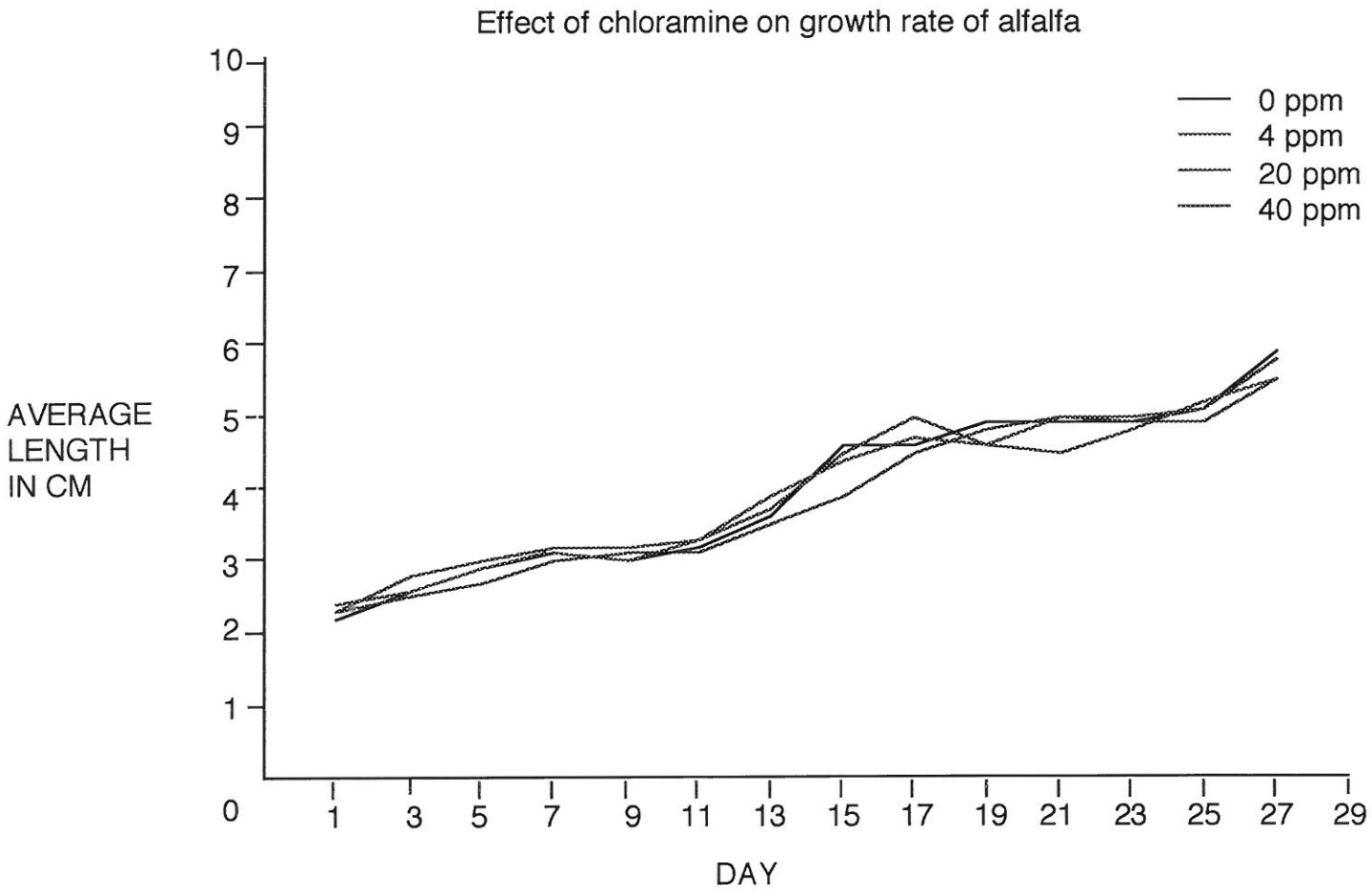

Effect of chloramine on growth rate of alfalfa
0 ppm
4 ppm
20 ppm
40 ppm
AVERAGE LENGTH IN CM
0
1
2
3
4
5
6
7
8
9
10
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
DAY

Figure 2.

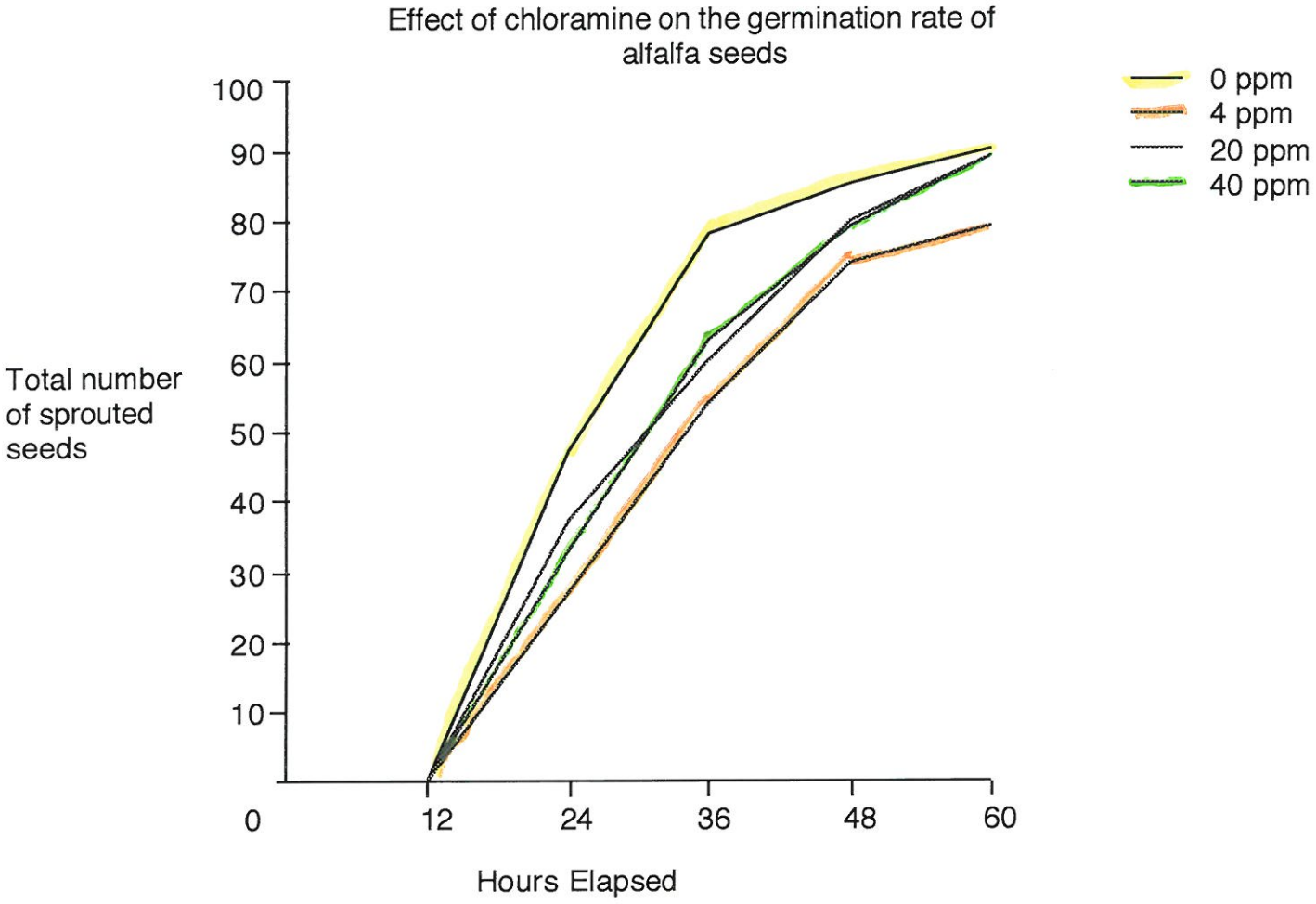
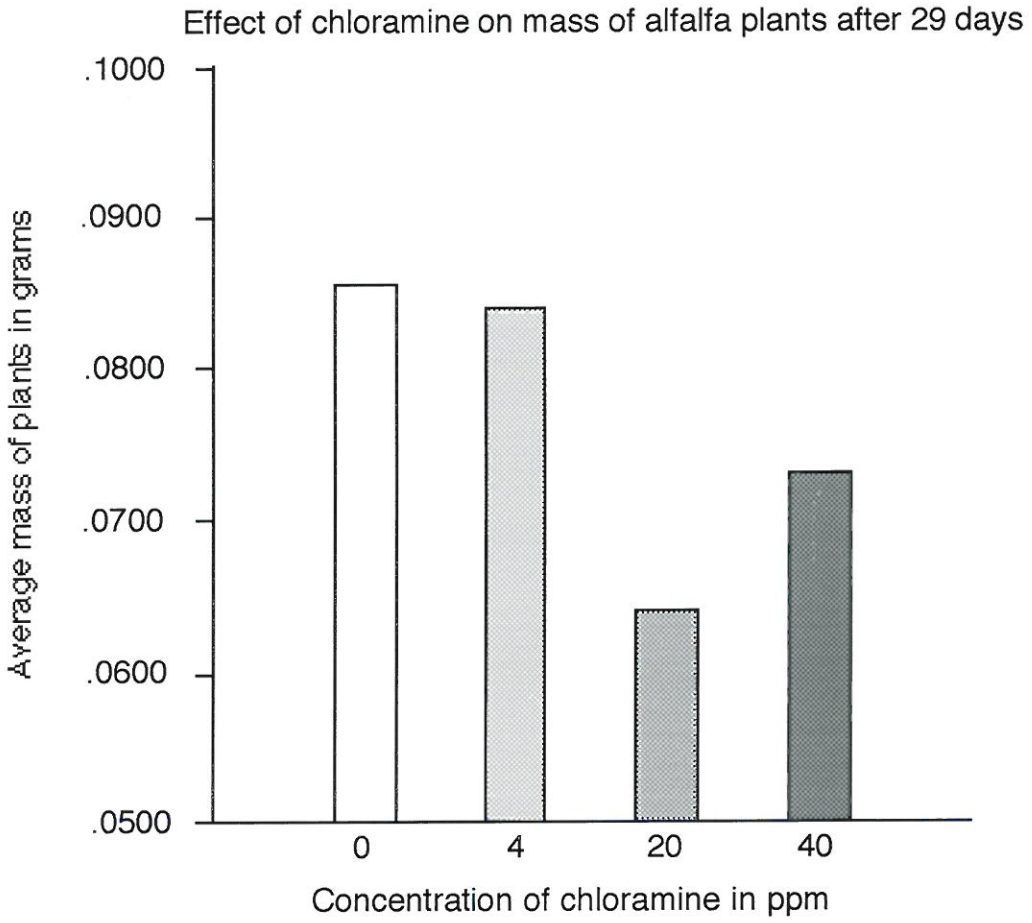

Effect of chloramine on the germination rate of alfalfa seeds
0 ppm
4 ppm
20 ppm
40 ppm
Total number of sprouted seeds
100
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
Hours Elapsed

Figure 3.


Effect of chloramine on mass of alfalfa plants after 29 days
Average mass of plants in grams
.1000
.0900
.0800
.0700
.0600
.0500
0
4
20
40
Concentration of chloramine in ppm