Fall 2022

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Recommended Citation

Smith, Cameron, "Alga of My Eye, Determining the Ability of Palmaria palmata to Bioaccumulate Metals" (2022). *WWU Honors College Senior Projects*. 627.
https://cedar.wwu.edu/wwu_honors/627

Algae of My Eye; Determining the Ability of *Palmaria palmata* to Bioaccumulate Metals

Cameron Smith

Abstract:

Algae, specifically macroalgae, have rapidly sprung into the spotlight as a valuable natural resource to serve many functions in recent years. Individual community members and foragers have found algae useful for home cooking and garden fertilizer, it can also be used commercially in dietary supplements, cosmetics, pharmaceuticals, and animal feed. *P. palmata* is a red algal species that grows naturally in Northwestern Europe and Iceland and is commercially grown in Japan, Maine, and recently, California and Washington. This study aimed to investigate the ability of *Palmaria palmata* to bioaccumulate chromium, cadmium, lead, and zinc at eight different concentrations over 48 hours and determine if it is safe for aquaculture. The four metals in this study are considered toxic to humans at certain doses, so have regulations limiting their presence in food. *P. palmata* was expected to have a low affinity for bioaccumulating metals regardless of metal concentration in the water. An ANOVA test determined the measured concentrations of each metal in the algal tissue (mg/kg) to be significantly different and increased for all four studied metals. No more than 15% of the initial metals were accumulated in the treatments, and the accumulation efficiency of cadmium by *P. palmata* decreased as the initial metal concentration increased. The low-efficiency bioaccumulation of metals by *P. palmata* could mean that this species is at low risk of reaching high levels of contamination when cultured near areas with above-average metal deposition into the water.

Introduction:

Algae, specifically macroalgae, have rapidly sprung into the spotlight as a valuable natural resource to serve many functions in recent years. *Palmaria palmata* is a red algal species that grows naturally in Northwestern Europe and Iceland and is commercially grown in Japan, Maine, and recently, California and Washington. *P. palmata*, along with other species of algae, have a high potential to be used in dietary supplements, cosmetics, pharmaceuticals, and animal feed (Lopez et al., 2019; Grote, 2019). The rate of expansion of farmed macroalgae industry has surpassed every other mariculture industry (mainly in Asia) in millions of metric tonnes (MMT) harvested in 2018 at 31.7 MMT, representing over 50% of the mariculture industry (Chopin & Tacon, 2021; Wells et al., 2016). Individual community members and foragers have also found *P. palmata* useful for home cooking and garden fertilizer (Grote, 2019). *P. palmata's* increased popularity has led to the necessity for its safety and feasibility for mass production to be studied. Recent research has revealed that farming macroalgae are a concern due to this group's ability to bioaccumulate heavy metals in their tissue (Chernova & Shulkin, 2019; Chernova, 2015). Algae are exposed to heavy metals from phosphate fertilizers, sewage and wastewater, and the by-products of burning fossil fuels. Metal-containing pollutants can make their way to the ocean via rivers, surface runoff from watersheds, atmospheric deposition, and purposeful dumping (Ali & Khan, 2019; Sánchez-Quiles, Marbà, Tovar-Sánchez 2017).

Many studies have focused on the biosorbent ability of brown and green algae, while few have pointed their attention toward red algae (Prasher et al., 2004; Ibrahim, 2011; Hamdy, 2000). The need for a complete analysis has sparked the question of which species of this group are the most sensitive to metals in the water and how suitable they are as a food source (Al-Shwafi & Rushdi, 2008). If algae are to be used as food products, further studies must be done on their ability to accumulate toxic metals in humans. This study focused on the accumulation of chromium, cadmium, lead, and zinc in the tissue of *P. palmata*. Zinc is an essential trace metal for human health, but too much in one dose can cause death, and long-term exposure may cause prostate cancer in men (Plum, 2010). Ingestion of chromium has been known to cause kidney, liver, and gastrointestinal damage, cardiovascular collapse, and cancer (CDC). Cadmium exposure has been observed to cause osteomalacia, osteoporosis, bone fractures, decreased bone density, anemia, and gastrointestinal issues (CDC). Finally, lead exposure is known to cause severe mental decline, cardiovascular and kidney diseases, decreased fertility, and cancer (Lustberg, 2002).

On the other hand, algae have the potential to act as ecologically friendly biosorbents for cleaning contaminated water, which could open new doors for algae that are exceptionally proficient at absorbing pollutants (Prasher, 2004). Measuring the sensitivity of *P. palmata* to different concentrations of metals could help to determine how safe it is to farm this species near population centers where metal deposition into the water is high. Algae with a high sensitivity to metals in the water are contenders to be efficient biosorbents for cleaning water (Hamdy, 2000). The downside to highly sensitive algae is that foragers and farmers must be far more cautious when considering these species as food sources. Meanwhile, algae with low sensitivity to metals in the water could be a safer option for farmers and foragers but would be ruled out as biosorbents.

This study aimed to investigate the ability of *Palmaria palmata* to bioaccumulate chromium, cadmium, lead, and zinc at different concentrations. *P. palmata* was expected to have a low affinity for bioaccumulating metals regardless of metal concentration in the water. Keep in mind that algae simply being insensitive to increases in metal concentration in the water does not permiss excessive metal deposition via anthropogenic causes. Work must still be done to mitigate metal deposition into the water to support aquaculture safety and to benefit marine species.

Methods:

2.27 kg of *P. palmata* were obtained from aquaculturist Diane Boratyn, owner of Sol-Sea Ltd. Before exposing the *P. palmata*, it was acclimated in four 5-gallon buckets in filtered and autoclaved seawater with air bubbling in the buckets to maintain healthy oxygen levels. Seawater also ran outside the buckets to keep the individuals cool. The water in the buckets was changed in 3-day intervals, and the water was supplemented with vitamins and minerals. Following the week-long acclimation process, we began the exposure portion. Zinc Chloride (ZnCl2), Lead (II) Perchlorate Trihydrate (Pb(ClO4)2·3H2O), Cadmium Chloride (CdCl2), and Potassium Chromate (K2CrO4) were chosen based on their presence in coastal water and the toxicity of the metals to humans (Hahn et al., 2022; Hamdy, 2000; Prasher et al., 2004). A stock solution of each metal was made using 30 mL of type II reagent water and 57.50 g of ZnCl2, 3.56 g of Pb(ClO4)2·3H2O, 1.56 g of CdCl2, and 2.63 g of K2CrO4. A second stock solution was also made by adding 2.777 mL of the first stock solution to 25 mL of type II reagent water (Table 2.). *P. palmata* samples were exposed to 8 different concentrations of metals with three replicates per treatment. Treatment 1 had the highest concentration of metals, and treatment 8 had the lowest, but due to a calculation error, only data from treatments 4-8 were used (Table 1.). The highest treatment level amounts used were based on 2x the highest concentration found in sites in the Salish Sea and were halved in each successive treatment. (Hahn et al., 2022).

There were three replicates for each of the eight exposure concentrations. A 6-gram sample of *P. palmata* was placed into a 1-liter plastic container with 800mL filtered and autoclaved seawater along with its designated metal concentration and left for 48 hours. Again, seawater flowed around the plastic containers to keep the samples cool. Shade cloth was secured over the containers to protect the *P. palmata* individuals from excessive UV damage.

Following the 48-hour exposure, The individuals' new weights were noted, and samples were inserted into 50mL centrifuge tubes and stored in a -80℃ freezer. Next, the samples were freeze-dried and ground into a fine powder. Next, HNO_3 and H_2O_2 removed the organic matter in each sample. The samples were finally run through an inductively coupled plasma mass spectrometry (ICP-MS) analysis to determine each sample's lead, zinc, chromium, and cadmium concentration.

An ANOVA test was used to determine if there was a significant difference in metal accumulation between treatments. The ANOVA test was followed up with a Tukey Post Hoc test to determine which treatments differed significantly.

Treatment	Chromium mg/L	Cadmium mg/L	Zinc mg/L	Lead mg/L
8	0	0	0	0
7 (0.25X)	1.52288944	0.90501851	33.2689228	2.06174894
6 (0.50x)	3.04577889	1.81003703	66.5378457	4.12349788
5 (1x)	6.09155778	3.62007406	133.075691	8.24699575
4 (2x)	12.1831156	7.24014812	266.151383	16.4939915

Table 1. The initial concentration of each metal added at the treatment levels was analyzed in this study. Treatment 8 had no metals added to the water, while treatment had 2x the concentration of these metals typically detected in the Salish sea.

Metal	Formula	Mass Added to 30 mL Water (g)	Stock A (g/mL)	Stock B (g/mL)
Zinc Chloride	$ZnCl_2$	57.50	1.917	0.213
Lead (II) Perchlorate Trihydrate	$Pb(ClO_4)_2 \cdot 3H_2O$	3.56	0.119	0.013
Cadmium Chloride	$CdCl_2$	1.56	0.0521	0.0058
Potassium Chromate	K_2CrO_4	2.63	0.0877	0.0097

Table 2. The mass of each salt was added to 30 mL of type II reagent water to create Stock A. 2.777 mL of stock A was then added to 25 mL of type II reagent water to produce stock B.

Results:

An ANOVA test of the total metal accumulation of each metal across treatments determined that there was a significant difference in metal accumulation between treatments for all metals (P-value: <0.001)(figure 1., figure 2., figure 3., figure 4.). However, upon further analysis, with a Tukey Post Hoc test, there were no significant differences in the accumulation of chromium by *P. palmata* between treatments 5 and 6 (P-value: 0.982), 5 and 7 (P-value: 0.298), 6 and 7 (P-value: 0.141), and 8 and 7 (P-value: 0.104). Likewise, there were no significant differences in cadmium accumulation between treatments 5 and 6 (P-value: 0.254), 5 and 7 (P-value: 0.821), and 4 and 6 (P-value: 0.417). As for zinc, the difference in metal accumulation was not significant between treatments 4 and 5 (P-value: 0.536), 6 and 7 (P-value: 0.291), and 7 and 8 (P-value: 0.183). Finally, the difference in lead accumulation was insignificant between treatments 4 and 5 (P-value: 1.000) and 6 and 7 (P-value: 0.997).

An ANOVA proved the percent accumulation of the total metals by *P. palmata* to be significant across all treatments (figure 5.). Upon further analysis, with a Tukey Post Hoc test of chromium percent accumulation, the only significant differences that occurred were between treatments 6 and 8 (P-value: 0.016) and 7 and 8 (P-value: 0.009). For the percent accumulation of cadmium, the only insignificant difference was between treatments 4 and 5 (P-value: 0.654). The only significant differences in percent accumulation for zinc existed between treatment 8 and the other four treatments (P-value: 4-8: <0.001, 5-8: <0.001, 6-8: <0.001, 7-8: <0.001). As for lead, there were insignificant differences between treatments 4 and 5 (P-value: 0.913), 4 and 6 (P-value: 0.455), and 5 and 7 (P-value: .096).

The total measured concentration of all metals in the algae increased as the initial metal concentration increased; this is supported by the significant difference between concentration measurements of treatments 7 and 4 for all four metals. Zinc was concentrated the most in *P. palmata*; even the concentration of zinc in treatment 7 was 500% higher than the next highest concentration that occurred in treatment 5 with lead (figure 4.). Although, zinc was also added in the highest initial concentration, about 14x higher than the other three metals. None of the metals were accumulated by *P. palmata* at an increasingly efficient rate as the initial concentration of metals increased (figure 5.). The efficiency of metal accumulation significantly decreased for cadmium and lead (figure 5.). Plus, *P. palmata* did not bioaccumulate more than 15% of the total metals in any treatments (figure 5.). The only reason the percent accumulation was significantly different from treatment 8 for any of the metals is that algae cannot accumulate metal that was not added to the water in the first place, so all values were zero.

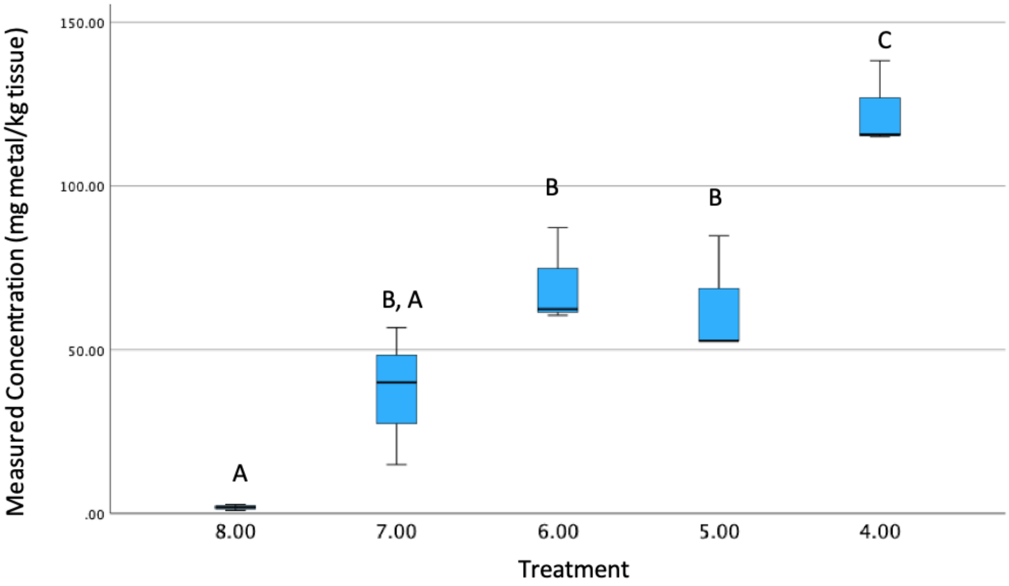

Figure 1. The measured concentrations of chromium accumulation in P. *palmata* samples 4 through 8 following 48-hour exposure. 4 is the highest concentration exposure, decreasing to 8 with no metal added. (P-value: <0.001, F-statistic: 25.250, n =15)

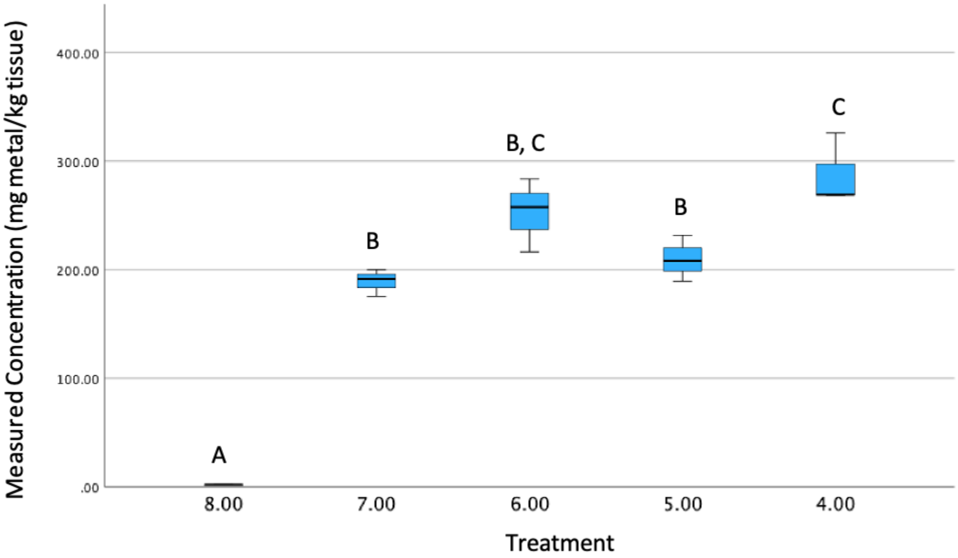

Figure 2. The measured concentrations of cadmium accumulation in P. *palmata* samples 4 through 8 following 48-hour exposure. 4 is the highest concentration exposure, decreasing to 8 with no metal added. (P-value: <0.001 F-statistic: 64.936, n =15)

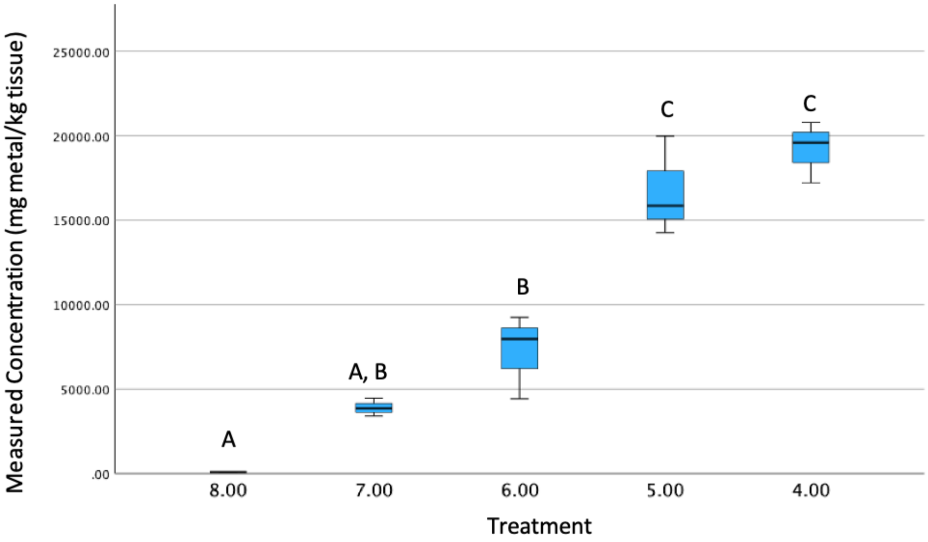

Figure 3. The measured concentrations of zinc accumulation in P. *palmata* samples 4 through 8 following 48-hour exposure. 4 is the highest concentration exposure, decreasing to 8 with no metal added. (P-value: <0.001 F-statistic: 54.733, n = 15)

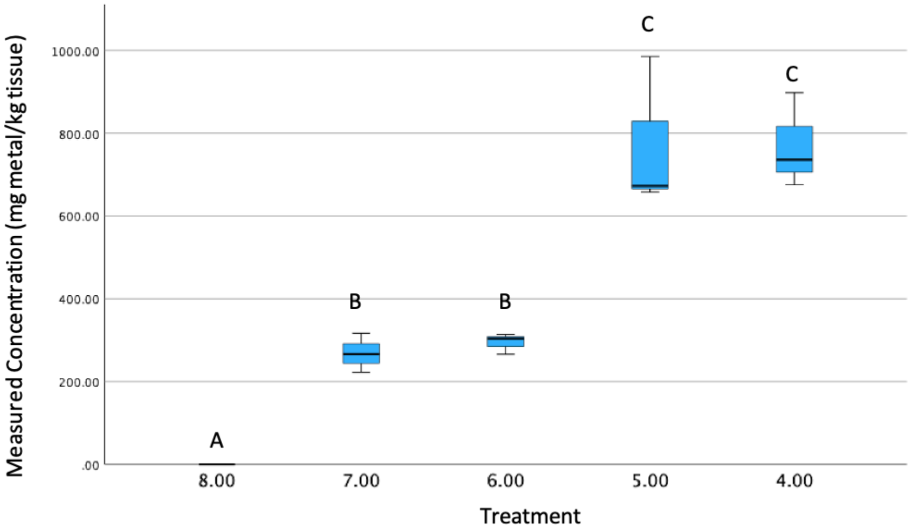

Figure 4. The measured concentrations of lead accumulation in *P. palmata* samples 4 through 8 following 48-hour exposure. 4 is the highest concentration exposure, decreasing to 8 with no metal added. (P-value: <0.001 F-statistic: 34.376, n = 15)

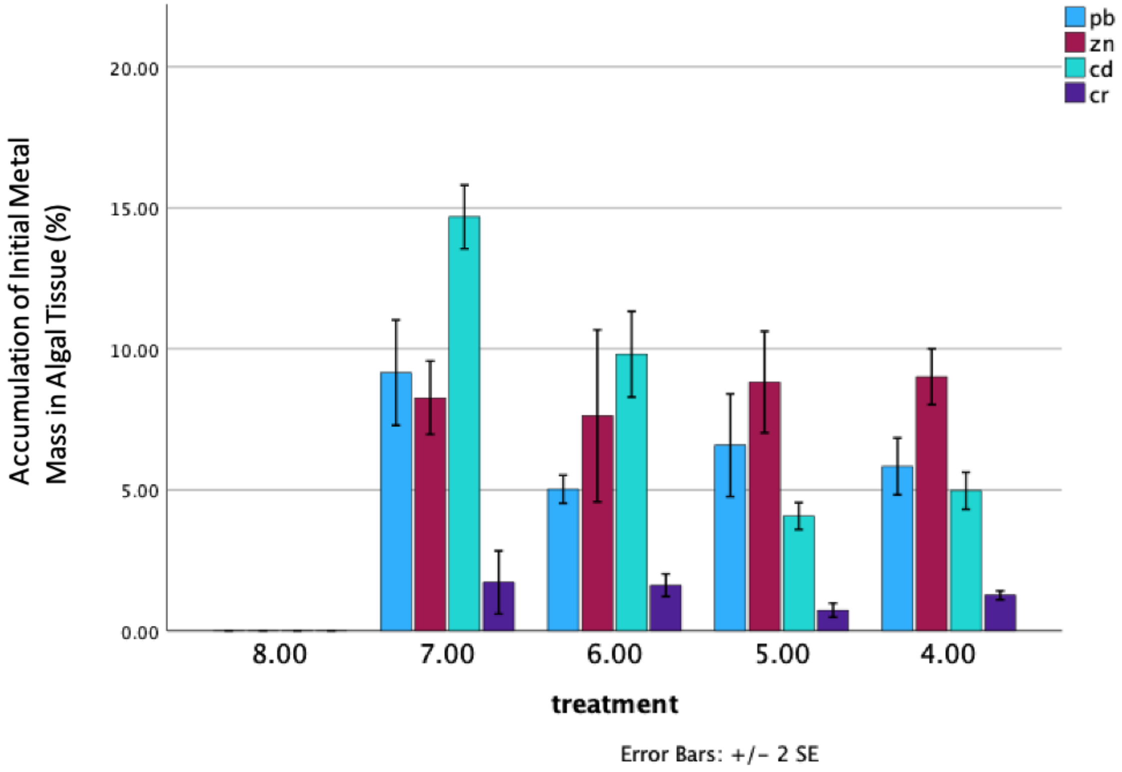

Figure 5. The percent total metal accumulated in each treatment in *P. palmata* following a 48-hour exposure. Lead is represented by the blue bar, zinc is the red bar, cadmium is the teal bar, and chromium is the purple bar. The error bars represent +/- two standard errors. (P-value for all Pb, Cd, and Cr are <0.001, P-value for Zn = .007, Cr F-statistic: 6.706, Cd F-statistic: 151.176, Zn F-statistic: 19.073, Pb F-statistic: 27.871, n = 15 for each metal)

Discussion:

From the data collected, *P. palmata* was not efficient at concentrating metals in its tissue compared to other algae specie. Regardless, the concentration of cadmium, zinc, and lead surpassed the upper daily limit (UDL) of the allowed intake in at least one of the treatments. A linear increase in the final concentration of the metals in the tissue of *P. palmata* was apparent, with the most evident patterns being chromium (figure 1.) and zinc (figure 3.). For most of the metals, there was a lack of significant differences between treatments 5, 6, and 7. The lack of significant differences between many treatments alludes to *P. palmata's* insensitivity to changes in metal concentration and could disqualify it as an efficient biosorbent of toxic metals. In nature, there may need to be significant differences in initial metal concentration to make a difference in accumulation in the tissue of *P. palmata*, which also means it could be a safe option for aquaculture. Not only was percent accumulation low for all metals, but it also remained stagnant for chromium and zinc and decreased for cadmium and lead (figure 5.). The decrease in percent accumulation with the increased metal concentration in the water could mean that *P. palmata* became saturated with cadmium and lead in its tissue before it did with chromium and zinc. However, further research is necessary to determine this.

A study by Prasher et al. (2004) investigated the ability of *P. palmata* to bioaccumulate metals but used a much more comprehensive range of treatment amounts. Hence, a complete analysis of the absorption ability of *P. palmata* could be obtained. The optimal conditions for maximum bioaccumulation by *P. palmata* were investigated and used, resulting in a much higher bioaccumulation efficiency than in this study. *P. palmata* in this study was 6x less efficient at absorbing cadmium and lead than the study done by Prasher et al. (2004), but the algae was not subjected to the same conditions as the other study (Prasher et al., 2004). Data about *P. palmata's* ability to absorb chromium was not found. However, a different red alga, Galaxaura *oblongata*, absorbed 85.2% of the initial chromium added to the water (Ibrahim, 2011). Another species of red algae, Laurencia *obtusa,* was able to bioaccumulate 98.6% of the chromium and 98.0% of the cadmium added to the water (Hamdy, 2000). Different methods of experimental setup could play a role in the differences in outcomes between these studies. Temperature, pH, sun exposure, algal species, and salts used to introduce metals to the solution all differ among these studies. In retrospect, it would have been wise to closely follow another study's methods to produce more easily comparable data.

An error that plagued this experiment occurred when we calculated the concentration of metals to mix into the stock A solution. Our initial intent was to treat the *P. palmata* with 4x the highest concentration found in algae in the Salish Sea (Hahn et al., 2022) and decrease each successive treatment by half. Unfortunately, metal concentration was calculated based on the dry weight of the algae instead of the wet weight. The concentration of metals in dry algae is about 6x higher because algae are about 85% water (Mouritsen et al., 2013). Ultimately, treatment 1 ended up being 26x higher than initially planned. After realizing our mistake, we decided only to use treatments 4 - 8. Treatment 4, being the highest, still represented a reasonable concentration at about 2x the concentration of the metals typically found in algae in the Salish Sea. Still, a lack of treatments deteriorates the robustness of the experiment and provides fewer data to make inferences about the ability of *P. palmata* to bioaccumulate metals in its tissue.

Based on the data collected, *P. palmata* is prone to chromium, cadmium, zinc, and lead in marine water. Aquaculturists should consider where they grow *P. palmata* for use as a food product. It would have been informative to see how exposing algae to metals over time, similar to their lifespan, would affect the concentration of metals in their tissue. This study only exposed the algae to the chosen metals for 48 hours, but in a real-world situation, the algae would likely grow for several months in contaminated waters. Also, there are few clear guidelines about the allowed metal concentrations in commercially grown algae, so it is difficult to know what amounts to consider unhealthy or dangerous other than the UDL state by the FDA and CDC. In conclusion, *P. palmata* is not nearly as efficient at bioaccumulating metals as other algal species (Hamdy, 2000; Ibrahim, 2011), making it a strong contender for aquaculture. Nonetheless, we should still concentrate on cleaning coastal waters and produce a system to prevent the deposition of toxins into the water.

Bibliography

Al-Shwafi, N., A. Rushdi. 2008. Heavy metal concentrations in marine green, brown, and red seaweeds from coastal waters of Yemen, the Gulf of Aden. Environmental Geology. 55:653-660.

Ali, H., E. Khan. 2019. Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs—Concepts and implications for wildlife and human health. Human and Ecological Risk Assessment. 25(6):1353-1376.

CDC. Toxicology Profile for Cadmium. https://www.atsdr.cdc.gov/ToxProfiles/tp5-c3.pdf. 11/29/30.

CDC. What Are the Physiologic Effects of Chromium Exposure? https://www.atsdr.cdc.gov/csem/chromium/physiologic_effects_of_chromium_exposure.html. 11/29/22.

Chernova, E.N. The biogeochemical background and trace metal accumulation by brown algae of the genus *Fucus* in coastal waters of the Sea of Japan, the Sea of Okhotsk, and the White Sea. Russian Journal of Marin Biol 42, 87–96 (2016).

Chernova, E.N., Shulkin, V.M. 2019. Concentrations of Metals in the Environment and in Algae: The Bioaccumulation Factor. Russian Journal of Marine Biology 45, 191–201. https://doi-org.ezproxy.library.wwu.edu/10.1134/S1063074019030027

Chopin, T., A. Tacon. 2021. Importance of seaweeds and extractive species in global aquaculture production. Reviews in Fisheries Science and Aquaculture. 29(2):139-148.

Deepak, J.R., A. Anbarasu, V. Prabu. 2022. Removal of Toxic Metal Presence in the Wastewater and Production of the Biomass from Microalgae *Chlorella* sp., Journal of Nanomaterials,vol. 2022, Article ID 8408462, 5 pages. https://doi.org/10.1155/2022/8408462

Grote, B. 2019, Recent developments in aquaculture of Palmaria palmata (Linnaeus) (Weber & Mohr 1805): cultivation and uses. Revolutionary Aquaculture, 11:25-41. https://doi.org/10.1111/raq.12224.

Hahn, J.L., Van Alstyne K.L., Gaydos J.K., Wallis L.K., West J.E., Hollenhorst S.J. Ylitalo G.M., Poppenga R.H., Bolton J.L., McBride D.E., Sofield R.M. 2022. Chemical contaminant levels in edible seaweeds of the Salish Sea and implications for their consumption. PLoS ONE 17(9): e0269269. https:// doi.org/10.1371/journal.pone.0269269.

Hamdy, A.A. 2000. Biosorption of heavy metals by marine algae. Current Microbiology. 41:232-238. DOI: 10.1007/s002840010126.

Ibrahim, W. 2011. Biosorption of heavy metal ions from aqueous solution by red macroalgae.

Journal of Hazardous Materials. 192(3):1827-1835.

Lopez, D., T. Melo, J. Meneses, M. Abreu, R. Pereira, P. Domingues, A. Lilleb, R. Calado, M. Domingues. 2019. A new look for the red macroalga Palmaria palmata seafood with polar lipids rich in EPA and with antioxidant properties. Marine Drugs. 17(533): 1-19.

Lustberg M, Silbergeld E. Blood Lead Levels and Mortality. *Arch Intern Med.* 2002;162(21):2443–2449. doi:10.1001/archinte.162.21.2443.

Mouritsen, O.G., C. Dawczynski, L. Duelund, G. Jahreid, W. Vetter, M. Schroder. 2013. On the consumption of red seaweed dulse (palmaria *palmata* (L.) Weber and Mohr). Journal of Applied Phycology. 25:1777-1791.DOI 10.1007/s10811-013-0014-7.

Plum LM, Rink L, Haase H. The essential toxin: impact of zinc on human health. Int J Environ Res Public Health. 2010 Apr;7(4):1342-65. doi: 10.3390/ijerph7041342. Epub 2010 Mar 26. PMID: 20617034; PMCID: PMC2872358.

Prasher, S.O., M. Beaugeard, J. Hawari, P. Bera, R.M. Patel, S.H. Kim. 2004 Biosorption of heavy metals by red algae (Palmaria *palmata*), Environmental Technology, 25(10:1097-1106. DOI:10.1080/09593332508618378

Sánchez-Quiles, D., N. Marbà, A. Tovar-Sánchez. 2017. Trace metal accumulation in marine macrophytes: Hotspots of coastal contamination worldwide. Science of the Total Environment. 576:520-527.

Spain, O., Plöhn, M. & Funk, C. 2021.The cell wall of green microalgae and its role in heavy metal removal. *Physiologia Plantarum*, 173(2):526– 535. https://doi.org/10.1111/ppl.13405

Wael, I. 2011. Biosorption of heavy metal ions from aqueous solution by red macroalgae. Journal of Hazardous Materials. 192(3):1827-1835. DOI: 10.1016/j.jhazmat.2011.07.019.

Wells, M., P. Potin, J. Craigie, J. Raven, S. Merchant, K. Helliwell, A. Smith, M. Camire, S. Brawley. 2016. Algae as nutritional and functional food sources: revisiting our understanding. Journal of Applied Phycology. 29:949-982.