Entry into the Stockholm Junior Water Prize 2024

# Investigating if Avocado Skin powder or Bacopa monnieri Can Alter the Amount of Heavy Metals in Aquatic Environments

Lily Rofail, PLC Sydney, NSW, Australia

## I. Abstract

Phytoremediation remains an under-utilised, cost-effective and safe mitigation strategy for reducing heavy metal contamination in aquatic environments. This study aimed to determine if Avocado Skin powder and Bacopa monnieri can improve duckweed survival outcomes in copper-contaminated aquatic environments. The results support the hypothesis that Avocado Skin powder and Bacopa monnieri (Water hyssop) improve duckweed survival outcomes in 0.1, 0.2, 0.4, 0.8, 1 and 2 ppm copper contaminated aquatic environments at 7 & 14 days. The ANOVA showed a significant difference in the duckweed survival with both Avocado Skin powder and Bacopa Monnieri on day 7 and day 14 ($p<0.001$ & $p<0.0001$ respectively). A Tukey test confirmed a significant difference in duckweed survival between the Base Case and both Avocado Skin powder ($p<0.01$) and Bacopa Monnieri ($p<0.01$). Further, there was a significant improvement in Duckweed survival with Avocado Skin powder compared to Bacopa monnieri on day 14 ($p<0.05$). Both Avocado Skin powder and Bacopa Monnieri can be implemented as phytoremediation strategies.

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## III. Key Words

Bacopa monnieri, water hyssop, avocado, avocado skin, copper concentration, heavy metal, aquatic environments, phytoremediation, bioremediation, fruit skin, colorimeter

## IV. Acknowledgements

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## V. Biography

Lily Rofail is a 16-year-old high school student (Year 10) at PLC Sydney, Australia. Lily has a passion for science research and innovation. Lily is an active member of her school's science community. She has won several awards in science research competitions including three major research projects. Her science research focuses on providing innovative, new and creative solutions to global issues. Lily Rofail is a respected member of her school community and she participates in a broad range of co-curricular activities such as Hockey, Volleyball, Speech, Public Speaking and Debating. She is also an avid member of her school sustainability group SEED (Sustainable Education & Environmental Development Group) as well as the Science Festival Committee which organises a yearly science-focussed student conference. She is part of her school's Student Representative Council (SRC) and strives to make a positive change to her school's environment. Lily's favourite subjects at school are Science and Maths and she hopes to pursue her love for STEM in future years.

## 1. Introduction

### 1.1. Problem

Dangerous quantities of heavy metals contaminate freshwater aquatic environments around the world. For example, six lakes in the Tasmanian Wilderness World Heritage Area are contaminated with heavy metal concentrations above maximum recommended levels (Australian National University, 2019). Heavy metals occur naturally as high-density metallic elements. Water runoff from agriculture, industry and factories is usually the main cause of heavy metal contamination in aquatic environments and drinking water (Izah et al., 2016, Kinuthia et al., 2020 and Masindi et al., 2018). Many organisms that inhabit these environments are therefore subject to absorbing heavy metals. For example, traces of heavy metal substances have been discovered in fish gills, liver and muscle tissues (Rajeshkumar et al., 2018). Interestingly, low concentrations of some heavy metals such as copper are essential for human health and plant photosynthesis. However, at medium to high concentrations, heavy metals like copper are extremely toxic. Furthermore, since heavy metals cannot be degraded, they often accumulate in living organisms, soil and water, injuring and, in extreme cases, killing plants, animals and humans due to toxicity. When released into the environment, ingested and absorbed; common heavy metals such as arsenic, cadmium, mercury and lead have been found to bioaccumulate in the human body which can result in death and disability due to diseases such as cancer. The monitoring and subsequent removal of heavy metals from water can be time-consuming and costly. There is a pressing need to discover efficient and environmentally friendly solutions to remove toxic heavy metals from water.

Copper is easily available and is non-toxic in low concentrations. It is commonly used in animal food supplements and in many industries such as textiles, petroleum and metal (Boone, 2012). It is also commonly used in agriculture as a fertiliser and to control algae, fungus and weeds (La Torre et al, 2018). Copper is highly soluble in water and thus poses a risk of toxicity due to bioaccumulation in living organisms. Therefore, the effect of copper toxicity on plants can be used to model the impacts of heavy metals in aquatic environments.

Bioremediation is a low-cost, natural and eco-friendly method that can remove heavy metals from water (Jain et al., 2016). Only a few studies have focussed on bioremediation and especially phytoremediation using plants or plant derivatives to remove heavy metals from aquatic environments (Rajeshkumar et al., 2018 and Samet et al., 2018). Duckweed (Lemna Disperma) makes the ideal plant to study the effect of phytoremediation as it is fast-growing and can double its size in the span of one to two days. It is a rapidly-spreading, small, free-floating plant that is native to Australia and many parts of the world. Each plant has 1 to 4 leaves grouped together in clusters (Conn, 2017). They float on the surface of still/slow-flowing freshwater rivers (Aquatic Technologies, 2022). Duckweed is used by environment control and mining regulation agents in Canada, the United States and the Organisation for Economic Co-operation and Development (OECD) countries (Park et al., 2021) to monitor heavy metal toxicity. Usually, the leaf count and/or dry weight are measured. Thus, Duckweed frond growth or the number of leaves alive can be used to measure the effectiveness of bioremediation strategies to remove heavy metals such as copper from water.

Discarded fruit skins such as avocado skin, make an optimal candidate for phytoremediation, as they can be repurposed for removing heavy metals instead of accumulating in landfill. Avocado skin powder may also possess the same properties for removing copper from water (Makhado, 2018). The production of Avocado in Australia has increased significantly since 2009-10 from 40,000 tonnes to 120,000 in 2020-21, with a predicted increase to about 170,000 by 2025/2026 (Avocado.org.au, 2022). They are produced all year round across eight major avocado farming regions in Australia. With this expansion in production comes the expansion in the amount of avocado waste that can be used for bioremediation. Mallampati et al (2015) developed a method of cleaning the surface of the fruit by boiling the fruit skin to remove soluble impurities. The skin was then dried and crushed before being used. In addition, Mallampati et al (2015) demonstrated that avocado skin can remove lead and nickel from water in the laboratory. Thus this method can be adopted to remove heavy metals in real-life aquatic environments. Furthermore, a recent dissertation (Makhado, 2018), used chemically treated avocado skin to remove arsenic, cadmium and chromium from water samples in South Africa. Thus, avocado skin could potentially remove heavy metals from water in real-life aquatic environments.

Other excellent candidates include native Australian water-loving plants such as Bacopa monnieri (Water hyssop) which are non-toxic and so can be used as natural tools for bioremediation. They will not only remove copper but support other native flora, fauna in particular marine animals.

Bacopa monnieri (Water hyssop) is a creeping evergreen herb with succulent small leaves (3.5-15 mm long). It is native to coastal NSW and Queensland and usually grows around the banks or under wetlands of freshwater ponds (Barker, 1992). It can also tolerate brackish water, poorly drained soil and various climatic environments. Sinha et al (1990) have shown that Bacopa monnieri has the distinctive ability to absorb copper. Hence, it can be used to remove heavy metals from aquatic environments. Mallubhotla et al (2016) showed that Bacopa monnieri can remove up to 40ppm of copper from soil because of its role as a micronutrient for plant growth. Similarly, Bacopa monnieri could remove or mitigate heavy metals in a natural aquatic environment.

Both Bacopa monnieri and avocado skin may be able to remove heavy metals in aquatic environments at minimal cost. Hence, measuring and comparing the effect of copper concentration, to represent heavy metal accumulation, on duckweed growth in water with the addition of Bacopa monnieri or avocado skin, may identify potential eco-friendly, renewable, safe and inexpensive bioremediation strategies for the removal of toxic heavy metals from aquatic environments.

### 1.2. Aim

This research aims to determine if Avocado Skin powder and Bacopa monnieri (Water hyssop) can improve survival outcomes of duckweed in 0.1 ppm, 0.2 ppm, 0.4 ppm, 0.8 ppm, 1 ppm & 2 ppm copper-contaminated aquatic environments.

### 1.3. Materials

- Deionised water
- Avocadoes, Duckweed & Bacopa monnieri plants
- Clear plastic/glass containers
- Copper Sulphate Pentahydrate (*25% Copper*)
- Electronic balance
- Mortar and pestle
- Sieve (aperture 1mmx1mm)
- Aquarium gravel substrate
- Stove or heating element
- HI7024 Pool Line High Range Copper Checker HC (handheld colorimeter)

## 1.4. Methods

### 1.4.1. Part 1: Preparing Copper Concentrations

**To prepare Control Environment -0.0 ppm copper concentration**

1. Three separate containers of 1000 mL deionised water were labelled "Control-Base Case", "Control-Avocado Skin Powder-Control" and "Control-Bacopa monnieri"

**To prepare stock copper sample and first dilution**

2. Four grams of copper sulphate pentahydrate containing 25% copper were added to a container of 1000 mL deionised water to create a 4 g/L (or 40mg/10mL) stock solution. The container was labelled "Stock Solution".

3. 10 mL of the "stock solution" were added to a separate container of 990 mL deionised water to make a 40 mg/L Copper Sulphate Pentahydrate solution (or 0.4 mg/10 mL) and labelled "Dilution 1 - 0.4 mg/10 mL"

**To prepare Pond Environment A - 0.1 ppm copper concentration**

4. 10 mL of the " Dilution 1 - 0.4 mg/10 mL" solution was withdrawn and added to each of three containers of 990 mL deionised water to make 0.4 mg/L Copper Sulphate Pentahydrate or 0.1 ppm copper solution.

5. A permanent marker was used to label the containers "Pond A-Base Case", "Pond A-Case 1 (Avocado Skin Powder)" and "Pond A-Case 2 (Bacopa monnieri)".

**To prepare Pond Environment B - 0.2 ppm copper concentration**

6. 20 mL of the "Dilution 1 -0.4 mg/10 mL" solution was withdrawn and added to each of three containers of 980 mL deionised water to make 0.8 mg/L of Copper Sulphate Pentahydrate or 0.2 ppm copper solution.

7. A permanent marker was used to label the containers "Pond B-Base Case", "Pond B-Case 1 (Avocado Skin Powder)" and "Pond B-Case 2 (Bacopa monnieri)".

**To prepare Pond Environment C - 0.4 ppm copper concentration**

8. 40 mL of the "Dilution 1-0.4 mg/10 mL" solution was withdrawn and added to each of three containers of 960 mL deionised water to make 1.6 mg/L of Copper Sulphate Pentahydrate or 0.4 ppm copper solution.

9. A permanent marker was used to label the containers "Pond C-Base Case", "Pond C-Case 1 (Avocado Skin)" and "Pond C-Case 2 (Bacopa monnieri)".

**To prepare Pond Environment D - 0.8 ppm copper concentration**

10. 80 mL of the "Dilution 1-0.4 mg/10 mL" solution was withdrawn and added to each of three containers of 920 mL deionised water to make 3.2 mg/L of Copper Sulphate Pentahydrate or 0.8 ppm copper solution.

11. A permanent marker was used to label the containers "Pond D-Base Case", "Pond D-Case 1 (Avocado Skin)" and "Pond D-Case 2 (Bacopa monnieri)".

**To prepare Pond Environment E - 1 ppm copper concentration**

12. 100 mL of the "Dilution 1-0.4 mg/10 mL" solution was withdrawn and added to each of three containers of 900 mL deionised water to make 4 mg/L of Copper Sulphate Pentahydrate or 1 ppm copper solution.

13. A permanent marker was used to label the containers "Pond E-Base Case", "Pond E-Case 1 (Avocado Skin)" and "Pond E-Case 2 (Bacopa monnieri)".

**To prepare Pond Environment F - 2 ppm copper concentration**

14. 200 mL of the "Dilution 1-0.4 mg/10 mL" solution was withdrawn and added to each of three containers of 800 mL deionised water to make 8 mg/L of Copper Sulphate Pentahydrate or 2 ppm copper solution.

15. A permanent marker was used to label the containers "Pond F-Base Case", "Pond F-Case 1 (Avocado Skin)" and "Pond F-Case 2 (Bacopa monnieri)".

### 1.4.2. Method Part 2 – Preparing Pond Environment Remediation Cases

**Base Case**

16. A cup of 125 g of aquarium gravel substrate was carefully placed at the bottom and 44 Green duckweeds were placed on the water surface of each of the containers labelled Control-Base Case, Pond A-Base Case (+ 0.1 ppm copper), Pond B-Base Case (+ 0.2 ppm copper), Pond C-Base Case (+ 0.4 ppm copper), Pond D-Base Case (+ 0.8 ppm copper), Pond E-Base Case (+ 1 ppm copper) and Pond F-Baseline (+ 2 ppm copper).

**Case 1: Avocado Skin powder**

17. Fifteen ripe avocados were rinsed in 3 L of deionised water.

18. The avocados' skin was carefully peeled and boiled in a saucepan in 2 L of deionised water for 30 min.

19. The boiled avocado skins were removed from the saucepan, placed in two large trays in a single layer, patted dry with a paper towel and left on the bench to dry for 3 days.

20. Once the avocado skin was dry it was ground into a fine powder using a mortar and pestle and passed through a 1 mm x 1 mm sieve to ensure that the particle size was consistent.

21. A cup of 125 g of aquarium gravel substrate, Avocado skin powder (5 g) and 44 duckweed leaves were added to each of the containers labelled Control-Avocado Skin Powder, Pond A-Case 1 (Avocado Skin Powder+0.1 ppm copper), Pond B-Case1 (Avocado Skin Powder+0.2 ppm copper), Pond C-Case 1 (Avocado Skin Powder+0.4 ppm copper), Pond D-Case1 (Avocado Skin Powder+0.8 ppm copper), Pond E-Case1 (Avocado Skin Powder+1 ppm copper), Pond F-Case1 (Avocado Skin Powder+2 ppm copper).

**Case 2: Bacopa monnieri-Water hyssop**

22. Three stems of Bacopa monnieri (6 nodes 14-16 leaves each) were carefully planted into each of seven cups of 125 g of aquarium gravel substrate.

23. The cups in step 24 were placed on the bottom and 44 duckweed leaves were placed on the water surface of each of the containers labelled Control-Bacopa monnieri, Pond A-Case 2 (Bacopa monnieri+0.1 ppm copper), Pond B-Case 2 (Bacopa monnieri+0.2 ppm copper), Pond C-Case 2 (Bacopa monnieri+0.4 ppm copper), Pond D-Case 2 (Bacopa monnieri+0.8 ppm copper), Pond E-Case2 (Bacopa monnieri+1 ppm copper), Pond F-Case2 (Bacopa monnieri+2 ppm copper).

### 1.4.3. Method Part 3-Definition of Alive duckweeds

24. Alive duckweeds were defined as being green and not having any chlorosis (lack of green colour) in any part of the leaf and/or not having brown discolouration indicating necrosis or death (Khellaf et al, 2010).

25. The number of duckweed leaves alive was recorded for each solution on days 0,1,2,3,4,5,6,7,8,9,10 &14.

### 1.4.4. Method Part 4-Colorimeter measurement of copper concentration(ppm) in solution

26. Copper concentration (ppm) was measured using a handheld colorimeter per manufacturer instructions for each solution on days 7 and 14.

### 1.4.5. Method Part 5

27. The whole experiment was repeated

### 1.4.6. Method Part 6-Statistical Analysis

28. A statistical Analysis of Variance (ANOVA) was carried out to find whether there was a significant difference between the Base case, Avocado Skin powder and Bacops monnieri environments

in duckweed survival at different copper concentrations. If a significant difference was found, a Tukey test was conducted, to identify which treatments were effective at reducing duckweed leaf loss.

## 2. Results

The results showed that the average number of duckweed leaves alive reduced as the copper concentrations rose in Base Case while the number of duckweed leaves alive increased or remained stable with both Avocado Skin Powder and Bacopa monnieri after 7 and 14 days as shown in Figures 1&2.

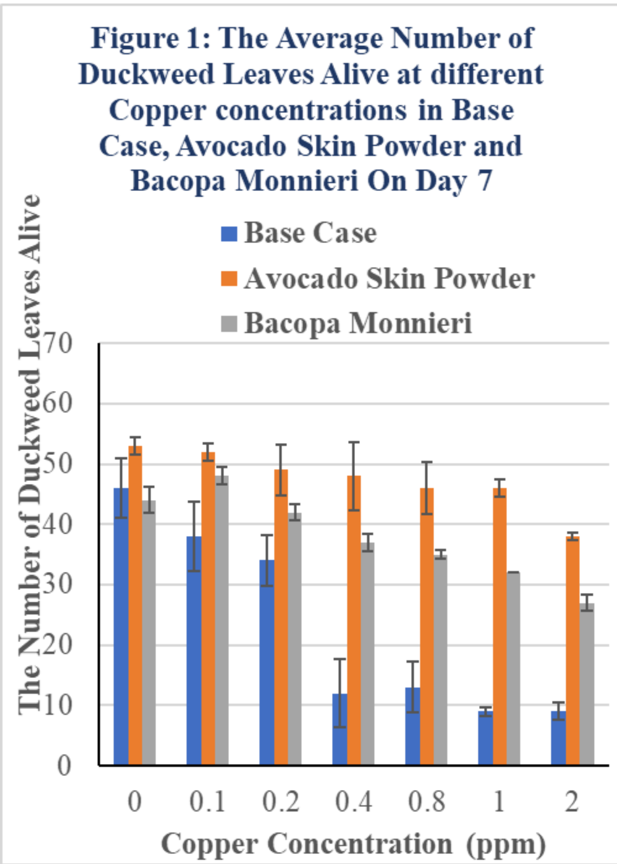


Figure 1: The Average Number of Duckweed Leaves Alive at different Copper concentrations in Base Case, Avocado Skin Powder and Bacopa Monnieri On Day 7

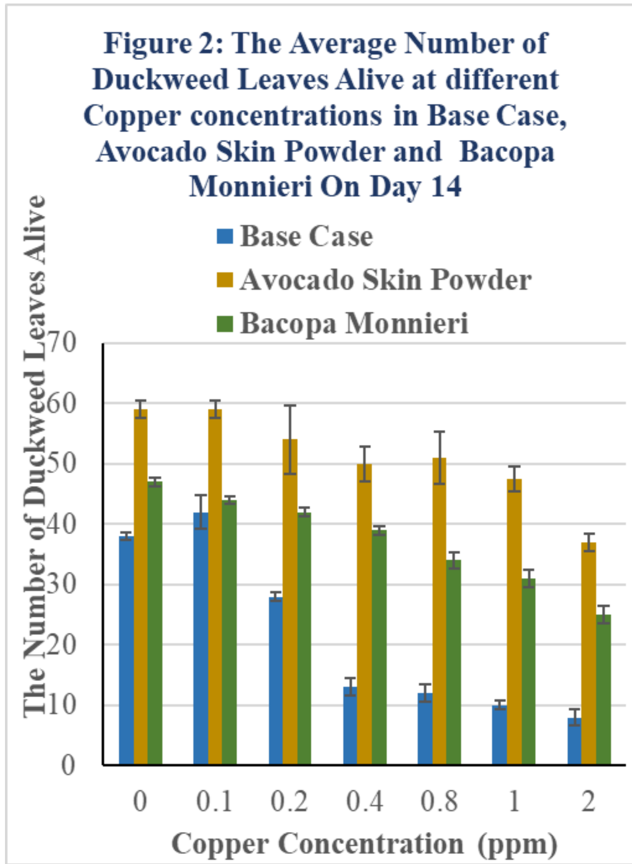
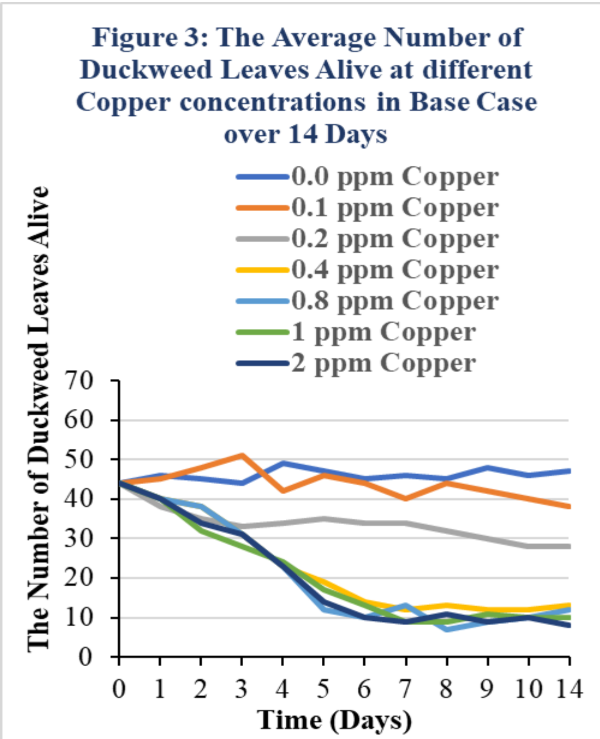
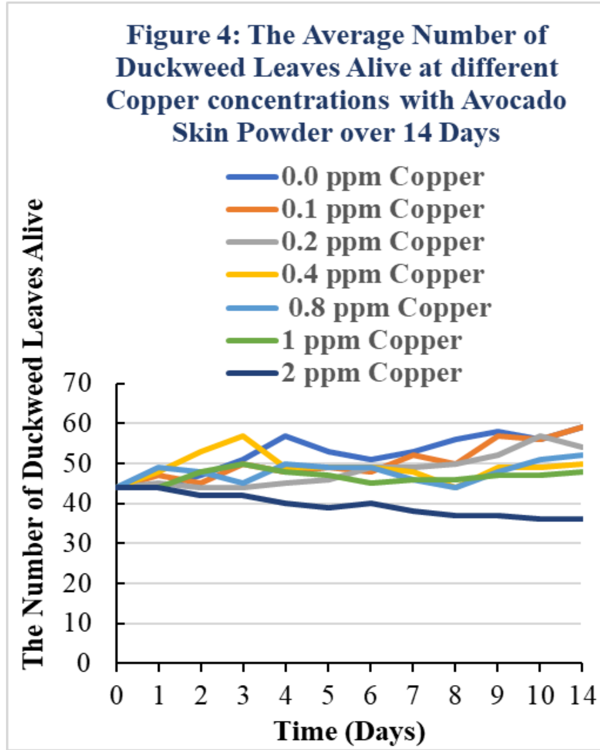
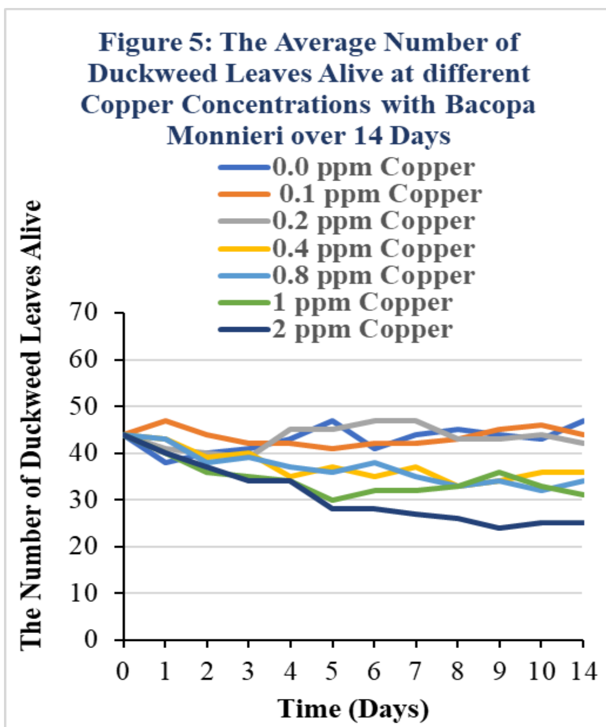


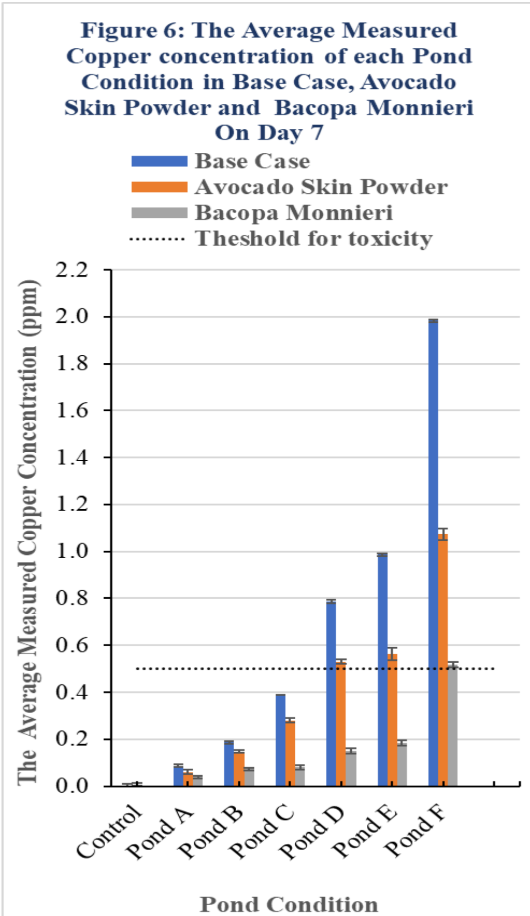
Figure 2: The Average Number of Duckweed Leaves Alive at different Copper concentrations in Base Case, Avocado Skin Powder and Bacopa Monnieri On Day 14

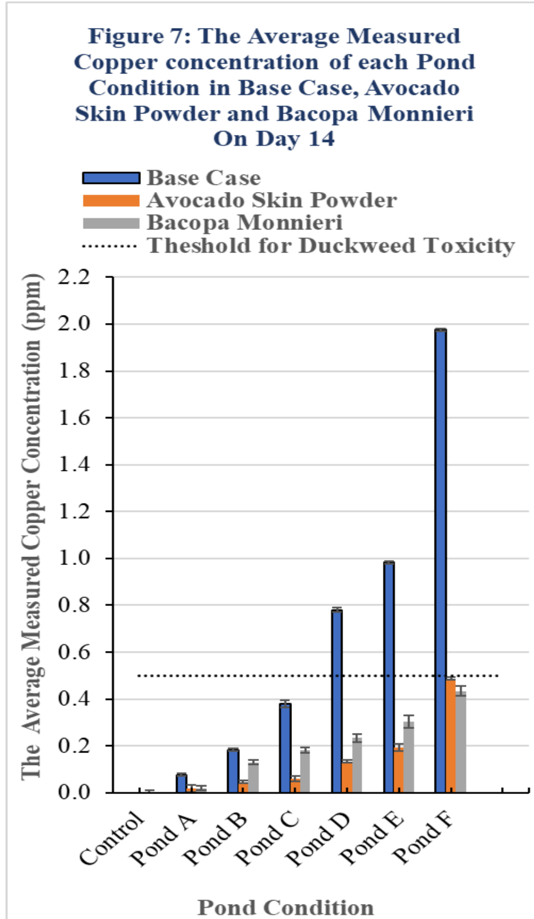
Figures 3 to 5 show the trends in the average number of duckweed leaves alive at different copper concentrations at Base Case, Avocado Skin powder and with Bacopa Monnieri over 14 days. At zero or low copper concentrations (0.1 ppm) the number of duckweed leaves alive remained stable or mildly increased in Base Case, Avocado Skin powder and Bacopa monnieri. However, at higher concentrations (0.2ppm-2.0ppm) the number of duckweed leaves alive were significantly lower at Base case compared to Avocado Skin powder and Bacopa monniera. A statistically significant increase in duckweed survival rate with Avocado Skin powder compared to Bacopa monniera was seen on day 14 but not on day 7. Figures 6& 7 show the average measured copper concentrations (with the calorimeter) were reduced with Bacopa Monnieri more than Avocado Skin powder on day 7 and were similar on day 14 where both treatments lowered the copper concentration to below that of duckweed toxicity(0.5ppm).






## 3.Discussion

The results support the hypothesis that both Avocado Skin powder and Bacopa monnieri (Water hyssop) improve survival outcomes of duckweed in 0.1, 0.2, 0.4, 0.8, 1 and 2 ppm copper-contaminated aquatic environments at day 7 and 14 (Figure 1&2). The ANOVA showed a significant difference in the duckweed leaf survival at Base case, Avocado Skin powder and Bacopa Monnieri on Day 7 & 14 (*p-value* <0.001 and < 0.005) The Tukey test confirmed a significant difference in Duckweed survival on day 7 and day 14 between the base case and the Avocado Skin powder (*p-value* <0.01 & <0.01 respectively) and Base case and Bacopa Monnieri (*p-value* <0.05 and < 0.01). Further, the number of duckweed alive was significantly higher in Avocado Skin powder compared to Bacopa monnieri on day 14 ( $p<0.05$) but not on day 7 ($p<0.23$).

This experiment has confirmed that Duckweed is a hardy aquatic plant, useful for comparison of aquatic heavy metal toxicity (Figure 8). The number of duckweed leaves alive were stable or increased at 0.0 ppm copper concentration with the Base Case, Avocado Skin powder and Bacopa monnieri (Figure 3) which confirmed that duckweed can be used to study heavy metal toxicity as it has a low leaf loss rate and can withstand low nutrient conditions over time. Further, very low concentrations of copper can support growth and/or prevent duckweed leaf loss as seen in Pond A (0.1ppm) in Base case (Figure 1,2 & 3). This occurred over the first few days. However, duckweed leaf numbers were reduced over time and with increasing copper concentrations (0.2 ppm, 0.4 ppm, 0.8 ppm, 1.0 ppm and 2.0 ppm) in Base case over 14 days (Figure 2&3). Consistent with the findings of Khellaf et al (2010), the results showed that copper stimulated the growth of duckweed leaf numbers at low concentrations between 0.0 and 0.2 ppm but caused chlorosis (lack of green colour) and/or necrosis (death) occurred at concentrations of 0.5 ppm and above (Figure 3). This proves that copper at low concentrations is essential for duckweed growth but is toxic at higher concentrations. However, with the addition of Avocado Skin powder, the number of duckweeds alive increased or remained stable in the higher copper concentrations (Figure 4). Similarly, with the addition of Bacopa monnieri, duckweed survival rates remained stable or mildly improved compared to Base case (Figure 5). Although the number of duckweeds alive was lower with the addition of the Bacopa Monnieri than Avocado Skin powder this only reached statistical significance on day 14 ($p$-value<0.05) and did not reach statistical significance on day 7 ($p$-value 0.2). This indicates that copper can be toxic to duckweed in higher concentrations and that Avocado Skin powder was more protective for duckweed survival than Bacopa monnieri over 14 days with a confidence level of 95%.

Avocado skin is cheap and readily available. It was shown to improve duckweed leaf survival and growth in this experiment. In the Avocado Skin powder condition, duckweed leaf numbers increased at all concentrations tested (0.1 ppm, 0.2 ppm, 0.4 ppm, 0.8 ppm, 1 ppm & 2 ppm). Laboratory-treated avocado skin has been shown to extract heavy metals such as lead from water over time (Mallampati et al 2015). It is likely that Avocado Skin powder had a high ability to adsorb copper in varying pond conditions and so protected the duckweed leaves from the effect of copper toxicity and so allowing the duckweed leaves to multiply (Figures 1, 2 & 4)). This multiplication also suggests that Avocado Skin powder may be providing micronutrients to support duckweed leaf growth. Thus, Avocado Skin powder could be beneficially used in nutrient-poor heavy metal-contaminated aquatic environments.

A high-range copper concentration calorimeter (copper resolution of 0.01 ppm and accuracy of +/- 0.05 ppm) was used in this experiment to assess copper concentration on days 7 and 14. The reaction between the copper in the sample and the sodium bicinchoninate reagent caused a purple tint in the sample. Using the Beer-Lambert law, where the colour intensity of the sample is proportional to the concentration, the copper concentration in the sample was determined using a photometer light source

(LED @ 575 nm) and a silicon photocell light detector. The results showed that both Avocado Skin powder and Bacopa monnieri reduced the measured copper concentration in Ponds A to F (Figure 6 &7). Bacopa Monnieri reduced the copper concentration in all pond environments to below 0.5 ppm which is the threshold for duckweed toxicity on both days 7 &14.

Avocado Skin powder reduced measured copper concentration to below 0.5 ppm at all concentrations tested in Ponds A to C on Day 7 and all ponds on Day 14. Bacopa Monniera reduced the measured copper concentration more than Avocado Skin powder in contrast to duckweed leaf survival where Avocado Skin powder was better than Bacopa Monnieri on day 14 (Figures 2 &7). Further supporting the evidence that Avocado Skin powder is likely providing nutrients to support duckweed growth separate from its ability to reduce copper concentration. Therefore, Avocado Skin powder could be implemented as a phytoremediation strategy especially in nutrient-deficient environments because it encouraged duckweed leaf growth and reduced copper concentration.

Avocado peel is rich in protein, sugars (glucose, xylose, arabinose, galactose) and lignan (García-Vargas et al, 2020). Lignan can be degraded by bacteria (Li et al, 2020) and fungi (Heeger et al, 2021) in aquatic systems. To prevent sludging or overaccumulation it could be packed into water-permeable sachets that can be applied to the water's edge or carried by floating gardens in ponds which could be easily removed once the nutrient levels are adequate.

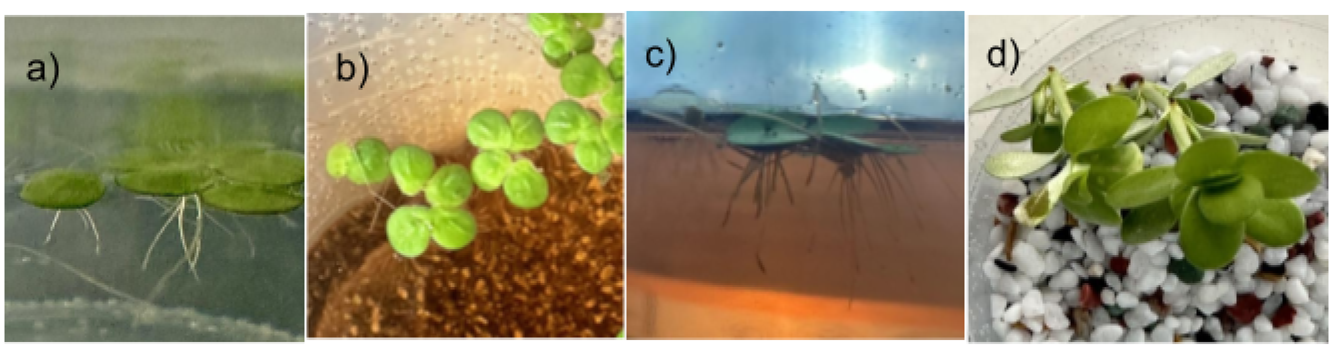



Figure 8: a) Duckweed floating on container surface, b and c) Duckweed floating n pigmented water in container with avocado skin, d) New growth in Bacopa Monnieri after 14 days

One notable issue with avocado skin powder is the discolouration of the water due to the tannins or phenol chemical compounds that form the pigment in the avocado skin (Figure 8 b & c). These compounds could bind to proteins and affect fish respiration (Rajeshkumar, et al 2018). Previous studies (Garcia-Vargass et, al, 2021) have determined the total phenolic content of treated avocado skin in the laboratory. The measurement of the phenolic compounds content of the water treated with avocado skin powder should be assessed to determine its possible effect on marine life before its implementation as a phytoremediation strategy.

Bacopa monnieri is a native to wetlands in many parts of the world including Australia, Europe, Asia and the America's. It is used in ponds and aquariums as an oxygenator and can be submersed to 20 cm. It can also support native marine life such as fish and tadpoles and is not harmful to the environment. Over the 14 days, Bacopa monnieri was also effective at preventing duckweed leaf loss compared to the Base case concentrations (0.1 ppm, 0.2 ppm, 0.4 ppm, 0.8 ppm, 1.0 ppm and 2 ppm) as shown in Figures 1, 2 & 5. Mallubhotla et al (2016), studied the effect of copper on Bacopa monnieri growth in soil and observed that copper sulphate induced Bacopa monnieri growth at a concentration of 20 ppm. Consistent with previous research, Bacopa monnieri should tolerate the copper concentrations (0.1, 0.2, 0.4, 0.8, 1, 2 ppm) used in this experiment.

Further, there was a significant difference in the number of duckweed leaves alive with Bacopa Monnieri compared to Avocado Skin powder on day 14, even though Bocopa Monnieri was shown to reduce the measured copper concentration more than Avocado Skin powder (Figures 6 & 7). Further, Figures 6 & 7 show that Bacopa Monniera lowered the measured copper concentration to at or below 0.5 ppm needed to reduce Duckweed toxicity. Thus the number of Bacopa monnieri plants and resulting surface area available was sufficient to effectively remove or lower the copper to protect the duckweed leaves. A possible explanation for the lower duckweed survival at high copper concentrations with Bacopa Monnniera compared to Avocado Skin powder on day 14 could be that there were too few other micronutrients available in the deionised water used, so Bacopa monnieri was competing for copper and other nutrients released by the dead duckweeds and compromising Duckweed survival. However, Bacopa Minnieri was still significantly more effective at preventing duckweed toxicity than the Base Case (Figures 1,2 and 5).

Further, new Bacopa monnieri leaves and growth in height were observed in the last few days of the experiment in all copper concentrations tested (Figure 8 d). With the absence of external micronutrients in the deionised water used, the growth of Bacopa monnieri may be further competing for copper and utilising nutrients released from the dead duckweed leaves, thus reducing duckweed growth and regeneration. This is seen in Figures 6 & 7 as the copper concentration with Bacopa Minnieri was lower than both the Base case and Avocado Skin powder suggesting that competition for nutrients rather than copper toxicity may be responsible for the duckwead leaf loss. This is consistent with the findings of Zhang et al 2020 that competition for nutrients could account for the reduced growth of duckweed. This may not be an issue in real-world conditions where there is a risk of duckweed overgrowth in nutrient-rich environments. The number of duckweed leaves alive with Bacopa monnieri was still higher than it was in the Base Case and frond and root growth of Bacopa Monniera were observed over the last few days of the experiment. Hence, Bacopa monnieri could be used for phytoremediation in nutrient-rich heavy metal-contaminated aquatic environments to lower copper concentrations and prevent duckweed overgrowth.

The number of duckweed leaves alive (frond reduction and cholrosis) is a valid method of assessing the copper concentration/toxicity in aquatic environments and has been used for standardised toxicity testing by many environmental control agencies around the world (Sharma et, el 2022). The following control variables were employed to enhance internal validity of the experiment: all duckweed plants were grown and sourced from the same environment, each plant was harvested from the same environment, each plant was kept under identical conditions before the experiment, the same number of duckweed leaves placed in all pond containers, the same amount of aquarium gravel, Avocado Skin powder and the same number of leaves and plants of Bacopa monnieri in each pond, the same total volume of copper solution for each pond, same stock solution, the same number of days (14) of observation, the same ambient conditions (temperature, sunlight etc.). To ensure accuracy, the number of duckweed leaves alive was counted three times for each copper concentration in all pond environments, the mean and standard deviations were represented in Figures 1, 2, 6 & 7. The accuracy of the copper concentration in each pond environment was further checked using a calibrated handheld colorimeter before the commencement of the experiment on day zero and a control was prepared for each of the copper concentrations for each pond modification. Further, the whole experiment was also conducted twice. Overall, the results obtained were valid, reproducible and accurate.

The study could be improved in several ways. Firstly, while deionised water was effective at demonstrating key trends, the study could have included real freshwater samples from surrounding reservoirs to better study the effect in real-world environments where other heavy metals can be found especially in nutrient-rich environments. Secondly, the nutrients, organisms and heavy metals that occur in a typical pond environment could have been added to examine whether they would compete for the adsorption. Thirdly, the amount of Avocado Skin powder and the number and size of Bacopa monnieri plants (number of nodes, fronds, size of roots) could have been varied to determine the optimal amounts needed per volume of polluted water to facilitate duckweed leaves' survival.

Furthermore, the study could be extended to examine alternative phytoremediation techniques using native plants and seeds. Mok et al (2013), studied six perennial Australian plants and found that Grevillea robusta, Acacia mearnsii, Eucalyptus polybractea, and Eucalyptus cladocalyx had the best performance in removing heavy metals from biosolids and potting mix. However, there is little research and minimal data on the use of native plants as biosorbents in aquatic environments. Several international studies have validated the use of other bioremediation methods such as fruit skin (Phuengphai et al., 2021) and seeds (Costa et al., 2020 and Edogbanya, 2013) as bioadsorbents of copper in water. Thus, the experiment could be repeated with native aquatic plants apart from Bacopa monnieri to assess their ability to control and remove heavy metal toxicity from water. The study could also be further enhanced by using other dependent variables such as duckweed root length. In fact, Gopalapillai et al (2014) concluded that duckweed root length is a sensitive measure of heavy metal water toxicity in mining effluent. Similarly, the experiment could be repeated using different independent variables such as Avocado Skin powder particle size, adding both Avocado Skin powder and Bacopa monnieri, and/or at varying pH and temperature conditions.

**Conclusions**

Both Avocado Skin powder and Bacopa monnieri (Water hyssop) improved the survival outcomes of duckweed in 0.1 ppm, 0.2 ppm, 0.4 ppm, 0.8 ppm, 1 ppm and 2 ppm copper-contaminated aquatic environments both on days 7 and 14. The number of duckweed leaves alive was significantly better with Avocado Skin powder compared to Bacopa Monnieri on day 14 but not on day 7. Further, both Avocado Skin powder and Bacopa Monnieri were successful in lowering the copper concentration in all pond environments tested supporting the use of Bacopa monnieri and Avocado Skin powder as natural, eco friendly and cost effective bioremediation strategies to tackle the threat of heavy metal water toxicity. Avocado skin could be implemented especially in nutrient-deficient environments as it encouraged duckweed leaf growth and lowered the measured copper concentration. Similarly, Bacopa Monnieri was also very effective as it lowered the measured copper concentrations to below the toxic level for duckweed (0.5ppm) and improved duckweed leaf survival compared to the base case. It could be safely and successfully used to reduce heavy metal toxicity in nutrient-rich environments where competition for nutrients would not have an effect on the local ecosystem and it may control duckweed and prevent overgrowth. Bacopa Monnieri is a native plant to many areas around the world so it could be harnessed with great success and minimal adverse environmental effects on the local aquatic ecosystem. Avocado skin needs more careful study to investigate strategies to reduce tannins and their possible effects on aquatic environments. Further, Avocado Skin powder can be introduced using permeable satchels on floating gardens to reduce sludge potential. This study has shown that Avocado Skin powder and Bacopa Monniera are two potential eco-friendly mitigation strategies that are under-utilised, cheap, readily available and have a great potential to support both nutrient-poor and nutrient-rich heavy metal-contaminated aquatic environments.

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