The Response of *Euglena gracilis* to Magnesium Chloride Exposure
Environmental and Agricultural Implications and Areas for Further Study

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Euglena gracilis exhibiting chloroplast bleaching, 100x

ABSTRACT

In response to environmental observations concerning the loss of diversity of plant species within the right of way on major mountain roadways, a study of magnesium chloride ($MgCl_2$) as a causative agent for this loss was performed. A preliminary experiment was performed on radishes and had an extremely high correlation between plant tissue damage and $MgCl_2$ exposure. The photoheterotrophic protist *Euglena gracilis* became the model organism for studying the effects of $MgCl_2$, due to the similar physiologic relationships that it has with various grain crops. *Euglena* was exposed to varying $MgCl_2$ concentrations from 0% (control) to 5.2% in increasing increments of 0.2% and a single temperature step of 5°C above ambient temperature. Magnesium chloride was dissolved into *Euglena* growth media and applied by injection into test tubes containing *Euglena* cultures. The procedure allowed for the investigation of the effects of $MgCl_2$ within the model and to also address concerns of global warming because of chloroplast bleaching. Results indicate that although $MgCl_2$ is an effective substance for preventing ice formation on roads, it also carries the potential to harm the environment over time. It was found that exposure to even small amounts (as low as 0.8%) could agitate *Euglena*, and concentrations as low as 3% were lethal within minutes. Because of global warming and cropland being lost due to the concentration of salts and minerals in irrigated soil, the results of this investigation indicate the need for further study into alternative, environmentally appropriate methods of preventing and removing ice from roadways.

LIST OF ABBREVIATIONS AND ACRONYMS

$MgCl_2$ – Magnesium chloride

SU – Survivors

USM – Under stress and displaying metamovement

IK – Instantly killed

CB – Chloroplast bleaching

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INTRODUCTION

In a nation where the automobile is the most common method of transportation, measures must be taken to ensure that transportation can remain both unobstructed and quick-moving. In mountainous regions, the snow and ice are major obstacles for road crews to surmount in order to maintain safe road conditions so that traffic can continue to move freely.

There is a history of how to keep roads safe, everything from closing the mountain passes that are too dangerous to drive on, to spreading gravel on the roads. In addition, prepared solutions of modern chemical compounds, such as Magnesium Chloride ($MgCl_2$) are used particularly in Colorado [2, 3, 4, and 6]. Chlorine is a highly electronegative element that has the ability to destroy organic matter with reports dating back to the early 1900's in nerve gases during World War I and in such infamous toxins such as DDT [1]. With this history, I could not help but wonder as to whether or not these chlorine atoms, when combined with magnesium, could be just as deadly as when they are combined with a carbon atom.

Initial observations came about while driving through Colorado's mountain passes where $MgCl_2$ is often applied on a daily basis during the winter months. Within the right of way, the area seemed disturbed and lacked plant diversity. Cedar trees seemed to be turning brown even though they are evergreens. Pines and other coniferous trees were also turning brown, both in their bark and in their needles. There were large ruts that ran along the highway that were full of $MgCl_2$ slurry. There must have been a certain amount that trickled out of the ruts and into the soil and waterways below the highway. Not only was $MgCl_2$ used on roadways, I also noticed that was available to the general public and sold in bulk in wholesale stores for de-icing driveways and sidewalks.

For this investigation, questions about $MgCl_2$ were developed into the following: 1. How does $MgCl_2$ solution effect the environment? 2. How do organisms respond to the introduction of $MgCl_2$? 3. How toxic, if at all, is $MgCl_2$ to plants?

In 1997, the State of Colorado began the implementation process for using $MgCl_2$ as a de-icer and as an anti-icer for roadways that are maintained by the state. This implementation process included a preliminary environmental, efficacy and safety report that was released in 1999 [6]. A literature search has indicated that since the release of the 1999 report, the Colorado Department of Transportation has not published further studies of the effects of $MgCl_2$ on the environment.

Another problem that fueled this investigation is related to agriculture. Studies from the United States Geological Survey and other groups have indicated that water used for irrigation can adversely affect cropland by concentrating salts and minerals from the water into cropland, ultimately making that land unsuitable for crop production [8]. Water in the west, including Colorado is used and reused many times for agriculture and community needs [9].

Furthermore, bleaching of chloroplasts has been observed in rye and cucumber plants when under heat stress [11]. *Euglena* are photoheterotrophic protists that have also demonstrated chloroplast bleaching when under heat stress. *Euglena* bleach their chloroplasts in a similar fashion to rye and cucumber cells. Chloroplast bleaching is caused by increased temperatures and with a rise in temperature, an awareness for global warming becomes evident. The modern scientific community has reached a general consensus that global warming is currently happening [7].

Euglena gracilis was used as a model organism to test the current effects of salt concentration and temperature on the growth of the organism. Because chloroplast bleaching has been demonstrated in agricultural crops, *Euglena* can be used as a test organism. The main goal of this study is to model one possible long-term outcome from the use of $MgCl_2$ as a de-icer and anti-icer by both state and local transportation agencies and as well as individuals.

A series of hypotheses were formed to investigate the effects of $MgCl_2$ in a range of environmental settings:

Preliminary Study:

H_{0P} (Null) Magnesium Chloride ($MgCl_2$) will not have an effect on the growth and survival of Early Scarlet Globe radish (*Raphanus sativus*) plants.

H_{1P} Magnesium Chloride ($MgCl_2$) will adversely effect the growth and survival of Early Scarlet Globe radish (*Raphanus sativus*) plants when applied to the soil at the recommended amounts on a bag of $MgCl_2$ purchased from a wholesale store (one cup per square yard) for each major storm system that passed through the local area.

Principle Study:

H_{0A} (Null) Magnesium chloride ($MgCl_2$) does not have an affect on the growth and survival of *Euglena gracilis* as measured by spectrographic analysis.

H_{1A} Magnesium chloride ($MgCl_2$) will have an effect on the growth and survival of *Euglena gracilis* at concentrations above 1g% as measured by spectrographic analysis.

H_{0B} (Null) Magnesium chloride ($MgCl_2$) does not have a synergistic effect with the heat on the growth, survival and chloroplast retention in *Euglena gracilis* as measured by spectrographic analysis.

H_{1B} Magnesium chloride ($MgCl_2$), at concentrations above 1.4% weight by volume, will decrease chloroplast retention, cell growth and survival of *Euglena gracilis* as measured by spectrographic analysis.

Follow-up Study:

H_{0F} (Null) Magnesium chloride ($MgCl_2$) will not effect the germination of Black Seeded Simpson lettuce (*Lactuca sativa*).

H_{1F} Magnesium chloride ($MgCl_2$) will adversely affect the germination of Black Seeded Simpson lettuce (*Lactuca sativa*) when applied at concentrations above 1.4% weight by volume as measured by percent germination.

MATERIALS AND METHODS: PRELIMINARY STUDY

First, a preliminary experiment was begun by planting radishes in potting soil, in three planter boxes, lined in rows of two, 11 plants deep. These were placed inside of Eagle Valley High School's greenhouse building, underneath fluorescent growth lights. Each planter box was watered as needed and regularly to ensure germination and growth and was kept in the same room under identical conditions. Two weeks after germination, $MgCl_2$ crystals were applied with every passing snowstorm by sprinkling crystals over boxes labeled as treatment at the rate of one cup per square yard. One box was treated entirely with $MgCl_2$. One box was untreated and served as the control. The third box was divided into equal halves along the short axis of the box, with one half of the box treated at the rate of one cup per square yard and the other half left untreated. The plants were watered as recommended by the seed manufacturer. Upon contact with moisture, the crystals would dissolve into the soil just as would be done if it were in nature or more precisely, on an icy sidewalk or driveway. Daily observations were made. At the conclusion of the study, all radish plants were removed from the soil, ensuring to keep as much of the root structure attached as possible and with removal of as much soil from the roots as possible. Specimens were set onto paper towels for drying over a period of three days. After the third day, the biomass of each plant was weighed using an electronic balance and recorded in grams. The mean mass of the plants in each of the three treatments were compared for differences.

MATERIALS AND METHODS: PRINCIPLE STUDY

In order to test the effects of $MgCl_2$ on *Euglena*, samples were cultured and placed in test tubes in groups of six. Each test tube was filled with 5 ml of *Euglena*/growth medium solution and placed in one of two categories: heated or unheated. Each group of six test tubes was treated with varying amounts of $MgCl_2$ solution, added in 1% increments from 0% to 15% by volume. The treatment consisted of 5 ml of $MgCl_2$ crystals dissolved in distilled water. These treatments were created in beakers that allowed 100 ml of solution to be created at a time, and 5 ml of solution in a syringe was injected into test tubes containing the *Euglena*. The result was 10 total ml of *Euglena*/growth medium and a specific concentration of $MgCl_2$ that varied with each group. This was left over night so that any changes would be clearly observed the next day.

The principle study was repeated and refined, using more precise concentrations of $MgCl_2$ at 0.2% increments rather than 1%. This experiment offered a much more precise control over just what the *Euglena* were exposed to. During this phase of refinement, pH tests were also done in order to discover if there were large fluctuations in acidity that could have influenced earlier tests.

In order to attempt to quantify the number of surviving *Euglena*, this experiment was done for a third time and incorporated the use of a spectrophotometer. The samples were tested for absorbance of light within the wavelength of 430 nanometers, since the wavelength that is involved in photosystem I (chlorophyll *a*) is 430 nanometers [11]. Visual observations of *Euglena* were also recorded. Using a pipette, one drop of test tube culture was placed onto a slide and then observed under a microscope at varying magnification.

MATERIALS AND METHODS: FOLLOW-UP STUDY

A follow-up study was then conducted in order to gather more data and to provide a connection to the preliminary radish experiment. Using a large germination chamber, 0.5 grams of lettuce seeds (*Lactuca sativa*) were spread across each germination tray covered in paper towels which would fold over the seeds as soil would. The paper towels were then moistened with $MgCl_2$ solutions ranging from 0 to 4% by volume, increasing in concentration in increments of .4%. Another germination tray was moistened with distilled water and served as the control. The germination chamber was set in excess of an ideal Colorado day at the time of sowing. Daylight was set in excess of nature to 13 hours, humidity was provided by an internal mister which would keep air moisture levels at 30%, and temperature thresholds were set at 5° C and 27° C. The seeds were allowed seven days to germinate under these controlled conditions.

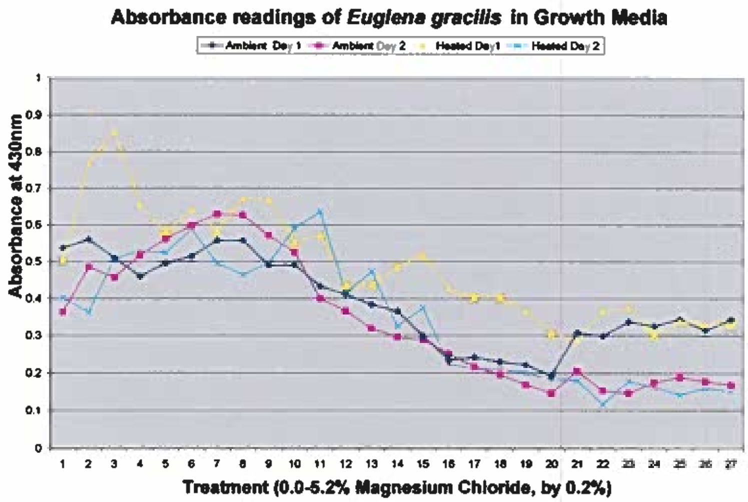
RESULTS

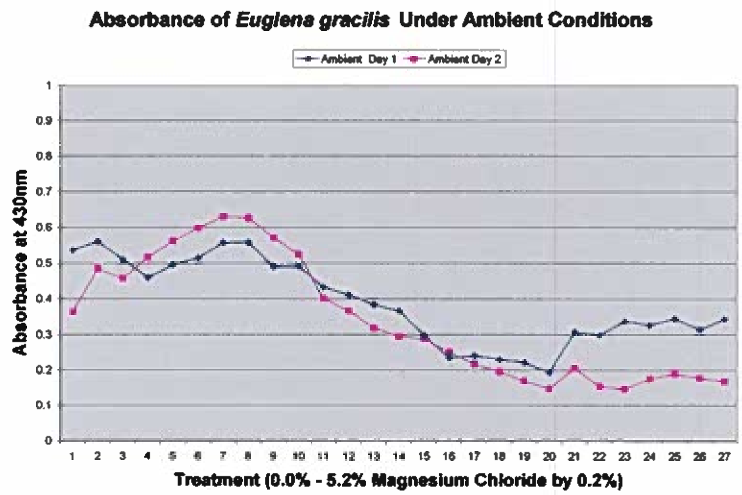
From the preliminary study, the average weight of the treated radish plants was .073 grams, the divided box of plants had an average weight of .065 grams, and the average weight of the control plants was .046 grams. Based on the results, the addition of $MgCl_2$ to the soil aided plant growth. These results ran counter to a key observation that was made, at three weeks in time, just after the fourth treatment (overnight): every plant that was exposed to the chemicals was wilted and contained patches of brittle yellow material. This condition had befallen the entire population of the treated plants, and exactly half of the plants that were in the divided planter. The half that was treated was hit heavily and the patches and other symptoms of adversity tapered as the rows of plants proceeded into untreated territory.

The first trial of the principle study showed that *Euglena* had been completely killed by 5% $MgCl_2$ concentrations, but at intervals of 1.0% the actual effects of heat and salt concentrations were not readily apparent. The first trial was run to get an initial testing of the hypotheses. After adjusting treatment intervals to 0.2% and the range from 0.0% to 5.2% the experiment was performed to obtain more precise and accurate results. There was no reason to go beyond 5.2% because of the results of the first trial. Visual observations of *Euglena* showed agitation as early as 0.6% represented by a decrease in movement and activity when compared to the control group. There was also a significant population decrease (up to 50% compared to control) at 2.4% $MgCl_2$ concentration. At concentrations of 3.8% $MgCl_2$ solution as few as 5.0% of the original population remained. Total eradication occurred at 5.0% in the unheated group and 3.6% in the heated group. These results are consistent with earlier tests, showing a damaging effect of $MgCl_2$ at relatively low concentrations.

The *Euglena* cultures in the test tubes containing a range of concentrations of $MgCl_2$ showed different pH values. Using broad-range pH paper to establish the general vicinity and fine-range pH paper to establish precision, the pH began at a slightly acidic 6.0 and slowly climbed to a slightly alkaline 7.4 at 5.2% $MgCl_2$ concentrations.

In the refined experiment incorporating the use of a spectrophotometer, the readings of the absorbance was highly variable. Even with high variability, the spectrophotometer readings did showed a decline in the absorbance readings at 430nm (absorbance of chlorophyll *a*) that corresponded with visual observations of the *Euglena*. Figure 1 demonstrated the declining trend of absorbance for all treatments across the increasing concentrations of $MgCl_2$. Using ANOVA, the interactions between increasing salt concentrations and increased temperature showed a synergistic trend (Fig. 2). ANOVA was done between the treatments at all $MgCl_2$ concentration levels.





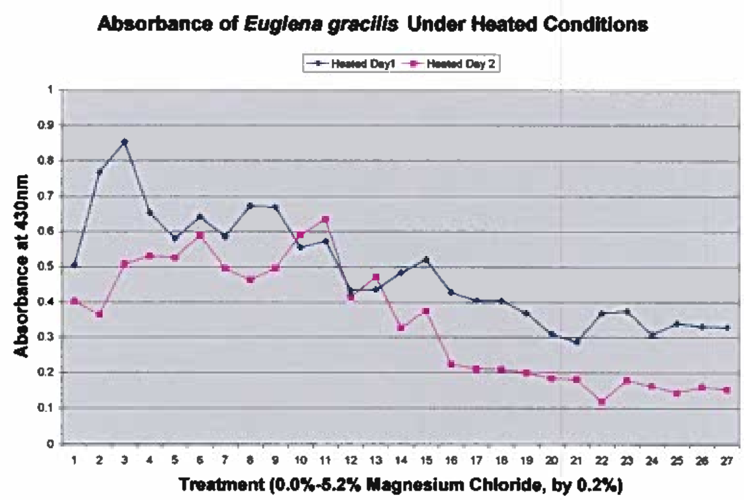
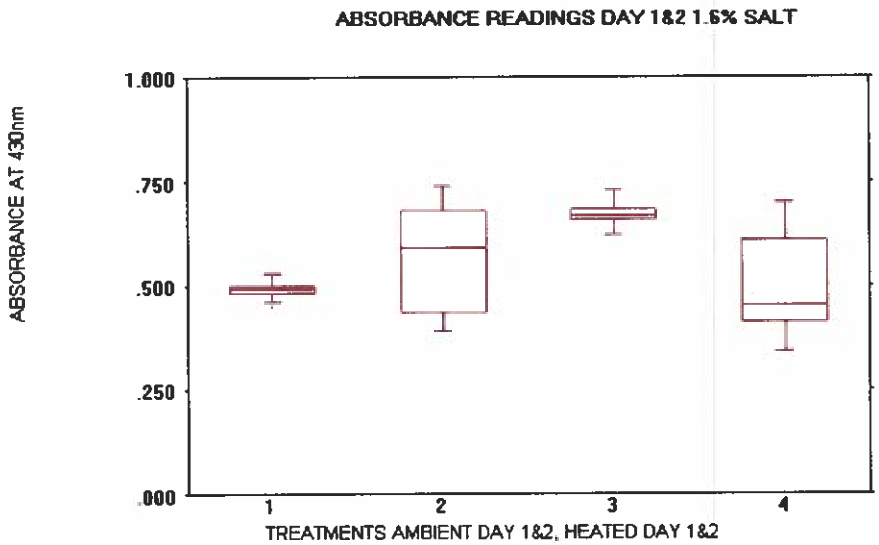


Figure 1. To obtain an approximation of the number of *Euglena gracilis,* spectrophotometer readings were taken at 430nm, the absorbance of chlorophyll *a*. Absorbance readings showed a decline that correlated with the visual observations of the number of active *Euglena.* Salt concentrations increase from 0.0% to 5.2% in increments of 0.2% and treatment 1 was the control at 0.0%. The above graphs summarize spectrophotometer readings for days 1 and 2. Graphs from top to bottom show all treatments, the ambient treatments, and heated treatments.



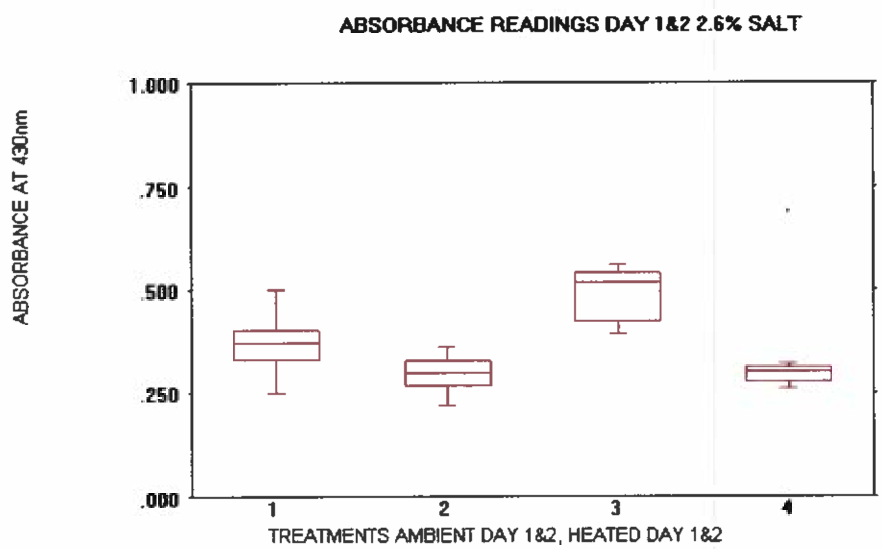


Figure 2. The above graphs represent the treatments at the salt concentrations of 1.6% and 2.6% $MgCl_2$. There was no difference between ambient treatments day 1and 2 at a confidence interval of 0.05. There was a significant difference between the heated treatments day 1 and 2 (treatments 3 and 4) at a confidence interval of 0.05. Therefore, a synergistic response was shown in the interaction of increasing $MgCl_2$ concentrations and increased temperature. ANOVA analysis was done for all salt treatment levels and is briefly represented by these two graphs.

Microscopic examination provided valuable information regarding *Euglena* behavior. Figure 3A demonstrates the normal appearance of *Euglena* under normal conditions. The *Euglena* demonstrated large effects at 2% solution including increased mortality. Concentrations as low as 3% $MgCl_2$ caused approximately 50% mortality after just one day of exposure or less, and above 5% $MgCl_2$ they were all killed. There was 100% mortality in the heated group at 8% $MgCl_2$ concentration. The *Euglena* also discharged their chloroplasts under stronger concentrations of $MgCl_2$ solution. The most frequent discharge was found in *Euglena* that were already dead, with their cell bodies identifiable, but without chloroplasts (Fig. 3D). In the heated samples, the *Euglena* discharged their chloroplasts, and many were found to still be motile. Those found to be moving displayed an alternative method of movement that did not utilize their flagella. This movement is called metamovement and occurs when *Euglena* are placed under any of various stressors (Fig. 3C). Metamovement can be best described as a contraction of cytoplasmic skeletons followed by an elongation of their bodies (amoeboid like). Hemocytometers were used to attempt to gather data on the number of survivors and their movement patterns (Fig. 3B). The high motility and large body size made counts unreliable because of the speed at which counts changed, so only qualitative assessment of surviving *Euglena* were obtained using light microscopy.

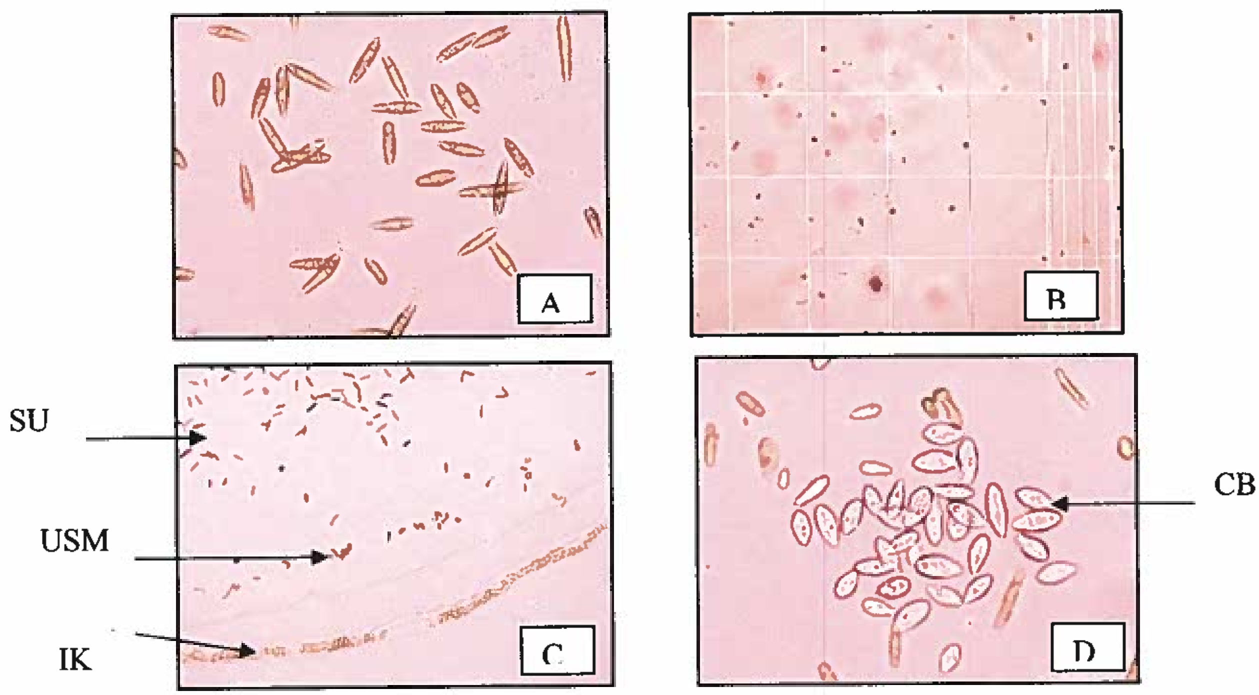


Figure 3. Microscopic observations of *Euglena gracilis*. (A) Untreated *Euglena* at 400x. (B) One grid section of hemocytometer at 100x with *Euglena* moving about demonstrates difficulty in obtaining accurate count of the organisms. (C) Three different shells of *Euglena* develop after exposure to $MgCl_2$ solution: survivors (SU), under stress and displaying metamovement (USM) and instantly killed (IK) organisms photographed at 100x. (D) *Euglena* demonstrating chloroplast bleaching (CB) photographed at 100x.

With reference to the follow-up study, the seed germination chamber was opened after seven days and the germination trays were counted for sprouted seeds. Two trays had sprouted seeds, the control watered with only distilled water, and the next level of treatment, at 0.4% $MgCl_2$ solution. Approximately 40% of the lettuce seeds in the control had sprouted by the seventh day and less than 5% of the 0.4% solution had sprouted. Every treatment above the 0.4% level showed no signs of germination. In contrast, the control had full cotyledons (the 0.4% had less developed cotyledons than the control).

DISCUSSION

Radish plants were started from seed in order to determine the effect of $MgCl_2$ on growing plants. In the preliminary study, the plants were allowed time to fully develop out of their sprout stage before they were treated, mimicking a plant that might be affected by the application of the anti-icer due to an untimely early snow.

Throughout the preliminary study, the control group of radish plants continued to remain green and healthy. This observation is counter to what the results infer. It is reasonable to assume that the plants would dry at an identical rate, at least if they were all left untreated. Magnesium chloride forms a film when wet and this film might prevent the even drying of all of the plants. This is the film that is observed on roads after recently sprayed with $MgCl_2$ slurry solution and makes the road extremely reflective of the sun. This inference is supported by another observation that was made during the growth period. Soil that had been treated with $MgCl_2$ held more moisture than the control group. In addition, the $MgCl_2$ treatment leeched across the divided planter box as the moisture levels followed a gradient that grew as treatments continued. From this observation, it is reasonable to suggest that there was indeed a film or an effect of the treatment that prevented the treated plants from loosing moisture as fast as the control plants.

Research reveals that $MgCl_2$ is indeed hydroscopic. Chlorine is extremely electronegative that it creates strong bonds with water molecules and can also pull water from a stable state into bondage [1]. When $MgCl_2$ is applied to the roads for ice control, it can pull moisture out of the air, actually making the road wetter, but still keeping it from icing. This research supports the speculation that during the drying process, the chemical film residually left after treatment could have kept water weight in the plants even after the control was dried out. The capability for $MgCl_2$ to hold water could have implications in its seemingly toxic effect on organic matter.

Another possibility for the loss of small but significant amounts of biomass can be accounted for in the amount of material being weighed. The control plants were extremely dried out and therefore, their root structures were extremely brittle. Every control plant had sections of root that broke off while dirt that was clinging onto them was cleaned off. The plants that had been treated all displayed more robust root structures, stalks, and leaves. Perhaps the moisture helped more dirt adhere to the fibrous structures, and fewer roots were broken off. These minute amounts of substance could add thousandths or even hundredths of a gram, tilting the biomass scales in favor of the treated groups.

Results from the preliminary study provoked further testing. There was too high a correlation of treated plants to unexpected plant death to be shrugged off as random error or improper procedure. Originally, testing was to be for difference in fruit size of healthy radishes against treated radishes. Unfortunately none of the test groups had favorable enough conditions to produce storage roots. This could have been from cold conditions, improper lighting, or malnourished soil; all were possible in trying to grow plants indoors during a Colorado winter. The experiment did not go as planned, but the results were still valid enough to extend this investigation through additional studies.

Overall, each phase of the principle study showed consistent results. These data indicate that $MgCl_2$ is harmful to *Euglena* at relatively low concentrations compared to what road crews put onto the highways to prevent ice. The State of Colorado Department of Transportation applies $MgCl_2$ at a concentration of 27-29% by volume [2, 3, 4, and 9]. No follow-up studies were performed since the initial environmental impact study was done in 1997. Since then, $MgCl_2$ has been applied to the roadways for the past nine years. It is worth considering the idea that there is a cumulative effect of the use of $MgCl_2$ over a time span of many years. This opens many other new avenues for research of the effects of $MgCl_2$ over time.

An external qualitative study also indicates that *Euglena* react to the introduction of salts. The external study was not conducted specifically for this investigation, but provides an additional source of related information. This study was preformed in conjunction with a local high school biology class who, studying microbiology, prepared slides of *Euglena* and then added salt solution to the samples and examined the cultures through a microscope at high magnification. The results from the entire class mirror that of studies conducted for this investigation [Williams, S., et. al., personal communication, November 28, 2006]. *Euglena* shows a severe inability to adapt to the introduction of salt solutions stronger than 2% concentration.

Errors in the reading of the spectrophotometer could have occurred as a result of its design. Although not precise enough to offer definitive results, data from using the spectrophotometer do show some correlation to the decrease in concentration of *Euglena* and the increase in concentration of $MgCl_2$. The spectrophotometer lenses were focused to obtain readings towards the tops of the cuvette glass. After mixing *Euglena* solutions, bubbles and foam rose to the top of the tubes, which may have affected light refractions. There were also films that formed on the solution surface that were a deeper green. The observable films only occurred in higher concentrations of treatments, and possibly contained discharged chloroplasts or cytosol from stressed *Euglena*. Light could have been refracted from portions of this film that could have disturbed the readings. Despite occasional extreme variations in readings, results were consistent over time, building credibility in these data despite seemingly random readings. This experiment offered strong evidence that $MgCl_2$ can be tied to destruction of organisms and plant matter.

The optimal pH for photosynthesis in *Euglena gracilis* is 6.0 and optimal pH for growth is 7.0 [5]. Due to the nature of *Euglena*, the pH swing did not affect their ability to survive in salt solution as it passed through their ideal pH range. This eliminates pH as a source of detrimental effects suffered by the *Euglena*.

Agricultural crops such as rye and cucumbers can experience similar physiological outcomes as *Euglena gracilis* when exposed to heat stress and at relatively low levels of soil salinization with $MgCl_2$ [5, 11]. Further studies that actually measure the quantities of $MgCl_2$ concentrations of plant germination in soil should be conducted. Parallel studies should also be performed using hydroponics and aeroponics to better isolate the effects of $MgCl_2$ on agricultural and native flora.

It is noted that salinization of soil can lead to growth problems in plants. Grand Junction, Colorado is experiencing salinization of irrigated soil, effecting crop and livestock development. These areas are not being treated with $MgCl_2$ though; instead, selenium salts are contaminating the soil [10]. Yet, essentially it is the salinization that is disturbing the soil. There is a wealth of information documenting this current dilemma, a dilemma that could soon be descending upon other areas that are known for their agricultural and environmental significance. It is imperative that more in-depth and precise studies be performed before further contamination in the form of excess salt deposits to the soil and water in these environments occur.

The follow-up botany study mimicked what might happen should the soil be left saline after a winter season of application of $MgCl_2$. This exploration was designed to try and control any tainting variables that might have affected earlier preliminary results. This study should be revised and tested further using seeds from various different crops and native flora and varying concentrations of $MgCl_2$ at narrower intervals such as 0.1% increments.

Due to the response of *Euglena gracilis* to $MgCl_2$ concentrations as low as 0.4% in heated samples it becomes evident that further study of the effects of $MgCl_2$ on native protists in both terrestrial and aquatic ecosystems is needed. Studies performed by the Colorado Department of Transportation measured salt concentrations after a season of use, at a time well past the thaw period for snow along the test roadways. Testing the effects of immediate discharge from the roads on the various below ground food webs, waterways that cross the roadways and closed aquatic environments need to be performed. Since there was a differential response of *Euglena gracilis* that appeared in the samples that experienced both salt stress and heat stress (Fig. 1 and Fig. 2), it is necessary to repeat this study with increments of 1°C to better determine the impacts of $MgCl_2$ on native aquatic and soil food webs that rely on protists as vital links in those trophic systems.

As development claims more agricultural land, being able to compare the effects of $MgCl_2$ on agricultural crops and on native vegetation using treatments that include varying salt concentration levels and varying temperature levels are necessary. Measurements of various soil and water concentrations of $MgCl_2$ should be gathered to correlate research data to conditions experienced in both agriculture and conservation. Global warming is accepted as an event that is occurring at the present time. The probability that minimal salinization coupled with increasing water, soil and air temperatures can negatively affect primary productivity of farmland and other terrestrial food webs indicates that further research expanding this study is immediately necessary. Moreover, salinization of streams and rivers that receive $MgCl_2$ discharge from roadways and city storm-drain systems during and after winter in the mountain west is a concern that needs to be addressed further. The use and reuse of water in the west for irrigation indicates that there is a real risk of cropland salinization when the water comes from a source that receives $MgCl_2$ effluent directly from roads or runoff. If $MgCl_2$ effluent is discharged into critical terrestrial or aquatic ecosystems there is also a high risk of causing further damage to threatened and endangered species.

Magnesium chloride has been in use by the Colorado Department of Transportation for the past nine years [2, 3, 4, and 9]. The effects of selenium salinization of soils from irrigation took time to become apparent. The results of this study indicate that salinization of soils and waterways are a serious concern for downstream users of water that receive discharge from roadways treated to prevent or speed the removal of ice on roads. That concern is increased with the use of $MgCl_2$ on residential and business sidewalks, driveways and parking lots due to its increased availability from retail sales. Residential and business application of $MgCl_2$ results in an amplified discharge of the salt into streams and rivers by community storm drains upon snowmelt. Wildlife managers should also be concerned about the effects of $MgCl_2$ on below ground and aquatic food webs that might result due to removal of protists as part of those unique trophic systems.

CONCLUSIONS

A meta-analysis of results from all phases of the studies indicates that the null hypotheses were rejected and the alternate hypotheses were accepted. The following conclusions arise from these studies:

1. In the preliminary study, evidence from the radishes demonstrates that $MgCl_2$ has detrimental effects on plant growth and survival after only four applications of $MgCl_2$. The alternative hypothesis for the preliminary study stated: Magnesium Chloride will adversely effect the growth and survival of Early Scarlet Globe Radish (*Raphanus sativus*) plants when applied to the soil at the recommended amounts on a bag of $MgCl_2$ purchased from a wholesale store (one cup per square yard) for each major storm system that passed

through the local area. The fourth application of $MgCl_2$ at the recommended levels resulted in plant death, which supports the alternative hypothesis.

2. In the principle study, statistical evidence supports that salt concentrations above 1.4% had a detrimental effect on growth and survival of *Euglena gracilis*. The P value was set at 0.05. Analysis by ANOVA further demonstrated that as early as 1.4% magnesium chloride concentration, a synergistic effect existed between increased temperature and salt concentration. Statistical analysis supports both of the alternative hypotheses of this part of the study.

3. In the follow-up study, the alternative hypothesis stated that $MgCl_2$ concentrations above 1.4% would adversely have an effect on germination of Black Seeded Simpson Lettuce (*Lactuca sativa)*. Results indicated that concentrations as low as 0.4% had an adverse effect on germination. Due to the fact that no seeds germinated at concentrations above 0.4%, the alternative hypothesis was supported.

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