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Dispersing Agents Prevent Negative Impact of Oil on Uptake of Zinc by Duckweed (*Lemna minor*)

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Summary

Oil spills have had extremely negative effects on the environment. The oil directly coats both animal and plant species living in and around the water, rendering these organisms unable to carry out normal life functions such as locomotion, in the case of animals, and nutrient uptake, in the case of plants. Long term, the oil contributes organic chemicals to the water, which may either dissolve or sediment. Duckweed is an aquatic plant that provides food and shelter to animals and removes pollutants, such as zinc, from the water in which it resides. It is an important plant in phytoremediation work, and an oil coating could interfere with this important function. Means of removing oil from water include both physical methods, such as skimmers, and chemical approaches. There is concern that chemical techniques, such as adding adsorbents and/or dispersants to the contaminated water, may also cause environmental problems and may interfere with zinc uptake by duckweed. Here, we confirm that the aquatic plant duckweed (*Lemna minor*) can remove zinc from its environment and that this process is impaired by the presence of oil in the water. Furthermore, we demonstrate that the negative effect the oil has upon zinc uptake by duckweed can be ameliorated by treatment with the dispersing agent, Dispersit™, and that the dispersant itself does not inhibit zinc uptake by duckweed. We conclude that treatment of oil-contaminated water by this dispersant may be a useful approach to maintaining the ability of duckweed to remediate polluted water.

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Introduction

Duckweed is a hardy aquatic plant that consists of a round, slightly oval-shaped body, called a frond. It is found in a wide range of marine and freshwater ecosystems, providing food and protective habitat for numerous animals. The common name was derived from the fact that these plants provide food for many species of waterfowl, especially ducks (1). Duckweed grows and produces biomass faster than many other flowering plants and is sometimes considered to be a menace, especially in the fishing community, because a thick surface layer of duckweed can prevent sunlight from reaching the deeper parts of the water column, inhibiting underwater plants and algae from photosynthesizing and producing oxygen. The lowered oxygen levels can stress or even kill fish and other aquatic species (1, 2). Nonetheless, duckweed is considered to be a good source of protein and is used as an abundant animal feed supplement (3-5). It also contains high levels of starch that can be fermented into ethanol and is being studied as a potential source of biofuel (6). Furthermore, because of its ability to uptake pollutants (e.g., heavy metals such as cadmium, lead, and zinc) from water, it is also being researched for use as a tool for phytoremediation (7-10). However, the duckweed must be healthy and have access to the pollutants to perform the remediation.

Duckweed removes pollutants from water, but the plant must contact these contaminants to do so. Chemicals such as oil, which will "coat" the plants, interfere with their ability to uptake impurities. It is estimated that nearly 3 million U.S. gallons of oil are spilled every year into U.S. waters as a consequence of accidents or unavoidable bad weather and geographical events (e.g. earthquakes) that occur during drilling and transport of oil (11). Intentional oil spills have occurred as acts of terrorism or war. For example, during the Persian Gulf War in 1991, an estimated 5 to 10 million barrels (210-420 million gallons) of oil were released from oil terminals and tankers near the coast of Saudi Arabia (12). Oil spills occur in both fresh and salt water and are damaging to the environment and the organisms that live in it (13-17). The initial effects are the most obvious because the oil stays on the surface for a time (depending on currents, weather, etc.) and clings to both sea birds and mammals, causing high mortality rates mainly because the animals are not able to move about properly (18, 19). As the oil mixes with the water and sinks, it can contaminate, and even decrease, fish populations by exposing them to chemicals such as polyaromatic hydrocarbons (15, 16). Finally, as the oil sinks to the bottom of the body of water, it can affect the populations of bottom dwelling organisms such as

lobster and shrimp (15). Oil spills in aquatic ecosystems can affect a wide range of organisms linked to humans through complex food webs as well as the quality of fresh water available to humans and other organisms (17); however, studies are now suggesting that even the most negative effects of oil spills decline with time as the oil disperses (13, 15).

There are now numerous ways oil can be removed from water biomes. Some options include traditional mechanical techniques (e.g., skimmers to contain and remove the surface oil) and newer methods such as the practices of *in situ* burning or using chemical agents to disperse or adsorb the oil (20, 21). Dispersing agents are compounds that reduce the interfacial tension between the oil and the water and break down the oil into small globules that can be dispersed so that the oil's effect is diluted. The small globules may also be degraded by "oil-eating" bacteria, which occur naturally, especially in environments that have had prior oil exposures (11). The use of dispersing agents has been controversial because of their potential effects on the environment: for example, it has been shown that even low levels of certain dispersants can decrease the fertility of sea urchins (22). Therefore, despite the fact that dispersants in water also dissipate with time (13), their application has been limited to cases approved by government agencies such as the Coast Guard (11).

Duckweed is an aquatic plant that serves as an important source of both food and shelter for aquatic organisms. Duckweed can also remove pollutants from the water in which it grows and is used for remediation of water, especially for removal of metals (7, 9, 10). Specifically, it is known that this plant will remove zinc from the environment when conditions are appropriate (8). Therefore, it is important to know if an oil spill would interfere with duckweed's ability to remediate and if the potential cleanup (e.g., a dispersant) would allow the plant to continue its uptake of the metal. In this project, we used a dispersing agent (Dispersit™; U.S. Polychem Corp., Chestnut Ridge, NY), which is a mixture of detergents used to treat oil spills, to determine if this method of cleaning up the spill ameliorates the damage done by oil contamination and if it does so without exacerbating the environmental problem. The experiments described here were performed to determine if dispersing agents (such as Dispersit™) are beneficial to plants whose environment has been contaminated by motor oil. We hypothesized that motor oil would interfere with zinc uptake by duckweed and that the dispersing agent Dispersit™ would disperse the contaminating motor oil and allow the duckweed to function more normally in spite of the oil contamination. We report that motor oil does indeed interfere with zinc uptake by duckweed and that this inhibition can be ameliorated by the dispersing agent. We also report that the dispersing agent alone does not inhibit zinc uptake by the plants. Our work strongly suggests that dispersing agents are a good way to prevent oil from smothering aquatic duckweed without harming its ability to remediate the environment. It suggests that these agents could possibly be safely applied more often than is currently practiced and strongly indicates that further research on dispersing agents as a means of cleaning up oil spills is merited.

Results

Study 1: Determination of zinc concentration absorbed by duckweed over time

Our experiments revealed that duckweed takes up zinc from its aquatic environment (Figure 1), which is consistent with the results of previous investigations (8, 10). The amount of zinc detected in the duckweed

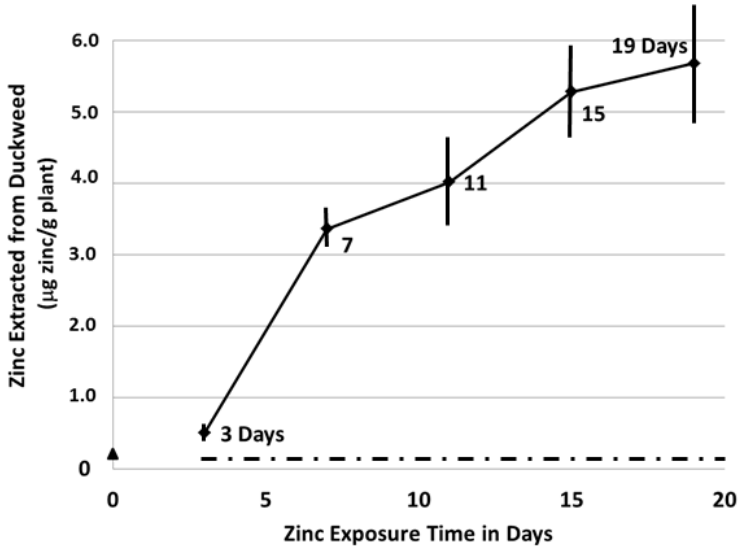

Figure 1. Zinc uptake by duckweed (Lemna) increases over time. n = 18, 3 replicates per time point. The solid line represents the change in zinc concentration over time. The solid diamonds represent the mean value and the error bars represent the standard error. The solid triangle signifies the detection limit of our assay, which was 0.25 μg zinc/g duckweed. The broken line represents that the concentrations in the control plants (no zinc treatment) were all below the detection limit.

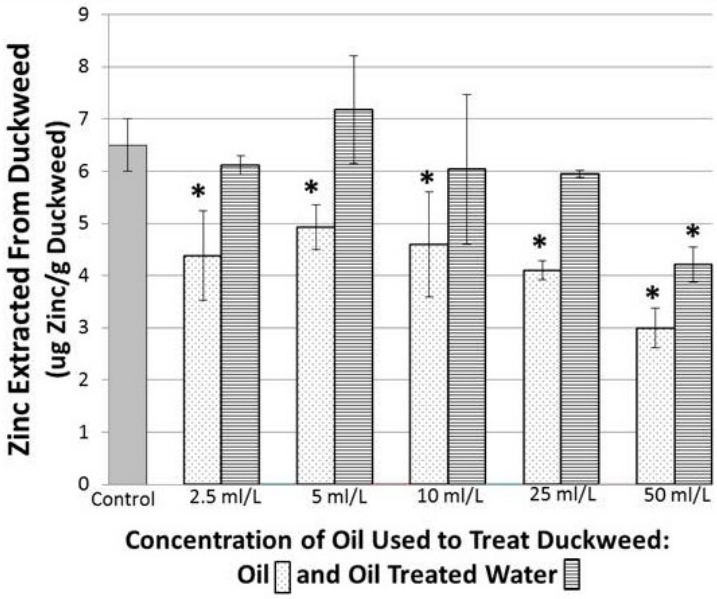

Figure 2. The highest concentration of motor oil and oil pre-treated water, 50 mL/L, significantly inhibited zinc uptake by duckweed (*Lemna*). n = 33, 3 replicates per 11 treatments. Bars represent mean. Error bars represent the standard error of the mean. * $p<0.05$ and indicates that the noted sample groups are significantly different from the control group.

exposed to 10 ppm zinc increased with exposure time, being barely above detection limits at Day 3 (0.5±0.05 µg/g plant) and increasing approximately 6.5-fold to 3.3±0.5 µg/g plant between Days 3 and 7. The accumulation continued through Day 19 to 5.6±1.7 µg/g plant for an approximately 11-fold increase overall, although the rate of increase slowed over time (as noted by the decreased slope in Fig. 1), suggesting that the amount of zinc taken up by duckweed would plateau shortly after 19 days. We determined that 7 days of exposure to 10 ppm zinc would give us an easily measurable accumulation of zinc, and we used this exposure timeframe for the remainder of our studies.

Study 2: Determination of motor oil concentration, which interferes with zinc absorption by duckweed over time

We needed to know what concentration of motor oil would inhibit zinc uptake by duckweed. Our data show that all of the tested concentrations of motor oil inhibited zinc uptake by duckweed and that this interference intensified as the concentration of oil increased (Figure 2). Indeed, zinc uptake decreased a significant 36.9% in plants treated with 25 mL/L motor oil, declining from control levels of 6.5±0.5 µg Zn/g plant to 4.1±0.18. Zinc uptake decreased a significant 53.8% in plants treated with the highest concentration of oil (50 mL/L), declining from control levels to 2.5±0.38 mg Zn/g plant. This demonstrates that the highest concentration of motor oil was sufficient to significantly inhibit zinc uptake. Interestingly, exposure to the oil-treated water (oil incubated in water with the oil physically removed after 7 days) also significantly decreased the uptake of zinc by 36.9% (a decrease from control levels of 6.5±0.5 µg Zn/g plant to 4.21±0.34), but only at the highest concentration of oil pre-treatment with 50 mL oil/L water. We concluded that a 50 mL/L motor oil in water concentration should be used in our next study because it induces a strong inhibition of zinc uptake by duckweed and creates a physical barrier that would better test the power of the dispersal agent.

Study 3: Determination of the effect of motor oil, dispersant and their combination on zinc uptake by duckweed

In this experiment, the 7-day exposure of duckweed to 50 mL/L oil significantly inhibited the uptake of zinc by 28.3%, decreasing the value to 18.0±1.5 µg Zn/g plant from a mean control value of 25.3±1.4 µg Zn/g plant (Figure 3). This inhibition of zinc uptake is not as great as that noted in response to 50 mL/L oil in Study 2 (53.8%, see above), although it is still statistically significant. We suggest that this difference may be explained by some variability in the plants used in Studies 2 and 3 or by the decreased amount of light to which the plants were exposed in Study 3. Interestingly, using recommended concentrations of the dispersing agent, we found that the mean zinc concentrations in duckweed treated with zinc only as control (25.3±1.4 µg Zn/g plant), with Dispersit™ and zinc (26.2±2.9 µg Zn/g plant), and with Dispersit™, oil and zinc (28.3±2.0 µg Zn/g plant) were not significantly different from each other, but were all significantly greater than the plants treated with the motor oil and zinc alone (18.0±1.5 µg Zn/g plant).

Discussion

Oil spills and the techniques used to remediate them

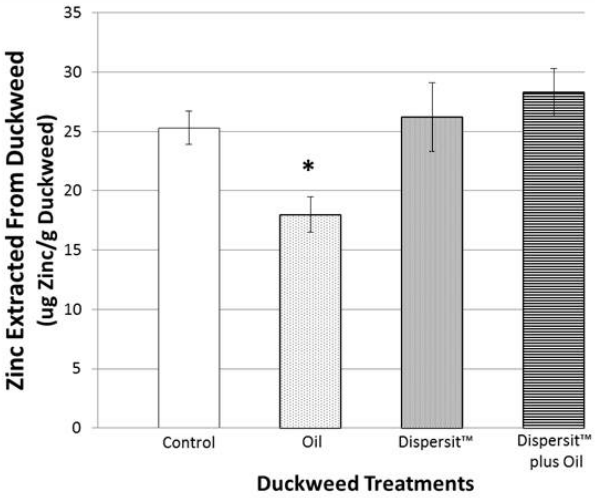

Figure 3. Motor oil treatment inhibits zinc uptake by duckweed, but this inhibition is ameliorated by treatment with dispersing agent. Bars represent the mean zinc concentrations of duckweed treated for 7 days with: 1) zinc only (control, 10 mg/L zinc); 2) oil (50 mL/L) and zinc (10 mg/L); 3) Dispersit™ (1.0 mL/L) and zinc (10 mg/L); and 4) Dispersit™ (1.0 mL/L), oil (50 mL/L) and zinc (10 mg/L). n= 16 samples, 4 replicates per 4 treatments. Error bars represent the standard error of the mean. * p≤0.05 and indicates that the oil-treated group removed significantly less zinc from the water than the other treatment groups.

can pose a serious threat to the environment. To explore the effect of oil spills and their clean-up by dispersants on duckweed, we exposed samples of this aquatic plant to separate treatments of oil, a dispersant, and a combination of oil and dispersant in the presence of 10 µg/mL zinc (in the sample water) and then assayed zinc uptake by these and control plants. We found that oil has a negative effect on the incorporation of zinc by duckweed, but that this effect is ameliorated by treatment with the dispersant Dispersit™ which, when exposed to duckweed alone, does not interfere with zinc uptake.

Initially, we determined that 7 days of exposure to 10 µg/mL zinc in water would allow duckweed to incorporate a reliably measurable concentration of zinc, yielding a good measure of duckweed health. Because the density of water is 0.99669 (~1.0) g/mL at 26.7°C, we can convert the zinc concentration to 10 µg zinc/g water and calculate a rough estimation of the amount of zinc removed from the water by the plant on a weight basis. After 7 days of exposure to the 10 µg zinc/g water solution, the plants had taken up 3.3 µg of zinc for every gram of plant. This estimate very roughly represents an uptake of 33% of the zinc from the water over time. Of course, this equates 1 g of water with 1g of plant and assumes that there was a constant concentration of 10 µg zinc/g water. Although we replaced the existing plant zinc-water with fresh zinc in water solution for this study at days 3, 4, and 6, the plants were not exposed to a constant concentration of zinc because we did not adequately correct for zinc removal by plants. The 10 µg/mL (10 mg/L) concentration of zinc is environmentally relevant because >200 mg/L levels of zinc have been detected in waters contaminated by human activities such as zinc mining and smelting (23). However, the chosen zinc concentration is roughly an order of magnitude higher than the 0.002 to 200 µg/L zinc that is estimated to exist naturally in various U.S. waters, depending upon the location and degree of mineralization of the specific aquatic environment (24). Thus, our work is more relevant to human-caused environmental contaminations.

As expected, the oil had a negative impact on zinc uptake, and this effect became worse as the concentration of oil increased. We suspect that zinc uptake is actually inhibited to some extent by a physical barrier created by the oil, because we observed that the oil did cling to the plant clusters, creating a tangible impediment between parts of the plant and the water. However, the plants did not appear truly damaged by the oil (within our study time frame) because, once they were rinsed with the Dawn™ and water solutions, they had a similar shape and only mildly muted color in comparison to the non-oil treated plants. We suggest that the decreased green color was caused by the blocking of some sunlight to these plants by the oil coating. Perhaps the decreased light exposure resulted in reduced levels of photosynthetic green pigments in the oil-treated plants. Indeed, duckweed grown in water pretreated with oil exhibited no obvious decrease in green color although the plants grown in the water pretreated with 50 mL/L oil did have a significant 36.9% decrease in zinc uptake (Figure 2). This suggests that the inhibition of zinc uptake is not completely a result of physical inhibition by the thick oil, but that there may be some water-soluble chemical extracted from the oil that has a negative effect upon duckweed's ability to extract zinc from water.

It is worth noting that the concentrations of zinc taken up by control duckweed samples differed between the three studies. When Study 1 (3.3±0.5 µg Zn/g plant) is compared to Study 2 (6.5±0.5 µg Zn/g plant), the difference in control zinc uptake is nearly two-fold. The concentration of zinc taken up by control duckweed in Study 3 (25.3±1.4 µg Zn/g plant) is even higher than those in either Studies 1 or 2. In fact, it is nearly 8-fold higher than that in Study 1. This discrepancy would be an issue only if we wanted to compare data between the studies rather than within study as we do here. That is, we do not statistically compare the data from one study to that from any other study. All data within a study is compared to that study control, so the differing control uptake levels do not interfere with our interpretation of the results. Nonetheless, the source of the difference in zinc uptake by control plants between studies is not clear. It has been shown that pH, temperature, and zinc source can affect the uptake of zinc by duckweed (8). Specifically, duckweed is reported to take up the highest concentration of zinc around 21°C, with uptake decreasing in temperatures above 21°C. Our first study was undertaken in slightly cooler temperatures than the second and third, thus we would expect the latter control plants to have incorporated less zinc; however, control plants from the second and third studies had the higher

zinc uptake levels, so it is not likely that temperature contributed to the differences in control plant zinc uptake between studies. It is also known that duckweed does not take up zinc as well in acidic conditions (i.e., pH < 6) (8). Unfortunately, we did not measure the pH of the water in which plants were grown in any study. It is possible that the differences in control plant uptake could be related to variations that may have occurred in pH; however, this is unlikely because deionized water was used in all cases and should have a pH of about 7.0. It is possible, however, that there was some differences in the water used, because the studies were performed over a nearly three year period and the sources of the deionized water were different for each study and were not monitored for parameters such as pH, conductivity, purity, hardness, etc. The zinc source was the same for studies 1 and 2, therefore, source does not contribute to the relatively small difference in zinc uptake between the control groups in these studies. Study 3 was conducted a year after Study 2 as a separate, but continuing project, so a number of factors could have contributed to the higher level of control plant zinc uptake in Study 3. However, because we switched the source of the zinc to a commercial standard rather than using the zinc chloride we made in the lab originally, we suspect the zinc source is the most likely explanation for the higher zinc uptake. It has been shown that zinc uptake by duckweed is less when $Zn(NO_3)_2$ is used, rather than zinc chloride or zinc sulfate (8). However, the supplier of the zinc standard was unable to reveal the source of zinc in the standard we used for Study 3; therefore, we cannot speculate further about the zinc supply.

Our work demonstrates that oil has a negative effect on zinc uptake by duckweed, suggesting oil contamination has a negative impact on plant health. The dispersant we used (i.e., Dispersit™) protected the duckweed from the negative impact of the oil and did not have an obvious negative effect of its own.

The results suggest that, when searching for a resource to clean environmental aquatic oil spills, dispersants could be beneficial in diffusing the oil without negatively impacting the health of duckweed. Indeed, it has been reported that, although dispersants have some mildly toxic effects on animals (25), it is very effective in dispersing oil (26). Furthermore, one study reports that in mixtures of oil and dispersants (Dispersit™ was not tested), any negative effect on duckweed growth appeared to be due to the oil rather than the dispersant (27). Our data support this result, showing that the dispersant Dispersit™ restores the ability of duckweed to take zinc up from an environment contaminated by oil.

Methods

Plants

Duckweed plants (*Lemna*) were bought from William Tricker, Inc. (Cleveland, OH) and cared for as recommended by Landolt and Kandler (28). The plants (0.5g) were placed in small Ziploc™ (Ziploc; Racine, Wisconsin) bowls containing 100-150 ml of deinoized water containing 2.65 mL/L of Miracle Grow™ house plant food. Study 1 was performed indoors in a controlled temperature (~21-26°C) environment with the plants being placed on a table in front of a large window. Studies 2 and 3 were performed in a well-ventilated garage (temp ~25-29°C) in front of a large window. Studies 1 and 2 were performed in June (of different years), so that the light:dark cycle was approximately 12:12 hrs. Study 3 was performed in September (of a third year), so the light:dark cycle was approximately 11:13 hrs.

Zinc Solution

In Studies 1 and 2, a 10 mg/L solution of zinc was made using zinc chloride (Sigma; St. Louis, MO) and deionized water. In Study 3, a 10 mg/L zinc solution was made by adding 25 mL of a 1000 mg/L zinc solution (Hach Company; Loveland, CO) to 2.5 L of DI water. A 2.65 mL aliquot of Miracle Grow™ (Scott's Miracle-Gro; Marysville, Ohio) was also added and mixed. The Miracle Grow label reports that it contains 0.06% zinc which, when diluted as above, added 0.64 mg/L zinc to the study solution. Although the amount increased the zinc content by 6.4%, it was not factored into the final 10 mg/L zinc concentration. Because it was added to all plant solutions, it does not affect the interpretations of the results.

Motor Oil

Exposure to Mobil Premium Motor Oil (Mobil Exxon, United States) was used to mimic the effect of an oil spill. The manufacturer reports that this product contains no water-soluble zinc.

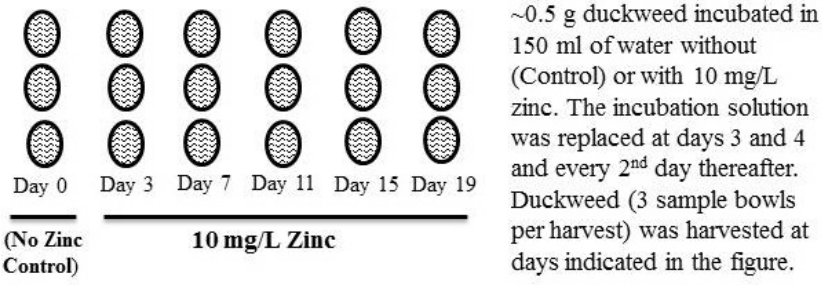

Figure 4. Study 1. Determination of zinc (μg/g plant) absorbed by duckweed over time.

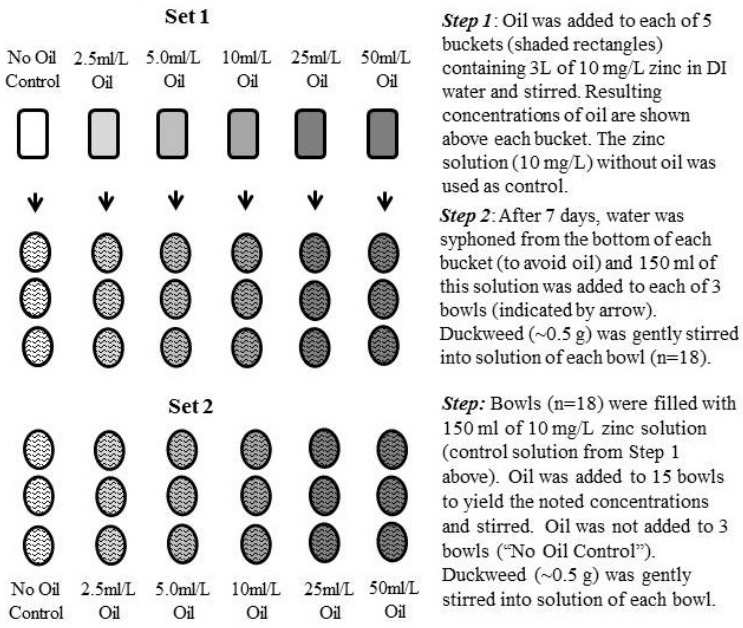

Figure 5. Study 2. Determination of motor oil concentration which interferes with zinc absorption by duckweed over time.

Oil Dispersive Additive

Dispersit™ by Poly Chem (Chestnut Ridge, NY) was added to disperse motor oil. This product contains no reported zinc.

Plant Digestion for zinc extraction

Sample plants were thawed at room temperature and approximately 0.5 gram of each sample was placed in individual, acid-washed test tubes. The exact weights of the samples were recorded. Aliquots of 65% suprapur nitric acid (5 mL) and 35% hydrochloric acid (6 mL) were added to each test tube. The samples were then heated at 93°C using a heating block (Fisher Scientific; Barrington, Illinois) under a ventilated fume hood until the samples were clear of plant material. Each sample was then diluted to 20 mL with deionized water and the pH adjusted to 4 using crushed sodium hydroxide pellets.

Zinc Assay

The zinc concentration of each sample was determined using a commercial Zinc Determination Kit (Hach Company; Loveland, CO). Zinc standards were prepared by diluting a 100 ppm zinc solution with deionized water to concentrations of 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ppm. A Zincover 5 Reagent pillow was dissolved in 10 mL of each standard. An aliquot (0.5 mL) of cyclohexanone was added and mixed for 30 seconds. After 3 minutes, the absorbances were read at 620 nm using a Genesys 5 Spectrophotometer (ThemoFisher Scientific; Waltham, MA). All standards were read against a treated blank. The standard concentrations and absorbances were used to create a line graph, and a simple linear regression was performed to determine sample zinc concentrations (extrapolation of sample absorbances against the standard curve). Zinc concentration per plant (µg Zn/g plant) was calculated using the weight of the starting plant material.

Experimental Set Up: Study 1. Determination of zinc concentration absorbed by duckweed over time (Figure 4)

Approximately 0.5 g of duckweed was added to 18 small Ziploc™ (Ziploc; Racine, Wisconsin) bowls containing deionized water and Miracle Grow™ (100 mL) as described above. All bowls sat next to a large kitchen window at about 21-26°C with roughly 12/12 light cycles. After 3 days, the water was changed in all bowls except for three bowls from which the plants were harvested as controls. The remaining 15 bowls received the solution containing 10 ppm zinc as described above; this solution was changed after 24 hours and then every 2 days to maintain a constant concentration of zinc. The plants were harvested at days 3, 7, 11, 15 and 19 after the initial exposure. All harvested plants were gently rinsed with deionized water using a wire sieve and then dabbed with paper towels. These were then placed in Ziploc™ bags and frozen at -20°C until digested and assayed for zinc (described above).

Experimental Set Up: Study 2. Determination of motor oil concentration which interferes with zinc absorption by duckweed over time (Figure 5)

Small buckets (n=6), each containing 3.0 L of 10 mg zinc/L DI water (with Miracle Grow™ as above),

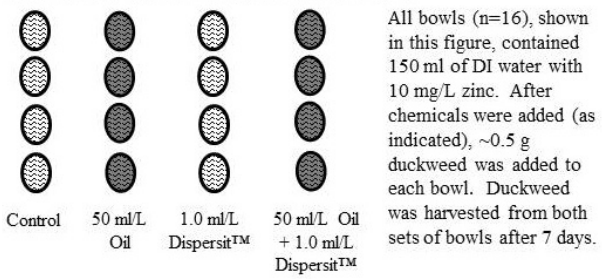

Figure 6. Study 3. Determination of the effect of motor oil, Dispersant™ and their combination upon zinc uptake by duckweed.

were placed in a ventilated, window-lit garage. Aliquots of unused motor oil were added to 5 of these to yield concentrations of 2.5, 5.0, 10, 25, and 50 mL/L motor oil in water. These were allowed to sit in the garage in front of a large window at ~25-29°C with roughly 12/12 light cycles. After 7 days, water was syphoned off the bottom of the bucket to avoid motor oil. Aliquots (150 mL) of this water were added to 15 small Ziploc™ (Ziploc; Racine, Wisconsin) bowls. The control 10 mg/L zinc in DI water (with Miracle Grow™ as above; 150 mL) was added to another 18 bowls. Three of these were used as controls. Aliquots of oil were added to the other bowls to yield 3 bowls of each: 2.5, 5.0, 10, 25, and 50 mL/L motor oil in water. Aliquots of duckweed (~0.5 g) were added to each bowl and all bowls sat in front of a large window as in Study 1. In this study, the water was not changed throughout the experiment. After 7 days, all plants were harvested as described above, except that the rinsing of the plants was slightly more vigorous in this study than in Study 1; this was necessary to remove the oil. All plants were first rinsed with deionized water containing Dawn™ detergent (5 mL/ L; Proctor and Gamble; Jackson, MO) and then with deionized water. All rinsed plants were dabbed and frozen at -20°C prior to being assayed for zinc.

Experimental Set Up: Study 3. Determination of the effect of motor oil, dispersant, and their combination upon zinc uptake by duckweed (Figure 6)

A 150 mL aliquot of the 10 mg/L zinc solution was added to each of 16 small Ziploc™ (Ziploc; Racine, Wisconsin) bowls. Four containers served as the control samples. A 7.5 mL aliquot of Mobil Premium Motor Oil (50 mL/L final concentration) was added to each of four other containers and stirred. To the third set of four bowls, 0.15 mL of the Dispersit™ (Poly Chem; 1 mL/L, according to manufacturer recommendations) was added to each container and stirred. Aliquots of 7.5 mL motor oil and 0.15 mL Dispersit™ were added to each of the four bowls that comprised the fourth set. Duckweed (0.5 g) was added to each bowl and allowed to sit in the containers in front of a large window at ~ 25-29°C for seven days (as in Study 2). The light:dark cycle was approximately 11:13 because the study was done in September. The bowls were agitated for 1 minute in the morning and again in the evening. In this study, the water was not changed throughout the experiment. After seven days, all duckweed samples were harvested onto a small screen and cleaned by gentle rinsing with a solution of water and Dawn™ dishwashing liquid and then water (as above in study 2). The plants were then dabbed and placed in appropriately labeled Ziploc bags and frozen (-20°C).

Statistics

Data were analyzed by ANOVA for a completely randomized design. When significant differences were found, means were separated by Fisher's Protected Least Significance Difference. All data were analyzed using the General Linear Model Procedure of SAS (SAS Institute Inc., Cary, NC). Statements of significance were based on $p \leq 0.05$.

References

1. Stutzenbaker, C. *Aquatic and wetland plants of the western gulf coast*. Austin, Texas: Texas A&M University Press. (2010). Print.
2. Sengupta, S., Medda, C., and Dewanji, A. "The impact of duckweed growth on water quality in sub-tropical ponds." *Environmentalist*. 30(2010):353-360. Print.
3. Zetina-Cordoba, P., Ortega-Cerrilla, M.E., Ortega-Jimenez, E., Herrera-Hato, J.G., Sanchez-Torres-Esqueda, M.T., Reta-Mendiola, J.L., Vilaboa-Arroniz, J., and Munguia-Ameca, G. "Effect of cutting interval of Taiwan grass (*Pennisetum purpureum*) and partial substitution with duckweed (*Lemna* sp. And *Spirodela* sp.) on intake, digestibility and ruminal fermentation of Pelibuey lambs." *Livestock Science*. 157(2013):471-477. Print.
4. Xu, Jiele, Cheng, Jay J., and Stomp, Anne-M. "Growing *Spirodela polyrrhiza* in Swine wastewater for the Production of Animal Feed and Fuel Ethanol: A Pilot study." *Clean – Soil, Air, Water*. 40.7(2012):760-765. Print.
5. Brown, W.Y., Choct, M., and Pluske, J.R. "Duckweed (*Landoltia punctate*) in dog diets decreases digestibility but improves stool consistency." *Animal Production Science*. 53(2013):1188-1194. Print.
6. Blackburn, Abe G., Carter, Cord M., Lemons, Ricky E., Wade, Nicholas J., Baze, Dianne, and Paiva, Nancy L. "Investigation of the potential for biofuels and other uses for duckweed in Oklahoma." *Amer Chem Soc*. 245(2013):446-CHED. Print.
7. Brooks, R.R., and Robinson, B.H. "Aquatic Phytoremediation by Accumulator Plants." *Kiwi Science*. Web. 17 Oct. 2011. http://www.kiwiscience.com/BookChapters/Chapter9.prf
8. Khellaf, Nabila and Zerdaoui, Mostefa. "Phytoaccumulation of zinc by the aquatic plant, *Lemna gibba L*." *Bioresource Technology*. 100(2009):6137-6140. Print.
9. El-Kheir, Wafaa Abou, Ismail, Gahiza, El-Nour, Farid Abou, Tawfik, Tarek, and Hammad, Doaa. "Assessment of the Efficiency of Duckweed (Lemna gibba) in Wastewater Treatment." *International*

Journal of Agriculture & Biology. 9.5(2007):681-687. Print.
10. Boniardi, Nicoletta, Rota, Renato and Nano, Giuseppe. "Effect of Dissolved Metals on the Organic Load Removal Efficiency of *Lemna Gibba*." Wat. Res. 33.2(1999):530-538. Print.
11. National Research Council, Committee on Understanding Oil Spill Dispersants. *Oil Spill Dispersants: Efficacy and Effects*. Washington, D.C.: The National Academies Press. (2005). Print.
12. Tutton, M. "Lessons learned from the largest oil spill in history." *CNN*. 2010. Web. (20 Oct. 2011.). http://www.cnn.com/2010/WORLD/meast/06/04/04/kuwait.oil.spill/
13. Fitzgerald, Timothy P., and Gohlke, Julia M. "Contaminant Levels in gulf of Mexico Reef Fish after the Deepwater Horizon Oil Spill As Measured by a Fishermen-Led testing Program." *Environmental Science & Technology.* 48(2014):1993-2000. Print.
14. La Peyre, Megan K., Eberline, Benjamin S., Soniat, Thomas M., and La Peyre, Jerome F. "Differences in extreme low salinity timing and duration differentially affect eastern oyster (*Crassostrea virginica*) size class growth and mortality in Breton Sound, LA." *Estuarine, Coastal and Shelf Science*. 135(2013):146-157. Print.
15. Sanchez, F., Velasco, F., Cartes, J.E., Olaso, I., Preciado, I., Fanelli, E., Serrano, A., and Gutierrez-Zabala, J.L. "Monitoring the Prestige oil spill impacts on some key species of the Northern Iberian shelf." *Marine Pollution Bulletin*. 53(2006):332-349. Print.
16. Kazlauskiene, N., Svecevicius, G., Vosyliene M.Z., Marciulioniene D., and Montvydiene D. "Comparative Study on Sensitivity of Higher Plants and Fish to Heavy Fuel Oil." *Wiley Periodicals, Inc.* 19(2004):449-451. Print.
17. Walker, A.H., Kucklick, J.H., Steen, A.E., and Fritz, D. "Oil spill chemical in freshwater environments: technical issues." *International Oil Spill Conference Proceedings*. 1995.1(1995):373-379. Print.
18. Monson, D.H., Doak, D.F., Ballachey, B.E., Johnson, A., and Bodkin, J.L. "Long-term impacts of the Exxon Valdez oil spill on sea otters, assessed through age-dependent mortality patterns." *Proceedings of the National Academy of Sciences* 97(1999):6562-6567. Print.
19. Piatt, J.F., and Ford, G.R. "How many seabirds were killed by the *Exxon Valdez* oil spill?" *American Fisheries Society Symposium* 18(1996):712-719. Print.
20. El-Zahaby, Ali M., Kabeel, A.E., Bakry, Ayman I., and Khaira, Ahmad M. "Effect of Disk Offset Angle on the Hydrodynamic Performance of Rotating Disk Skimmers During Oil Spill Recovery." *Environmental Engineering Science*. 28.2(2011):113-119. Print.
21. Allen, Erik W. "Process water treatment in Canada's oil sands industry: II. A review of emerging technologies." *Journal of Environmental Engineering and Science*. 7.5(2008):499-524. Print.
22. Hagström, B.E., and Lönning, S. "The effects of Esso Corexit 9527 on the fertilizing capacity of spermatozoa." *Marine Pollution Bulletin*. 8(1977):136-138. Print.
23. Agency for Toxic Substances and Disease Registry. "Toxicological Profile for Zinc, 2005." Toxic Substances Portal. Web. 04 Dec. 2014. http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=302&tid=54
24. International Zinc Association. *Zinc in the Environment: An Introduction, 2nd edition*. Brussels, Belgium: The International Zinc Association. (1997). Print.
25. Environmental Protection Agency. *Comparative Toxicity of Eight Oil Dispersant Products on Two Gulf of Mexico Aquatic Test Species*. Washington, D.C.: US EPA. (2010). Print.
26. Environmental Protection Agency. *US Environmental Protection Agency Report*, 2013. Web. 13 Jan. 2013. http://www.epa.gov/oem/content/ncp/products.dispersi.htm
27. Lockhart, W.L., Billeck, B.N., and Buchko, G.W. "Phytotoxicity testing with common duckweed (Lemna minor)." *Canadian Technical Report on Fisheries and Aquatic Science* 1368(1985):297-298. Print.
28. Landolt, E., and Kandeler, R. "Growing duckweed." *Tips for Growing Duckweed*. 1996. Web. (17 Oct. 2011.). http://www.mobot.org/jwcross/duckweed/growing-duckweed/growing-duckweed.htm