Water Lettuce and Apple Snails' Ability for Bioremediation of Copper

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ABSTRACT

Central Florida's waters are in critical need of restoration. Recognizing which plants and animals that help in restoring our waters will provide a realistic and cost efficient solution. Our research focused on the capacity of Water Lettuce (Pistia stratiotes) and Apple Snails (Pomacea insularum), which are both classified as invasive, to improve water quality in lake systems contaminated with Copper. To perform this project, we created an environment suitable for the animals involving a recirculating system. Overall, the system contained 8 tubs; a control, plants, snails, and plants and snails. Out of the six weeks of expected research only four weeks were conducted due to the initial spraying of Cu and lack of health of the snails and water lettuce. After reestablishing the tubs we were able to conduct four weeks of data. We assessed the efficiency of bioremediation by testing the levels of Cu weekly within each of the eight tubs. The water from each tub was tested for the concentration of Cu and was read using an YSI 9,500 photometer. The results showed that P. insularum was most efficient at bioremediation of copper than P. stratiotes and P. Insularum together and together. Further research is required to determine if the classified invasive species P. stratiotes and P. insularum are sufficient at the removal of Cu in lake systems.

INTRODUCTION

Florida's water quality is becoming an increasing issue. Nitrate rich pesticides have been intentionally introduced into Florida's freshwater ecosystem. The increasing use of these pesticides are threatening the balance and causing environmental pollution. The adding of nutrients, such as copper, nitrogen and phosphorus, is one of the most common pollutants affecting the Florida's freshwater. It accounts for more than 50% of reported water quality impairment (Huang, 2009). A large portion of these waters and the surrounding area are used for agriculture treated with fertilizers and pesticides, including Cu (Hoang, 2009). Over 45% of Florida's lakes are considered impaired from pollutants and stressors, such as an over abundance of nutrients, metals, excess algae growth, and pesticides (Quality of America's Lakes, 2000). The toxicity of all fresh water bodies are interconnected, the runoff from fertilizer laced lawns eventually trickles down and pollutes the Floridan aquifer. This causes excessive algae growth around the mouth of many springs which in turn slows down the flow and leads to even more algae growth. Spraying toxic chemicals to treat unwanted aquatic plants such as hydrilla is common practice in Florida waterways. It is uncertain what kind of affect the chemicals are having on the native wildlife in the spring runs and rivers due to lack of research. The vibrant green of algae is the environment's response to nutrient runoff which can be made worse by heavy rainfall ("Florida waters alive", 2013).

Invasive species are defined as a nonnative species that out competes a native species for resources (USDA, 2006). Water lettuce and island apple snails are two nonnative species that plague central Florida's river systems. Island apple snails are known to grow larger than the native Florida apple snail and are thought to be a source of frustration to the threatened snail kite (Kitchens, 2010). Water lettuce is known to form dense mats that shut out sunlight to the submerged plants below, as well as cause problems for boaters and swimmers who are used to a clear path in a certain part of their creek (Ramey, 2001). The fact of the matter is that many invasive species are here to stay, and while chemical-laced means of disposal are culling the population in certain areas many of these species are producing quickly and hindering the growth of native species.

There are many invasive species of flora and fauna that can be used to our advantage instead of being destroyed by harmful chemicals. If an invasive species cannot be contained, and is thriving in our ecosystems despite extensive measures to get rid of it we should begin planning ways to use them to our advantage, because like the plants and animals that share an ecosystem with invasive aquatic species, we need to learn to adapt and grow.

The apple snail is one of the many invasive species in Florida. Apple snails are large freshwater gastropods that are native to tropical and subtropical areas around the world (Barker, 2002). They are commonly known as mystery snails, or golden mystery snails in the pet trade (Barker, 2002). Many species of apple snail inhibit slow moving rivers and lakes, and are perfectly at home in marshes and ditches (Barker, 2002). All apple snails have both lungs and gills which makes it possible for them to live in a dried lake bed for 9 months to a year (Barker, 2002). There are five species of apple snail in Florida, and only one of them is native. The most prominent species of apple snail in Florida is the Island Apple Snail, *Pomacea insularum*, originally thought to be released into the wild by aquarium hobbyists who became aggravated by the snail's ability to uproot and destroy a planted tank in a very short amount of time.

While *P. insularum* is considered to be an invasive species the reasoning is vague or inaccurate. Many cite the plight of the threatened Florida Snail Kite as a reason to rid the state of these creatures. The snail kite feasts almost exclusively on apple snails and studies have shown that juvenile snail kites have trouble opening the larger *P. insularum*, and therefore expend more energy than acquired by eating the snail (Kitchens, 2010). However limpkins also eat the apple snails and they have no problem opening the larger snails. By having *P. insularum* in the same population there is an abundance of food available for both species of birds.

P. insularum are voracious herbivores and are known to wreak havoc on aquatic plants and habitats. While they do devour native aquatic plants they are also extremely partial to hydrilla (*Hydrilla verticillata*), a highly invasive aquatic plant that is choking our spring runs and pushing out native species of plants (Mitchel, 2004). By culling invasive plant populations, and being reproductively successful so more snails are available to be a primary food source for native birds, one could argue that *P. insularum*, while nonnative, are not as invasive as once considered and should not be actively destroyed with chemicals that could potentially harm the species around them.

Water lettuce (*Pistia stratiotes*) is a floating aquatic plant with large soft leaves that form a rosette and roots that hang submersed beneath the floating leaves. It is considered a weed in Florida because it can form large thick mats that clog water ways (Ramey, 2001) and also has the potential to reduce biodiversity in a water way by blocking gas exchange at the surface, which in turn reduces oxygen and kills fish (Ramey, 2001). The large mats of water lettuce also block light which can kill submerged plants, and can potentially crush the plant communities. Water lettuce, while it has its faults, can be used to clean up a river system as water lettuce is known for its water purifying ability (Reddy et al., 1982). It can outcompete with algae for nutrients in the water and thereby prevents algae blooms (Pirie, 1960). Compared to native plants, this invasive species shows significantly higher nutrient removal efficiency with their high nutrient uptake. They have a large capacity, a fast growth rate, and a big biomass production (Reddy and Sutton 1983). Water lettuce can double their number and size in one to two weeks (Lu and He, 2010). The success of water treatments using aquatic plants depends on the growth rate of the specific plants, and how they utilize solar energy as well as the available nutrients (Reddy and DeBusk, 1984).

While these plants are considered highly invasive, they can be very useful to society. Pirie studied water lettuce, and another invasive water purifier called water hyacinth and deemed that both species could be used as fertilizer, livestock feed, or even human food (Pirie,1960) if we put the time and effort into harvesting the plants instead of attempting to destroy them with chemicals.

Copper is an element that can be found for all biota, however anthropogenic sources for copper can cause water pollution, and toxicity to aquatic organisms (Gendy, 2009) Copper sulfate is example of an herbicide that is commonly used to rid lakes and ponds of an overgrowth of algae called “algae blooms.” Specifically within the Central Florida area, Cutrine, an anthropogenic copper source with 9% of the element as an active ingredient is dispersed throughout fresh water bodies. It is used to control many species of algae as well as hydrilla, which as we discussed earlier is highly invasive in the central Florida area. The following can be found on the Cutrine label: that water treated with this pesticide is safe for swimming, drinking, animals, and watering lawns (MSDS No. D7120, 2007). The label sounds relatively safe; however Cutrine can affect the entire ecosystem as well as non-targeted organisms (Deoliveirafilho, 2009). Over the past two decades, copper from anthropogenic sources have caused toxicity to aquatic organisms and has contaminated freshwater ecosystems (Deoliveirafilho, 2009). When exposed to heavy metals, such as copper sulfate, aquatic invertebrates have developed ways to regulate internal copper concentrations and essentially build up copper in their systems (Hoang, 2008). Studies have determined that the apple snail can accumulate copper from a variety of sources in its environment such as food, soil, and water (Hoang, 2008), however it has been shown that copper transfer through the food chain was the most important route of uptake as compared to water or soil exposure (Hoang, 2008). This raises the question about apple snail predators such as the snail kite and limpkins and how much of the pesticide they are being exposed to when they eat the apple snails (Hoang, 2008). This pesticide has been developed to poison aquatic plants therefore Cutrine has devastating consequences for water lettuce. The copper in Cutrine triggers oxidative stress in rooted aquatic plants and equally harms floating and immersed plants (Upadhyay, 2009) short term copper exposure has been studied to have an effect on the biochemistry and physiology of aquatic plants (Upadhyay, 2009). Exposures and effects from anthropogenic copper sources (Cutrine) can be studied in living organisms known as biomarkers (Gendy,2009).

Sustainability is often defined by three pillars: environment, economy, and equity. From an ecological point of view, the relationship between aquatic organisms and their ecosystem is being affected by the application of chemicals into the pond systems to treat nuisance plants such as invasive species and algae blooms. This addition of chemicals harms the system by messing with the ph and chemical levels. The biodiversity of organisms will not tolerate heavy metals; it will lead to the eventual demise of keystone species from heavy metal build up due to preying on chemical-laden invertebrates and fish. When a keystone species is lost, it affects the hub and therefore the metapopulations. By focusing on restoration of a hub, more species can be positively influenced and the ecosystem's health can be improved.

Environmental pollutants and toxins come at a huge economic cost along with a cost to the environment. According to the U.S. Geological survey, "Cleaning up existing environmental contamination in the United States could cost as much as $1 *trillion* dollars" (U.S. Geological Survey). Bioremediation is shown in Figure 1 and is defined as, "the use of either naturally occurring or deliberately introduced microorganisms or other forms of life to consume and break down environmental pollutants, in order to clean up a polluted site" (Baker and Herson ,1994).

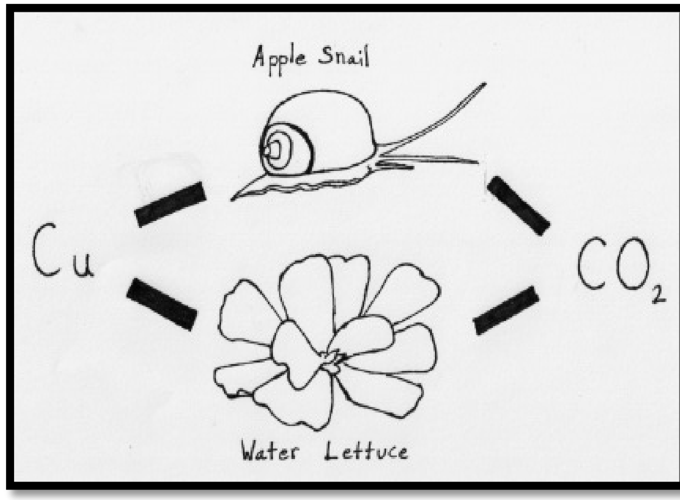

Figure 1 Bioremediation of copper with water lettuce and apple snails

It works as a cost effective solution, because it has the ability to treat toxins in place, reduce environmental stress, and harness natural processes (U.S. Geological Survey). Bioremediation also addresses restoration instead of displacement and therefore further helps the environment.

Invasive species are also a great economic cost to the government. Apple snails and water lettuce are both invasive species and understanding these species that are already present at polluted sites could address the aspect of bioremedation by harnessing natural processes and treating toxins in place. If invasive species could be better understood, they could possibly be used as helpful measures to clean the pond systems instead of adding more chemicals and economic burden to the ponds. Florida's water systems are a major source of tourism. If the water ways cannot be cleaned up, it will adversely affect the communities around them.

Clean water has an increased economic impact for the state of Florida, especially when it comes to tourism. Many people travel here to experience the white sand beaches, or hang out with friends on the lawns beside cool springs. Florida's ecosystems attract more than $67 billion in tourism and recreational spending each year (Guest, 2012). However, algae outbreaks can have devastating impacts on water-dedicated tourist destinations (Guest, 2012). Even one harmful algae outbreak may cause visitors to avoid a region, or even the state of Florida as a whole, which could spell disaster for cities, and towns that rely on eco-tourism to survive (Guest, 2012).

HYPOTHESIS

Based on previous research, it was predicted that a large biomass would provide a greater bioaccumulation and therefore a greater bioremediation. This study presented the hypothesis that the combintation of *P. stratiotes* and *P. insularum* will bioremediate the copper treated water more efficiently than *P. stratiotes* or *P. insularum* alone.

METHODS AND MATERIALS

The experimental design changed in the first two weeks of data collection due to trial and error. The experiment was originally designed to use PVC pipes with holes drilled into them that would harbor the water lettuce and collect water pumped from the tubs so it ran through the PVC pipes, over the roots of the plants, and trickle back into the tub to be reused. It was observed that the water lettuce was not able to thrive in this system so the experiment was redesigned without the PVC pipes and the plants were placed directly in the water in the tubs. Refer to Figure 2.

Figure 2 Top original set up with the PVC pipes; Bottom final design with water lettuce floating

The experiment was performed in a garage to avoid predation of the plants and snails. From 8 am to 8 pm the garage door was opened for sunlight exposure. 8 tubs were filled with 10 gallons of water obtained from pond nine on UCF campus, refer to Figure 3.

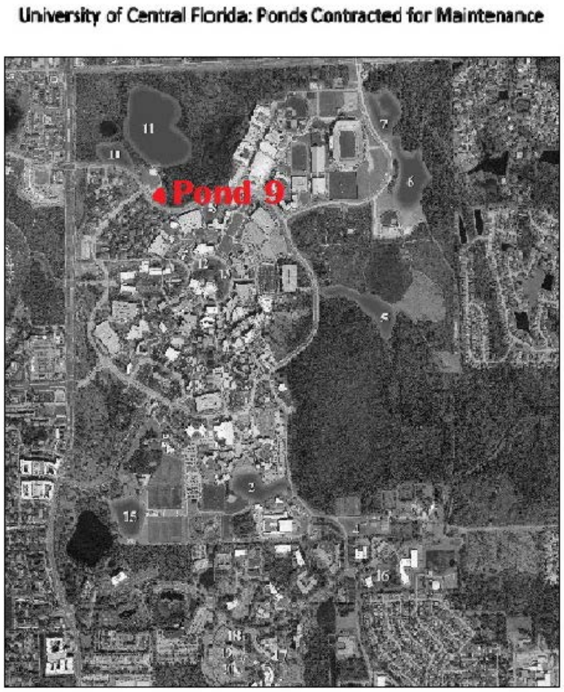

Figure 3 UCF Pond map, highlighting pond 9 in red

A water pump that circulated the water was installed in the tubs. Each tub was exposed to the same amount of sunlight and external environment. Several various sized snails were collected from treated ponds on campus and a dozen various sized water lettuces were collected from the Wekiva River. Snails and water lettuce were divided so that the biomasses were equal. The tanks with the combination of apple snails and water lettuce contained smaller apple snails than the tank that housed apple snails alone. Two tubs contained only snails, two tubs contained only water lettuces, two tubs contained both snails and water lettuces, and the two control tubs contained only pond water. Submerged plants that were found in the treated ponds were used to feed the snails.

The water was tested before the experiment using an urban water testing kit for bacteria, chlorine, dissolved oxygen, hardness, iron, nitrate, pH, phosphate, and temperature. These tests were simple tests that involved the sampling of the water in a test tube, inserting a tablet for the appropriate test and waiting five minutes to compare the results to a provided color scale. Copper was tested using a 9500 YSI Photometer. Once a week for 4 weeks the water was tested using the urban water testing kit and photometer.

RESULTS

Table 1 displays the average amount of total copper in parts per million (ppm) per manipulation across four weeks. These results are interpreted in Figure 4. Figure 4 shows the trends of total copper in ppm over a four week time period. In the four different trials the amount of copper went down each week and got the lowest in the tank with only *P. insularum* and remained the highest in the control. The initial drop is thought to be due to being tested from the tanks for the first time. The second point is a week after the tanks had been set up and the systems started working on the small scale of the tanks rather than the whole lake. Figure 5 displays the standard error and that there is no significant difference between some of the results. The tanks that contained the apple snails showed a significant difference compared to those that did not, but not between each other. The other tests performed were found to show no significant results. It is also important to note the condition of the *P. stratiotes* over the four weeks; the water lettuce was browning, wilting and showing signs of dying. In addition to the water lettuce dying some of the apple snails showed decreasing health.

Table 1 The averaged results of the 9500 YSI copper test

Total Copper (ppm)	Week 1	Week 2	Week 3	Week 4
control	0.22	0.06	0.09	0.14
P. stratiotes (water lettuce)	0.22	0.05	0.10	0.10
P. insularum (apple snail)	0.22	0.02	0.01	0.01
water lettuce and apple snail	0.22	0.03	0.02	0.02

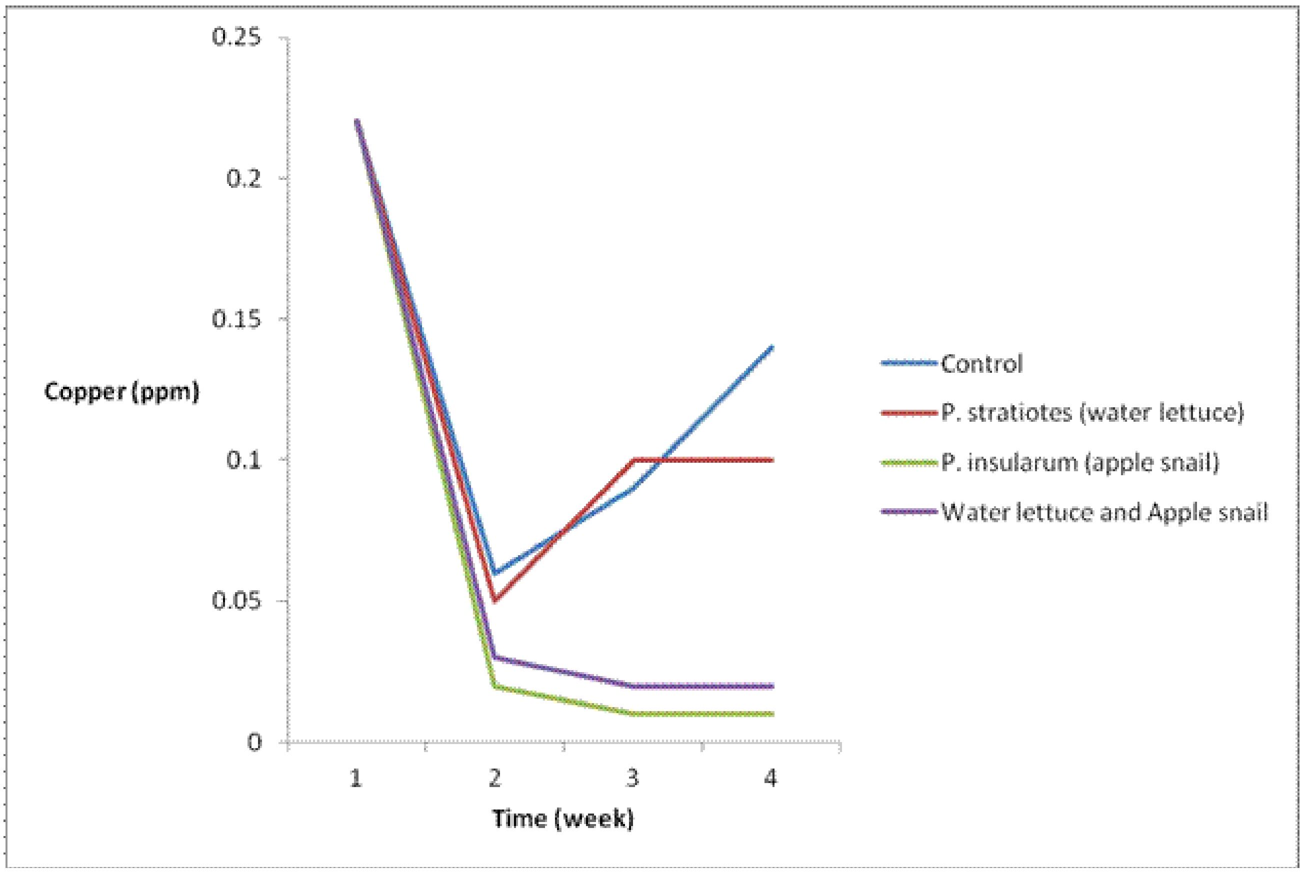

Figure 4 Trends of the loss in copper over four weeks

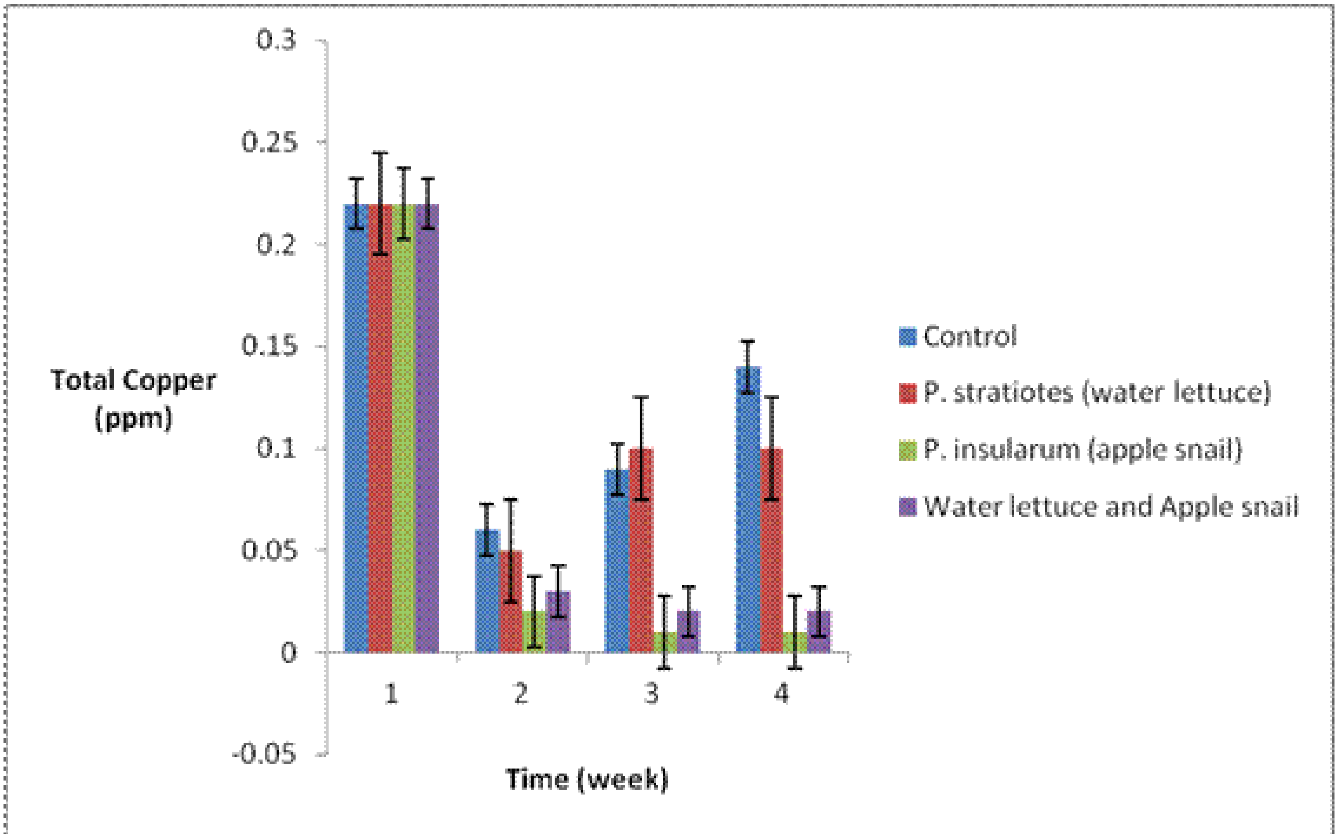

Figure 5 Loss of copper over four weeks with standard error bars

DISCUSSION

Water lettuce and apple snails did affect the concentration of copper in the tanks. The apple snails were more efficient than the water lettuce at the removal of copper and together they were less efficient than apple snails alone. These results do not provide support for the hypothesis. The combination of water lettuce and apple snails together was not the most efficient at the removal of copper.

There may be several reasons why the combination of apple snails and water lettuce was not most efficient at the removal of copper. One aspect of influence could be the YSI 9500 machine used to test the solution of copper displayed inconsistent readings. The machines inconsistency resulted from the amount of copper settled in the test tube when the reading was done. The method used in order to receive the most reliable copper reading was shaking the bottle so the copper was evenly distributed throughout the test tube and then taking a reading after 2 seconds. There is also the inherent variability when multiple lab partners measure the same thing.

Another reason could amount to the poor health quality of the water lettuce and apple snails that possibly contributed to their potential to bioremediate. The poor health of the water lettuce could be from the lag time between their original habitat and the tanks. The experiment was 6 weeks in total and after the first 2 weeks the experiment had to be restarted because the apple snails and water lettuce died after being sprayed with

Cutrine. During this time period of 2 weeks, there was leftover water lettuce from the initial collection from Wekiva Island that then replaced the water lettuce that was impaired from the copper spray. The water lettuce displayed signs of wilting, discoloration, and was subject to predation.

The poor health of the apple snails is attributed to where they were living previous to being collected. The apple snails were obtained from the UCF lake that was subject to copper sprays. There was a significant amount of dead snails present when the only visibly remaining apple snails were collected for research. This is also why the tanks were not sprayed with copper after they were reestablished because the water collected from lake already had copper present. Studies concluded that the bioaccumulation of copper in apple snails was most significant in dietary uptake (Hoang 605) or soil (The Pomacea Project). It is possible that the diet of the apple snails also contained copper because their food source was also collected from the lake that was sprayed and there was no significant soil accumulation present at the bottom of the tanks.

Consideration of the locations where the apple snails and water lettuce were collected also could have influenced the potential to bioremediate copper. The reason the apple snails were more efficient than water lettuce could also be because they had more experience with copper. The water lettuce was being controlled at Wekiva Island with salt sprays and the apple snails were being controlled with copper sprays. Since the apple snails were subject to the sprays it is possible that they become more efficient at the bioremediation of copper. According to Upadhyay, when water lettuce is exposed to copper it has a, “a remarkable effect on the biochemistry and physiology, induced oxidative stress in water lettuce characterized by the initiation of lipid peroxidation that inhibited growth and disintegration of major antioxidant systems” (Upadhyay 623). The oxidative stress could hinder the capacity for water lettuce to bioremediate copper.

Another consideration for the potential to bioremediate is the amount of carbon dioxide released by each organism. According to a study by the U.S. Geological Survey studies, “had shown that microorganisms naturally present in the soils were actively consuming fuel-derived toxic compounds and transforming them into harmless carbon dioxide” (U.S. Geological Survey). Plants are only able to release carbon dioxide at night for a relatively short period of time during respiration when photosynthesis is not taking place and the apple snails release carbon dioxide throughout the day while breathing. Millar explains that, “Mitochondrial respiration in plants provides energy for biosynthesis, and its balance with photosynthesis determines the rate of plant biomass accumulation” (Millar 2011). The potential for species to bioremediate could be influenced by their ability to bioaccumulate in correlation to their biomass. The difference in size of the apple snails is also worth noting, as the tanks that only housed apple snails contained larger snails than the tanks that held both apple snails and water lettuce. This could explain why the tank that contained both the water lettuce and apple snails was not as efficient as the tank that contained the apple snails alone.

Further research is required to determine if water lettuce and apple snails are sufficient at the removal of copper. Both apple snails and water lettuce are classified as invasive and further research could indicate if there is a relationship between polluted water that allows for a species to flourish leading to its invasive classification. Copper is a dangerous additive to an ecosystem and more research should be done before widespread use is continued. The harmful effects of copper in the environment were seen the first two weeks of research when the apple snails and water lettuce died or significantly suffered in health immediately after the initial spray of copper. Further research could indicate what species are able to bioremediate pollutants most efficiently in order to improve ecosystem health.

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