IdeaFest: Interdisciplinary Journal of Creative Works and Research from Humboldt State University

Volume 3 *ideaFest: Interdisciplinary Journal of Creative Works and Research from Humboldt State University* Article 7

2019

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Recommended Citation

Tusei, Cristina (2019) "The Effects of Citric Acid on pH and Nutrient Uptake in Wheatgrass (Triticum aestivum)," *IdeaFest: Interdisciplinary Journal of Creative Works and Research from Humboldt State University*: Vol. 3 , Article 7.
Available at: https://digitalcommons.humboldt.edu/ideafest/vol3/iss1/7

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Acknowledgements
Many thanks to Dr. Casey Lu, who this study was generated under and who provided the procedures for the AAS and Kjeldahl chemical assays. A big thank you to Dr. Dave Baston, for continued support and use of the Core Research Center. I would also like to express my appreciation for Susan Wright, for allowing the use of select equipment and for aiding in waste removal.

The Effects of Citric Acid on pH and Nutrient Uptake in *Triticum aestivum*

Cristina L. Tusei[1*]

KEYWORDS—*citric acid, chelate, wheatgrass, soil contamination, nutrient uptake*

INTRODUCTION—Citric acid is a metabolite in plants and plays an important role in photosynthesis and cellular respiration. It contains three carboxylic acid (COOH) functional groups (FIG 1) and can deprotonate to form a negatively charged citrate molecule.

The loss of external hydrogen atoms enhances citric acid's ability to act as a chelate and form complexes around positively charged ions such as metals, effectively removing these cations from solution. Citric acid has a different binding constant for various cations (Goli et al. 2012). The molecule's difference in affinity for positively charged molecules affects which metals it will complex and the rate at which this process will happen. This is an important concept to consider when studying the removal of heavy metals from the environment, a prominent environmental concern. Anthropogenic activities have increased the presence of elements that are harmful to plants and animals in concentrations above threshold levels (Hussain et al. 2019), and methods for their removal are currently being researched. A novel technique called phytoremediation has become a method of interest to decrease the concentrations of heavy metals in the environment. Phytoremediation is the ability of plants to sequester select compounds by secreting a chelating agent; the plant is then able to metabolically convert the element in question into something less harmful (Gong et al. 2018). Prior experiments suggest that citric acid aids elemental uptake in plants by forming a complex around the cation in question, whether it be beneficial or potentially harmful (Senden et al. 1994; FIG 2). This effectively increases both essential and non-essential nutrient availability to plants (Zhang et al. 2018) but also carries the potential for toxicity to occur within plant tissues if the threshold dose level is surpassed. Plants require macronutrients such as calcium (Ca), magnesium (Mg), and potassium (K) for biochemical processes, as well as micronutrients like copper and iron. Their ability to uptake metals also means they will draw harmful elements such as lead and cadmium through their roots, which can be detrimental after prolonged exposure. Metallic species present in the environment in close proximity to vegetation and wildlife often depend on soil composition and surrounding establishments. However, the general trend suggests that areas situated by increasing urbanization and industrial processes have experienced an increase in the concentrations of such metals. Various studies on removing detrimental elements from the environment have been conducted and phytoremediation shows promising results (Gong et al. 2018).

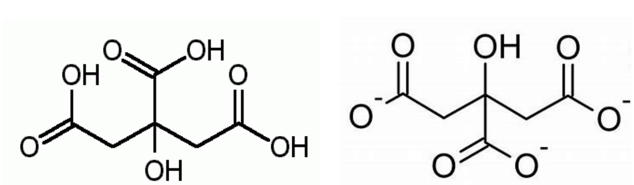

FIGURE 1. A comparison of a citric acid molecule (A) and citrate molecule (B).

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Tusei, C. L. 2019. The effects of citric acid on pH and nutrient uptake in *Triticum aestivum. ideaFest Journal: Interdisciplinary Journal of Creative Works & Research from Humboldt State University* 3: 31–35.

Assorted chelating agents, such as ethylenediaminetetraacetic acid (EDTA) and citric acid, have been utilized in phytoremediation research. A key component in determining functional ligands is examining their effects on the plants themselves. For example, EDTA is effective in complexing metals but is toxic to plants and biodegrades slowly (Hussain et al. 2019). Citric acid

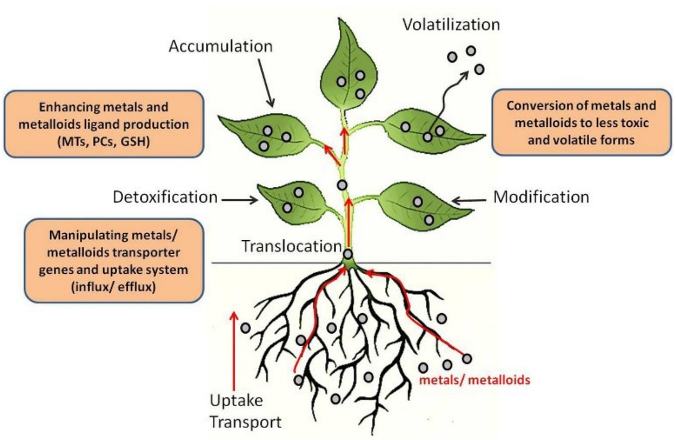

FIGURE 2. The transport of metals through a plant and their conversion to a less toxic form (from Mosa et al. 2016).

degrades in the environment naturally and is excreted by plants themselves, so it is suggested that using this molecule is more beneficial than utilizing other chemical species (Hussain et al. 2019). The use of plants in removing harmful elements from the environment is a fairly new and promising technology. However, because it can take years to restore a landscape by phytoremediation, it can only be used in sites that have low to medium contaminant concentrations. The effectiveness of phytoremediation also depends on the plant species and the heavy metals present in the environment.

Triticum aestivum, or common wheat from the family Poaceae, is a widespread plant that has established itself nationwide and proliferates in all seasons. It is highly tolerant in various environments and grows rapidly, making it the ideal plant to study. Due to citric acid's ability to chelate metals and be absorbed by plants, it is hypothesized that exposure to it will increase the ability of wheatgrass to absorb macro- and micro-nutrients, as well as heavy metals, from soil. This experiment will specifically examine the elements calcium (Ca), magnesium (Mg), potassium (K), and nitrogen (N); certain limitations of this

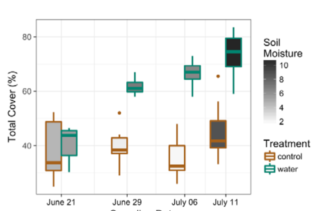

FIGURE 3. Citrate as chelate for calcium

inquiry made the application of heavy metals to solution not possible. The aforementioned nutrients are vital to plant growth and biochemical processes and results obtained can be used to predict how heavy metals such as lead and mercury would be absorbed by a plant similar to wheatgrass. Given that citric acid has a pH of 2.2, it is also hypothesized that prolonged exposure of wheatgrass to the organic compound will increase the acidity of the soil.

METHODS—*Triticum aestivum*, also known as common wheatgrass, was selected as the test subject due to its versatility in growing conditions and rapid growth rate. Seeds were purchased from Pierson's Garden Care Center in Eureka and germinated in Humboldt State University's experimental greenhouse. Six seeds were sowed per pot, with two replicate pots for each of four treatments and two replicate pots for the control. Plants were kept in a naturally lit area and were grown in a standard potting mix containing compost, perlite, alfalfa, and crushed basalt, among other nutrient-containing materials. The control group received 200 mL of deionized water (diH_2O) per pot. Treatment groups also received 200 mL of diH_2O per pot in addition to the following concentrations of citric acid: 5 mM, 10 mM, 15 mM, and 20 mM. Each replicate contained six plants and treatments were administered three times a week for three months.

Chemical assays. The pH was measured every two weeks initially, then increased to once a week after a month. Results were obtained by capturing water drainage from the soil and analyzing the solution with a pH meter. Macronutrients Ca, Mg, and K were analyzed using an Atomic Absorption Spectrometer (AAS). Solutions for AAS were prepared by drying and ashing plant material and filtering with 20% hydrochloric acid (HCl). After being treated with HCl, samples were diluted to 50 mL with diH_2O and centrifuged. Further serial dilutions were made with 2% HCl in order to obtain the proper range of readings on the AAS; otherwise the concentrations of each element would have been inaccurate. Serial dilutions were factored into calculating the concentration of each element.

Nitrogen content was measured using Kjeldahl digestion and titration, in which organic N was converted into ammonia following a reaction with sulfuric acid during a digestion period. The solution was reacted with 40% sodium hydroxide and distilled into 50 mL of a boric acid solution containing bromocresol green, methyl red, 100% ethanol, p-nitrophenol, and 4% boric acid. This solution

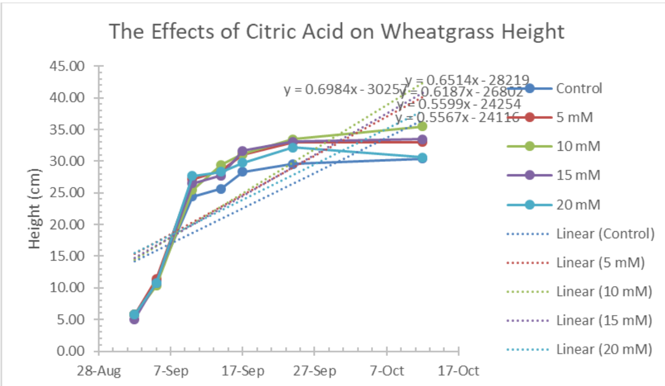

FIGURE 4. The effects of citric acid on wheatgrass height.

was then titrated with 0.1 M HCl until the endpoint was reached, indicated by an orange-red color.

RESULTS—An initial increase in height occurred for all treatments, including the control. The most efficient way to determine if one treatment grew more than another was through computing the slope of each line; the steeper the slope, the greater the growth rate of that particular treatment. Examining the slopes, it is clear that neither the control nor any of the treatments decreased in height and that the 10 mM wheatgrass experienced the largest growth rate, with the steepest slope of 0.6984. The control, with a slope of 0.5567, experienced the lowest growth rate.

The pH of the treatment groups steadily decreased (FIG 6). The control group varied so it is possible that other factors besides the addition of citric acid affected the pH of the soil. Observed decreases in pH suggest that citric acid may have caused a drop in pH; this was also visually apparent during the experiment, as water outflow was noticeably paler with increasing citric acid concentration (FIG 5). Statistical analyses were limited to R^2 values that were calculated based on linear modeling of pH over time. All treatments, including the control, had R^2 values greater than 0.50, indicating that these data fit the model better than by chance. In particular, the 10 mM treatment had an R^2 value of 0.807, indicating that this treatment best fit the model, in which there was a linear decrease of pH during the course of the experiment. The 5 mM treatment also had a high R^2 value (R^2 = 0.769), indicating that this treatment also experienced a decrease in pH over time. While I cannot comment on the significance of these effects, these effects were strong for 5 and 10 mM treatments in particular.

FIG 7 displays the effects that varying molarities of

FIGURE 5. A visual representation of the effects of citric acid on the outflow of water from wheatgrass plants. Molarity increases from left to right.

citric acid had on the concentrations of Ca, Mg, and K. Calcium decreased slightly at 10 mM and again at 20 mM. Potassium had a sharp dip at 15 mM but increased again at 20 mM; magnesium stayed constant. As indicated by the very low R^2 values for these linear models, the strength of the effect of citric acid concentration on the concentration of Ca, Mg, and K was extremely weak.

FIG 8 portrays the concentration of N analyzed through a Kjeldahl digestion. The percentage of N present in approximately one gram of sample was consistently higher in citric acid treatments relative to the control. Given an R^2 value of 0.4847, the data do not closely fit the linear model of increasing percentage of N with increasing citric acid concentration.

DISCUSSION—Plants often secrete organic acids from their roots in order to complex free metallic ions and draw the element up to their shoots (Wang et al. 2017). This includes both beneficial and harmful chemical species that can impact the plant's metabolic processes depending on the concentration of the element absorbed. Factors that determine metal uptake include the species of plant and the type of organic acid present. It is suggested that plants are able to convert toxic metals into nontoxic forms (Mosa et al. 2016). This occurs to an extent, until the metals become toxic to the plant itself and adversely affect it.

Favored chelating agents include EDTA, nitriloacetate

(NTA), diethylenetriaminepentaacetate (DTPA), and, more recently, citric acid (Hasegawa 2012; FIG 9). Biodegradable compounds such as citric acid are utilized more often due to the fact that they will break down in the environment instead of persisting like the aforementioned chemicals.

This study hypothesized that after being exposed to increasing increments of citric acid concentration, wheatgrass plants would contain higher amounts of macronutrient elements and the soil in which they reside would have a lower pH. The results obtained suggest that pH will decrease as molarity increases; however, this cannot be supported statistically and can only be inferred from FIG 5 and R^2 values. There is no strong evidence to support that nutrient metal uptake is influenced by varying molarities of citric acid.

Although results of this experiment were inconclusive, inquiries of the same nature have been conducted elsewhere with promising results. A study concerning the nutrient profile of wheatgrass at Marathwada Agricultural University in Parbhani, India, suggests that macronutrient concentrations increase linearly over time (Kulkarni et al. 2006). The study analyzed a total of fifteen elements by use of instrumental neuron activation analysis. Wheatgrass was grown for a total of twenty days in three different treatments: tap water, tap water with nutrients, and soil and tap water. All treatments were watered with 200 mL and kept in an unfiltered air and light setting. The main differences between this experiment and the referenced study lie in the varying treatments the plants were placed in and the amount of water they received. It is a possibility that a solution already treated with nutrients would increase the likelihood of there being a greater concentration of the nutrients within the plant already. While it was anticipated that macronutrient concentration would increase over time, I did not observe this in this study. This may be due to other contributing factors that were not incorporated into the initial reasoning, such as physiological strain or interactions with other chemical species.

The methods used in this experiment can be improved upon. The trial was run for two months, and this may not have been sufficient time to observe a difference in the effects of citric acid on elemental absorption. A more productive treatment window would have been six months to a year. The plants were grown in a greenhouse, but no special precautions, such as temperature and light control, were incorporated. It is possible results would have

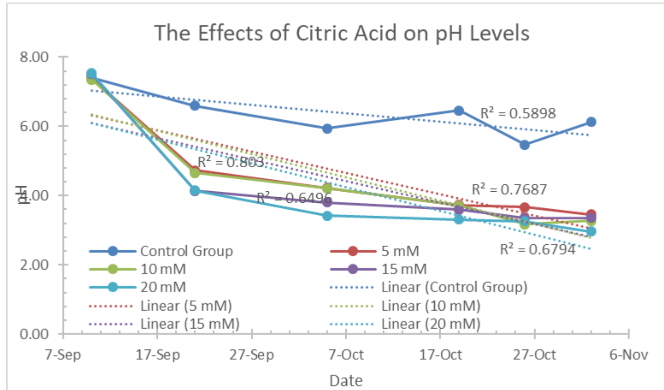

FIGURE 6. The effects of citric acid on pH.

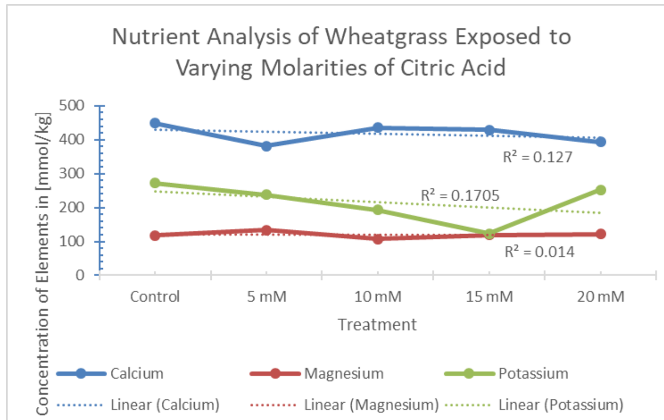

FIGURE 7. The variation in the amounts of nutrients in mmol/kg for each treatment of citric acid.

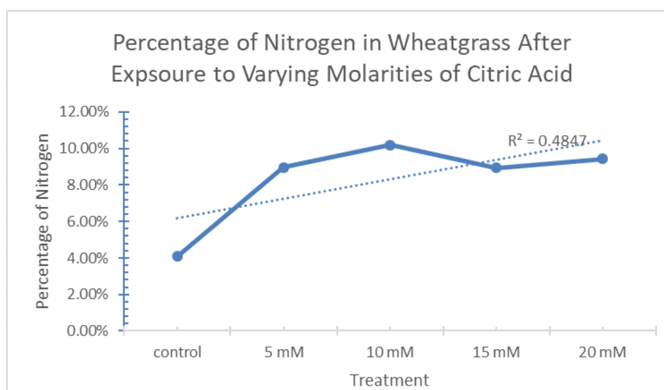

FIGURE 8. Percentage of nitrogen present in varying molarities of citric acid.

been significant had these factors been accounted for. Instrumental error could have also occurred but replicates of the analyses would need to be carried out in order to determine this. In the future, this experiment should be performed for a longer amount of time within an enclosed environment. Multiple replicates of instrumental

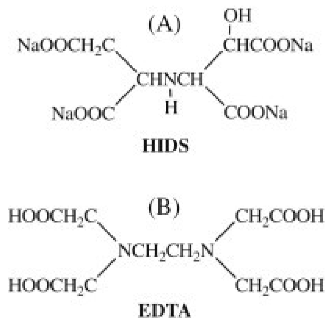

FIGURE 9. The chemical structures of two chelating compounds, HIDS and EDTA (from Hasegawa 2012).

analyses should also be performed with a greater number of treatment pots.

This experiment suggested that addition of citric acid to solution will decrease soil pH. Results are not conclusive as to whether it affects elemental absorption and thus it is difficult to ascertain its ability to improve phytoremediation ability of plants. Had results been more conclusive, the utilization of citric acid as a chelate and its ability to remove metals from solution would have been an indication for further experiments to determine its effectiveness in phytoremediation. It is recommended that future studies be conducted in order to better determine the effect of citric acid on metal and nutrient absorption in plants. Possible experimental improvements include prolonging the treatment period, growing plants in more controlled conditions, and spiking each treatment with a different nutrient element, such as Mg or Ca, and analyzing the plant for that particular metal. Solidified outcomes hold implications for future treatment of plants in nutrient deficient or contaminated soils.

ACKNOWLEDGMENTS—Many thanks to Dr. Casey Lu, who supervised this study and provided the procedures for the AAS and Kjeldahl chemical assays. A big thank you to Dr. Dave Baston, for continued support and use of the Core Research Center. I would also like to express my appreciation for Susan Wright, who allowed the use of select equipment and aided in waste removal.

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