Neutralisation and zinc extraction from acid mine drainage: a comparative study of oyster shells and marble chips

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ABSTRACT

The present study investigated the applicability of oyster shells and marble chips in raising pH and extracting heavy metal zinc from acid mine drainage (AMD). A shortcoming of the long-standing mining industry is concerned with the environmental degradation of AMD - a by-product of mining. To investigate treatment options, a hybrid passive treatment involving constructed wetlands was used, with nine batches - three containing oyster shells and acid mine drainage, three containing marble chips and AMD, and three controls - one with oyster shells and tap water, one with marble chips and tap water and one with only AMD. Results show that oyster shells had higher neutralization capabilities than marble chips. Oyster shells and marble chips raised the pH to 7.5 and roughly 7.17 respectively, and changed the resulting zinc concentration to an average of approximately 1.33 mg/L and 5.83 mg/L respectively. Surprisingly though, the marble chips were observed to be more absorbent. The conclusion that oyster shells generally performed better allows for increased recognition and ubiquity of this product in water bodies impacted by historic mine damage.

Keywords: oyster shell; marble chip; pH; heavy metal zinc; acid mine drainage; hybrid passive treatment; constructed wetland

INTRODUCTION

Acid mine drainage - defined as the flow of acidic water that occurs because of mining practices, has been a recurring and problematic phenomenon within the mining industry, devastating water bodies and aquatic life for thousands of years (1). According to Oxfam Australia, the mining industry greatly boosts Australia's economy, as metal and mineral exports account for approximately 70% of total export revenue - worth $413 billion in 2021 - 2022 (2,3). Australia's mining industry also provides an annual wage of roughly $141 thousand, reflecting the demanding nature of the industry - with mines being located far from existing infrastructure (4,5). Regularly being separated from family and friends has contributed to the Australian mining industry's ambitious goal of accomplishing zero fatalities (4). The industry's fervent commitment to a high standard of workplace safety desires every worker to return home safe and healthy (4). That high standard of empathy towards the health and safety of workers, has not been extended towards the industry's environmental considerations (4).

While the modern mining environmental practices are more accountable, highly overseen and better implemented than ever before, there is emphasis on the pursuit for sustained improvement - encompassing water use, biodiversity conservation and mine rehabilitation (4). It has been universally acknowledged that through initiatives and regulations to protect the environment that mining has serious environmental effects (2). This does not only involve the copious amounts of construction leading to deforestation, but with the harmful substances mines release even after being decommissioned (2). When sulfur-bearing minerals, which are mostly iron sulfides, are in contact with atmospheric oxygen, moisture and acidophilic iron-oxidising bacteria, sulfuric acid and dissolved iron is formed (6). The sulfuric acid leaches heavy metals present from surrounding rock to form acidic pH solutions (6). This heavy metal-laden and acidic substance infiltrates into the ground, thus polluting water bodies (7). Sulfuric acid, when diluted, readily donates two hydrogen atoms for each molecule, a process known as dissociation (8). Dissociation is a process where a molecule or compound breaks a part, and in this instance through the increased number of hydrogen atoms, causes a direct increase in acidity (9).

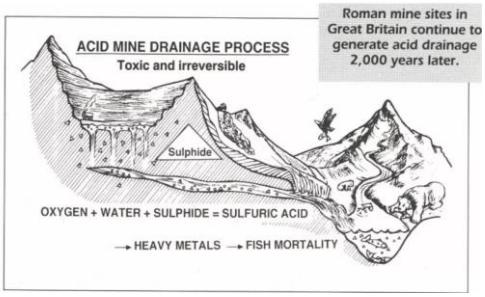

Fig. 1 A diagram showing the chemical processes involved in creating acid mine drainage and how it flows into water bodies. Image from the website Earthworks, 2023.

The formation of acid from this process results in acidic water, formally known as acid mine drainage (AMD) as shown in figure 1 (10). Its acidic quality generates changes towards the solubility of nutrients and metals, compromising the availability of crucial elements of aquatic life (10). Additionally, bioaccumulation, which explores the idea of heavy metals contaminating the tissues of aquatic life, decreases populations of animals (10). Sedimentation and erosion from the AMD can reform natural flow patterns, further impacting aquatic populations and vegetation. In regards to long-term effects, human health is jeopardised when they consume these contaminated life forms (10). Therefore, discounting the environmental effects of AMD contradicts Australia's effort to employ, "...strategic investment to support long-term development outcomes." (4). To investigate these ecological consequences, this study has researched the Captain's Flat Mine in the ACT.

Captain's Flat Mine is an inactive site which produced many metals, primarily copper, zinc, lead, silver and gold (11). It is important to note that while operations ceased in 1962 for this mine, comparably to numerous other sites, surrounding soil and water bodies can nevertheless be contaminated (11). The Molonglo River - a contributor to the water supply in Canberra and Queanbeyan, Australia - has been affected by this phenomenon (12). The Molonglo River is a freshwater inland river which flows close to the Captains Flat mine, and has thus become contaminated with three primary heavy metals including zinc, copper and lead, with heavy metals arsenic, manganese, gold, silver and cadmium having less contents but still present (11). The pH of the Molonglo River according to the ACT Water Report in 2010 - 2011 by the Australian government has reportedly been around 6.7 - 7.3 (13). But according to Professor Patrick De Deckker from the ANU Research School for Earth Sciences, there are some locations in the Molonglo river where the pH was as low as 2 (14). And while this site is under remediation, the fact that the mine is still closed today exhibits the stagnant and suboptimal quality of the contaminated water, making it ideal to experiment with treatment processes.

These treatment processes involve guidelines and specific goals established by the Australian Water Quality Guidelines for Fresh and Marine Water (1992) (15). The deterioration in the quantity and quality of Australian waters has been acknowledged by the Australian and New Zealand Environment and Conservation Council as well as the Australian Water Resources Council (15). This recognition has allowed the production of the National Water Quality Management Strategy to be implemented in 1992, in which the Australian Water Quality Guidelines for Fresh and Marine Waters would be used as a reference tool (15). In its implementation, the objective is not to observe immediate changes, but for communities to embed specific management goals tailored to individual issues (15). Additionally, mindfulness towards environmental, social and financial costs is central (15). In consideration of this, the below informed decisions were made for this study's method to abide by these guidelines.

There are two types of treatments which have been known to treat AMD - these being active and passive (16). Active treatment involves using alkaline substances such as limestone to chemically neutralise the acidity caused by AMD (16). Passive treatment involves biological

processes through constructed wetlands which involve microbial communities to treat AMD (16). This experiment would focus on passive treatment. Passive treatment, while less complex compared to active treatments, is more suitable for water systems affected long-term by abandoned mines (17). The use of passive treatment was also supported by the Australian Water Quality Guidelines for Fresh and Marine Waters, which seeks, "... long-term goals…achieved by a concerted management program." (15). Passive treatment involves chemical, biological and physical processes instead of only involving chemical ones like in active treatment (18). It can be categorised into two types: biological and geochemical types (19). Biological passive treatment stimulates microbial sulfate reduction and neutralises acidic water through absorbing heavy metals (19). An example of this is with constructed wetlands (19). Geochemical passive treatments have the AMD in direct contact with alkaline materials such as lime, limestone, marble and chalk to neutralise and filter the AMD (19). This experiment will employ a hybrid passive treatment, through the implementation of constructed wetlands and direct contact of AMD and alkaline substrates. This investigation extends on a 2022 paper titled, "Removal of Heavy Metals from Acid Mine Drainage with Lab-Scale Constructed Wetlands Filled with Oyster Shells".

Constructed wetlands (CWs) involve having cylindrical funnels with an inlet and outlet which treats wastewater (20). They have also been recognised as a potential green technology for mine drainage treatment (21). Additionally, the low cost, simple maintenance, effectiveness and high biodiversity value displays the many benefits to employing this method (21). These benefits also further conform with the Australian Water Quality Guidelines for Fresh and Marine Waters (16). To further signify the increased potential of this technology, this investigation has implemented CWs via the use of nine cylindrical separating funnels each placed on retort stands. Eight of these funnels were filled with 50 mL of one-of-two alkaline substrates to examine which could raise the pH of AMD and extract heavy metal zinc more effectively.

Another research gap stems from being unaware of how alkaline natural waste products such as oyster shells (OSs), interact with AMD in CWs. Using OSs as a substrate for treating acid mine drainage is an original way to recycle and thus limit environmental burdens and provide economic value (21). Seeing that it is an inventive and new approach, the precise effectiveness of OSs compared to less sustainable commercial calcium carbonate materials, especially ones like marble which are less utilised than limestone for AMD remediation projects, remains a standing question. Acknowledging this, this study has accumulated marble chips (MCs) and OSs to serve as the substrates which will be compared.

The research question is thus plainly presented as, "Which substrate, oyster shells or marble chips is more effective in neutralising pH and decreasing the zinc concentrations in AMD within a simple constructed wetland?"

It is hypothesised that through using a hybrid passive treatment, the sustainable OSs would be more effective than the less sustainable MCs. That is, OSs would likely bring the pH of the AMD closer to the standard set by the Australian Water Quality Guidelines for Fresh and

Marine Waters of 6.5 - 9.0 and the zinc concentrations to 0.008 mg/L (15). To preface, this treatment system works by having the calcium ions from calcium carbonate ($CaCO_3$) react with the acidic hydrogen ions from sulfuric acid (H_2SO_4) in AMD to form carbonic acid (H_2CO_3) (22). The products includes the salt - calcium sulfate ($CaSO_4$) - water (H_2O) and carbon dioxide (CO_2), a process that neutralises the acid (22) and can be visualised in the chemical equation below:

$$CaCO_3(s) + H_2SO_4(aq) \rightarrow CaSO_4(s) + H_2O(l) + CO_2(g)$$

While calcium carbonate accounts for more of the composition on MCs compared to OSs, this being 99% and up to 98% respectively, the porous quality of the OSs accounts for why it is predicted to be more effective (23,24,25). The irregular texture and more porous structure provides an increased surface area for chemical reactions (25). This allows increased contact points between the OSs and AMD, which would result in a faster reaction compared to the crystalline and compact nature of the MCs (25). It is thus also expected that the OSs would absorb more of the AMD than the MCs, and that zinc concentrations would lower with both substrates but decrease more with OSs.

If the results substantiate the hypothesis, then water bodies impacted by historic mining activity can benefit from a more sustainable substrate (i.e. OSs) being used in treatment systems. Marble demands energy-intensive quarrying and processing, which requires electric and thermal energy sources and mass detonation (26). Comparably, OSs are extracted in a more benign way through recycling the shells used after oysters are consumed (27). Therefore, raising awareness to the positive environmental impacts of OSs, which is not limited to its neutralising capability, can gradually decrease the myriad of OSs that end up in landfills each year (27). Its popularity can decrease the less sustainable commercial calcium carbonate materials used for neutralising AMD. Ultimately, it would become a forward-looking change which supports the National Water Quality Management Strategy that aims to enhance Australia’s water resources’ quality while maintaining economic and social development (15).

METHOD

Obtaining & Preparing Substrates

Since this study would employ a hybrid passive treatment with CWs, the use of substrates (i.e. OSs and MCs) would act as the independent variable. OSs were donated by the Tasty Fish Market in Belconnen and the Compa restaurant in Canberra Centre. Upon asking the former for these OSs, they immediately supplied them at no charge, while the Compa restaurant asked for a week to collect OSs at no charge again. Recycling OSs that would otherwise be discarded reflects consideration of both financial and environmental impacts. To increase the surface area of OSs, which as stated in the introduction would increase its neutralising capabilities, they were first scrubbed using the green abrasive side of a sponge. Then they were placed into a pot of boiled water and dishwashing liquid to ensure any debris would dissipate for 1 hour. A pair of dishwashing rubber gloves were worn throughout this whole process. At the 30 minute mark, the oven was set to 175 ℃. All the shells were then dried with a towel and spread evenly on a baking tray with baking paper and put in the oven for 20 minutes. Upon checking the OSs after 20 minutes, they appeared to not be brittle enough to crush, so they were placed back into the oven for another 30 minutes. After a total of 50 minutes, the OSs were taken out of the oven and at first, were all put into a metal pot. However, whilst crushing them with the wooden side of a rubber hammer, it was found that the OSs would fly out of the metal pot and create an increasingly hazardous environment even whilst wearing safety glasses. Therefore, the OSs were separated into five batches, which were individually crushed and put into a sieve. It sifted through all the very fine powders, which would have been a much smaller size compared to the MCs. The experiment aimed to use OS and MCs with similar dimensions. To continue, the OSs were then stored in a plastic bag for later use.

The marble chips were obtained from Mungga-iri Jingee (the southern, main location for the Academy of Future Skills) and were approved for order. Their dimensions were roughly 7 mm - 9 mm and grey in colour.

Pre-conditioning Substrates in CWs

On Tuesday 23rd of September, nine equally sized cylindrical separating funnels were attached at even levels to nine retort stands. Four of which were filled with 50 mL of OSs with dimensions ranging from 1 mm - 12 mm, another four were filled with 50 mL of MCs (7 mm - 9 mm). All nine batches were filled with tap water (TW) from University of Canberra Senior Secondary College - Lake Ginninderra. This step was crucial, as it allowed for the pre-conditioning and stabilisation of the substrates. Microbial communities established under these conditions due to the removal of harsh chemicals, ensuring that the substrates are hydrated and neutralised more effectively when not initially exposed to the highly acidic nature of AMD (28). The neutral pH of the TW provides a standard pH and chemical composition for each substrate, facilitating the biochemical processes and increasing their absorption ability (28).

On Wednesday 1st of October, the TW from each cylindrical separating funnel was drained using the outlet. The nine retort stands and these cylindrical separating funnels, with rubber lids covering the top of each funnel, were placed carefully into boxes and transported to the meeting room at Erindale Business Park.

Collecting AMD

On Thursday 2nd of October, at approximately 9:30, we departed Canberra for a ~1 hour trip to Captain's Flat Mine. Arriving at 10:30, we found a location at a bridge just beyond a sign stating, "Molonglo River", which was the spot indicated by the University of Canberra students which had previously done research in that area of the Molonglo River (see Fig. 2).

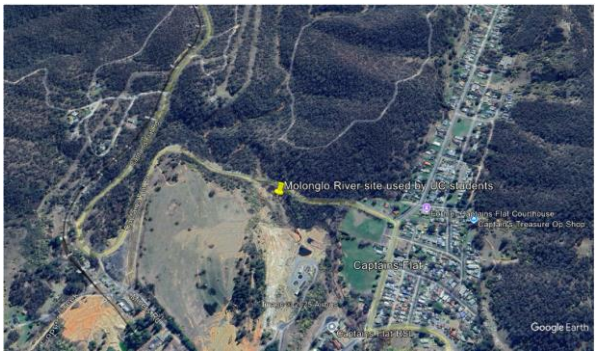

Fig. 2 Picture of the site used by UC students where the AMD was collected.

It was here that we tested the pH of a runoff close to the bridge which had surrounding rock coloured a deep orange hue and the vegetation nearby was lifeless (see Fig. 3). The pH strip was placed into this runoff for 1 - 2 seconds before being shaken. After 30 seconds, the pH value was marked to be around 5 - 6.

Fig. 3 Bui, A. (2025). Picture of the spot which has a pH of 5 - 6. In possession of the author.

While this value remains to be acidic and is under the Australian Water Quality Guidelines for Fresh and Marine Waters, which aims to have freshwater like the Molonglo River to be at a pH range of 6.5 - 9.0, we searched the area to try to find a more acidic water body. It is then that we found a pond which, after using the same pH gathering technique, was found to be at a pH level of 4 (see Fig. 4).

Fig. 4 Bui, A. (2025). Picture of the spot which has a pH of 4. In possession of the author.

It was here that eight bottles of this contaminated water, each containing roughly 1 L of the AMD were collected and stored in a bucket. We left for Erindale Business Park at around 11:00.

Operating CWs

Arriving at Erindale Business Park at around 12:30, each cylindrical separating funnel found in the boxes transported the day before, was set up at similar heights using the retort stands (see Fig. 5). They were set up from left to right in the order where the first three batches would contain OSs and AMD, the next three would contain MCs and AMD, and the last three would be the controls, the first containing OSs and TW, the next containing MCs and TW, and the last purely containing AMD.

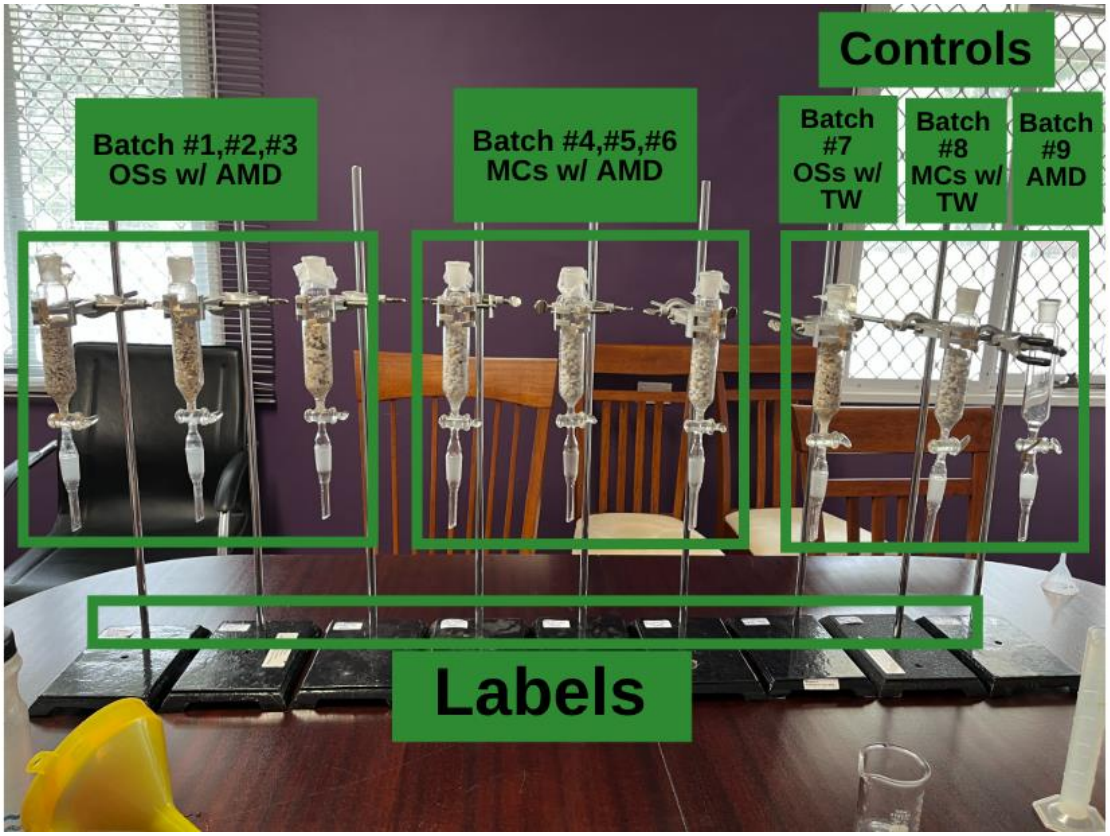

Fig. 5 Bui, A. (2025). Picture of the set up CWs with only the substrates. No AMD or TW. In possession of the author.

Each batch was poured in numerical order starting from Batch #1 and ending with Batch #9, with 40 mL of their respective liquids (AMD or TW) and labelled as shown below (see Fig. 6). By having each CWs set in the same room, the temperature of them could be controlled to ensure that if any of the liquid evaporated, they would evaporate similarly and not hinder results greatly.

a) Picture of Batch #1 OSs w/ AMD on day 0. In possession of the author.

b) Picture of Batch #2 OSs w/ AMD on day 0. In possession of the author.

c) Picture of Batch #3 OSs w/ AMD on day 0. In possession of the author.

d) Picture of Batch #4 MCs w/ AMD on day 0. In possession of the author.

e) Picture of Batch #5 MCs w/ AMD on day 0. In possession of the author.

f) Picture of Batch #6 MCs w/ AMD on day 0. In possession of the author.

g) Picture of Batch #7 OSs w/ TW right after pouring the TW. In possession of the author.

h) Picture of Batch #8 MCs w/ TW right after pouring the TW. In possession of the author.

i) Picture of Batch #9 AMD on day 0. In possession of the author.

Fig. 6 Bui, A. (2025). Pictures of the set up of all nine batches/CWs treatments on day 0. In possession of the author.

After finishing at 13:00, a meeting was then set at 15:00 on Wednesday 8th of October to collect the results.

Sampling

Arriving at Erindale Business Park at 15:00 on the specified date, it was found that Batch #6 (MCs w/ AMD) had leaked and only contained a minuscule amount of liquid left. This was taken into account when the amount of liquid absorbed by their respective substrates was calculated. The first dependent variable measured the amount of liquid left after using the outlet and a measuring cylinder. At 16:00, measurements were recorded in a sequential manner, commencing from Batch #1 and concluding with Batch #9. This method was implemented to warranty that each batch underwent an equivalent duration of substrate liquid absorption, which would be ~147 hours or ~6.125 days.

After placing the measuring cylinder underneath each outlet, the amount which flowed out was recorded. After which, this value was subtracted from the initial volume of AMD or TW used, which was 40 mL for every batch, to determine the volume absorbed by the substrate. To determine the percentage absorbed, this value was multiplied by 2.5, seeing that the product of 2.5 and 40 is 100 (see equation below):

$$\text{Absorption (\%)} = 2.5\ (40 - \text{outlet liquid volume (mL)})$$

Per the instructions of the Quantofix zinc test kit, 5 mL of each liquid, again going in sequential order, was poured into a cylindrical vial with 10 drops of the reagent. This was gently swirled with the hands, after which a test strip from the kit was placed into the solution for 1 - 2 seconds. It was then shaken with the hand before leaving to dry for exactly 30 seconds. Its colour was compared with the colour comparison chart on the side of the container with zinc test strips. A picture was taken of this. This solution was poured into the sink and the vial was rinsed with lukewarm tap water and dried with a paper towel for it to be used eight more times. The liquid from the measuring cylinder was put into a small plastic container also labeled with the appropriate batch number and title (i.e. Batch #7 OSs w/ TW).

Similarly, the pH strips were dipped into the pure liquid solution in this small plastic container (not the one containing the reagent) for 1 - 2 seconds before being shaken and leaving to dry for 30 seconds. A photo was also taken of the colour compared to the colour comparison chart on the side of the packaging. This whole process was repeated eight times for every batch. Timing the amount of time each test strip got to dry was a controlled variable essential for maintaining the integrity and credibility of results, because leaving them to dry for longer will change the hue of the pigment.

The following is a summary of the preprocessing steps applied prior to analysis. Three combo charts were produced using the original pH, original zinc concentration (mg/L), the percentage of liquid absorbed by each substrate, resulting pH and resulting zinc concentration (mg/L) (see Fig. 7 - 9). The initial volume of AMD or TW (40 mL) was equal across all CWs and thus excluded from these charts for clarity. Initially when this data was added, there was a noticeable disparity between the location of this point and all other data. Using a logarithmic scale to minimise this gap led to miniscule values not being visible, since logarithmic scales never reach zero. That is why the initial volume of AMD or TW was excluded from the graphs and linear scales were used.

The equation referenced above was used to calculate the percentage of liquid absorbed by each substrate. Furthermore, all the columns in the chart were clearly labelled for coherency and readability with average values. That is, when any test strip produced a colour equally similar to two of the boxes in the colour comparison chart, the average values were shown in the chart.

Figure 7,8 and 9 contained columns including the percentage of liquid absorbed OSs (blue), resulting pH (orange) and resulting zinc concentration (mg/L) (dark green). There are also two lines which are used to compare changes to the original pH (pink) and the original zinc concentration (mg/L) (light green). The right y-axis represents the percentage of liquid absorbed OSs, while the left y-axis displays all other measured values.

In figure 8, one column is marked as N/A because Batch #6 had leaked, which means the percentage of liquid absorbed by MCs would have been misleading.

In figure 9, one column is marked as N/A because Batch #9 had decreased its volume by 3.75% despite containing purely AMD with no substrates.

Risk Assessment

Risk	Level of risk	Control measures	PPE Required
AMD contacts body	**Medium**	**Follow close instructions, handle AMD carefully**	**Funnels, adult supervision**
Shards of oyster shells hits eye	**High**	**Use safe eye wear and crush oyster shells in small batches to avoid injuries**	**Gloves, glasses**
Glassware (i.e. cylindrical separating funnels, vials, beakers) breaking and causing injury	**Low**	**Handle glassware carefully, ensuring that the retort stands have a firm grasp on every cylindrical separating funnel**	**None**
AMD contaminating objects	**Medium**	**Any surfaces in contact with AMD will be thoroughly rinsed or wiped down**	**Water**

Ethical Considerations

This investigation adhered to ethical guidelines ensuring responsible behaviour prioritising safety and scientific probity. The oyster shells, marble chips and acid mine drainage were ethically sourced. No form of trespassing occurred at the Molonglo River near Captain's flat when testing for pH. All materials were handled with care adhering to the risk assessment, with appropriate personal protective equipment being worn when suitable.

The Tasty Fish Market and Compa restaurant were asked politely and professionally on whether or not they could donate oyster shells. Schedules to pick up oyster shells at the Compa restaurant adhered to their arrangement and any amount was accepted without complaints. Sourcing these substrates involved a parent being present at all times. The purchase of marble chips was confirmed by an authoritarian figure. Furthermore, the acid mine drainage was collected in areas confirmed by Professor Patrick De Deckker from ANU Research School of Earth Sciences to have sites previously for research purposes used by students.

No data was manipulated, and anomalies were discussed transparently. It was made clear when many interpretations were provided for unforeseen data and when explanations were inconclusive. References were made to credit original authors and uphold scholarly practice using the IEEE style of referencing.

RESULTS

The experiment measured the change in pH, zinc concentration (mg/L) and the absorption abilities across nine batches or constructed wetlands (CWs) with three containing oyster shells (OSs) and acid mine drainage (AMD), three containing marble chips (MCs) and AMD, and three controls - one with OSs and tap water (TW), one with MCs and TW and one with only AMD.

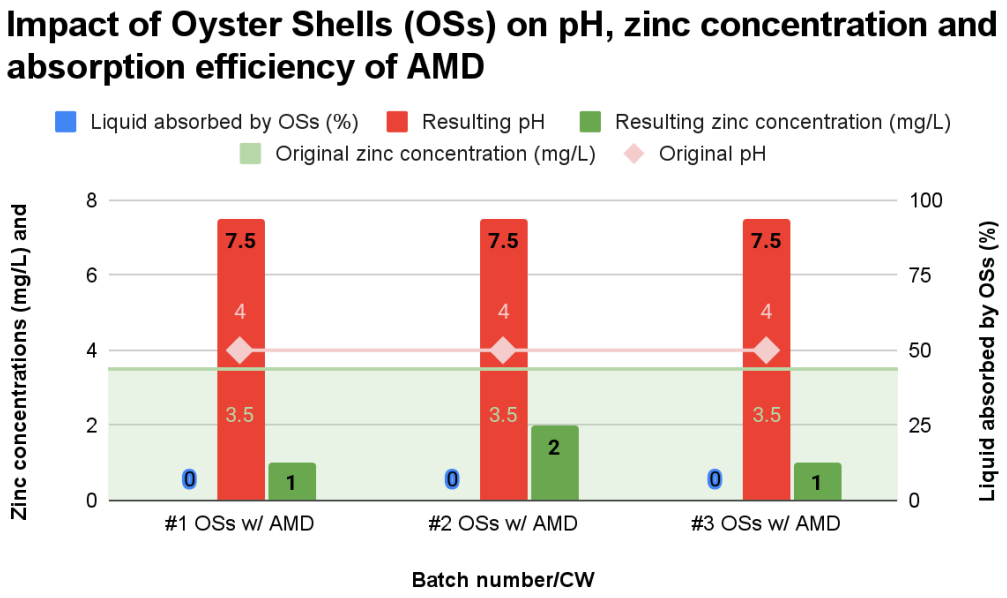

Fig. 7 Impact of oyster shells (OSs) on pH, zinc concentration and absorption efficiency of AMD.

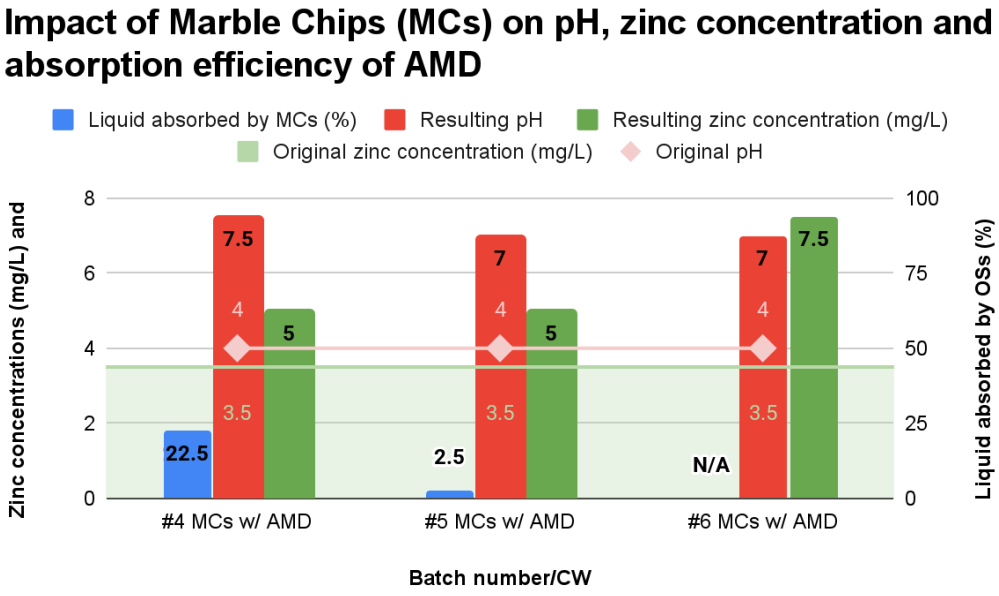

Fig. 8 Impact of marble chips (MCs) on pH, zinc concentration and absorption efficiency of AMD.

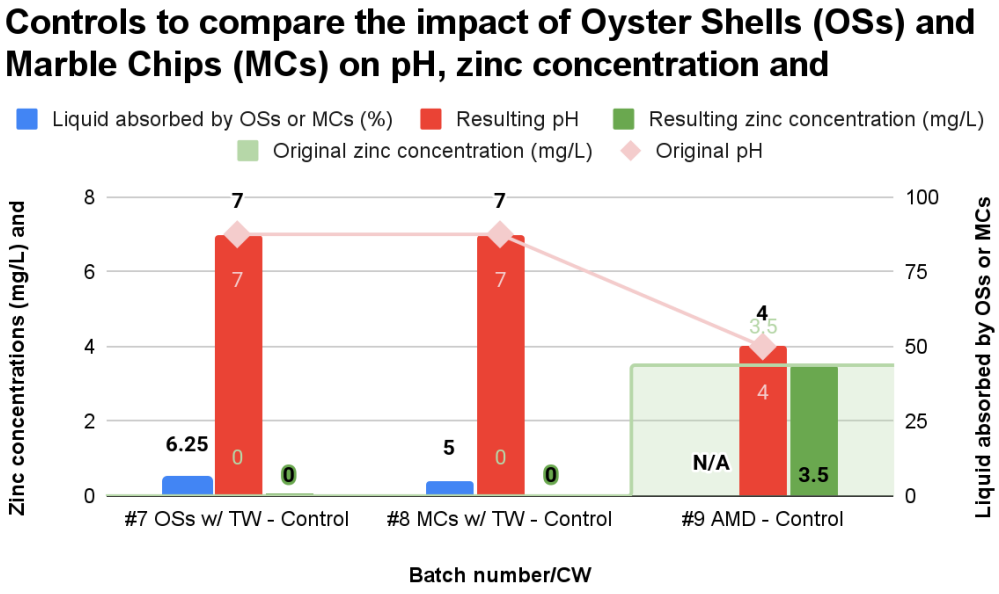

Fig. 9 Controls to compare the impact of oyster shells (OSs) and marble chips (MCs) on pH, zinc concentration and absorption efficiency of AMD.

Fig. 7 exhibits the consistency between every batch containing OSs and AMD. The resulting pH was averaged at all three batches to be 7.5, showing that from the original pH of 4,

the AMDs pH increased by 3.5. The original zinc concentration (mg/L) was at 3.5, and batches #1, #2 and #3 were all able to decrease this value to 1, 2 and 1 respectively. It was surprising to find that whilst all three batches were able to efficiently neutralise the AMD and extract the heavy metal zinc, the percentage of liquid absorbed was calculated as 0%. This means that the volume of 40 mL of AMD initially poured into these CWs remained when drained.

Fig. 8 displays the varied results involved with batches #4, #5 and #6 - which contained 50 mL of MCs and 40 mL of AMD. Batch #4 was able to increase the pH of AMD to 7.5, which was again, 3.5 more than the original pH. Batch #5 and #6 both increased the pH to 7. Unexpectedly, the MCs from Batch #4 had absorbed 22.5% of the AMD, the highest percentage across all nine batches and 16.25 more than the second highest. However, resulting zinc concentration was recorded as 5, higher than the original zinc concentration. Interestingly, Batch #5 also has a resulting zinc concentration of 5, yet its MCs only absorbed 2.5% of the 40 mL of AMD. The surprising increase from the original zinc concentration was emphasised as Batch #6 had a resulting zinc concentration of 7.5.

Fig. 9 presents the data for the controls. Batch #7 and #8 had similar results, with the TWs pH remaining at a neutral 7, and the zinc concentrations staying at 0. The percentage of absorption of the OSs from Batch #7 and the MCs from Batch #8 were 6.25% and 5% respectively. Seeing that the zinc test kit utilised Batch #9 to test for the original level of zinc, it is plausible that the zinc concentration for Batch #9 averaged at 3.5 (the original zinc concentration). Predictably, the pH level for this batch remained at 4.

DISCUSSION

Purely from these data from figures 7 - 9, it can be concluded that OSs created a slightly more effective CW to neutralise the AMD and decrease zinc concentrations. Fig. 8 shows that rather than decreasing the zinc concentration, the MCs increased heavy metal concentrations. Therefore, OSs was the only substrate able to decrease the zinc concentration and increase the pH at a fairly consistent level.

Interpretations

The hypothesis proposed that implementing a hybrid passive treatment would enable OSs to adjust the pH of the AMD to the standard set by the Australian Water Quality Guidelines for Fresh and Marine Waters of 6.5 - 9.0 (15). It is also expected to reduce zinc concentrations to 0.008 mg/L more effectively than MCs (15). The hypothesis was largely upheld by results; however, certain anomalies persist and will be further addressed. The results support the research on the rationale for OSs being effective compared to MCs. The irregular texture and higher porosity of OSs increases the surface area accessible for chemical reactions (25). This allows increased contact points between the OSs and AMD, resulting in a faster reaction compared to the crystalline and compact nature of the MCs (25). The frequency of collisions between reactants is decreased when they occur mostly on the surface of MCs, rather than in the interior (25). This quality of the OSs outweighs the fact that MCs contain more calcium carbonate - reacting with the acidic hydrogen ions from sulfuric acid (H_2SO_4) in AMD to neutralise the AMD (22).

An anomaly is presented when looking at figure 8, encompassing batches #4, #5 and #6 containing MCs with AMD. The resulting zinc concentration for batches #4, #5 and #6 were 5 mg/L, 5 mg/L and 7.5 mg/L respectively. These values average at approximately 5.83 mg/L, 2.3 mg higher than the original zinc concentration. Seeing that the values were consistently above the original zinc concentration, it is unlikely that those findings were through human error. Rather, this result could be due to the MCs containing heavy metals such as aluminium, arsenic, cadmium, chromium, lead, mercury, cobalt and nickel (29). In a 2024 research paper, they stated that the high accumulation of heavy metals contaminated within marble waste impedes on the structure and distribution of microbial communities (29). In this research, these microbial communities are established in agroecosystems - defined as a human-managed agricultural system where plants/animals interact with modified physical and chemical environments (30). This definition is almost adjacent to that of the constructed wetlands used in this study, which to reiterate, is a human-managed agricultural system where substrates treat contaminated water bodies via producing microbial communities (31). Therefore, it can be concluded that contaminated marble may affect the microbial communities within constructed wetlands to increase AMDs zinc concentrations.

The MCs used in this study could have been contaminated to cause a consistent increase in the zinc concentrations. This could have occurred during the quarrying and refining

process, which releases 40% of marble waste into the atmosphere (29). This waste could have potentially aero-transmitted into the extracted MCs used for this experiment (32). Thus, the changed chemical processing in the hybrid passive treatment, may inhibit increased zinc concentrations.

Another explanation to the zinc concentrations in batches #4, #5 and #6 increasing, pertains to the test method. The Quantofix zinc test kit is a dithizone test, where zinc ions react with a dithizone reagent to fashion a coloured zinc-dithizone complex (33). The dithizone can additionally react to other heavy metals including lead, cadmium and copper to produce inaccurate results (33). It is thus necessary to remove these interfering ions from the contaminated water before precisely determining zinc concentrations (33). As this consideration was not employed, the heavy metals within MCs could have caused the zinc concentrations to appear higher.

Both explanations to the anomaly in figure 8 do not overlook the fact that OSs also contain heavy metals (34). However, the reaction between each substrate, the AMD, and test strips differ for OSs and MCs. The specific chemistry, especially between the substrates and test strips, was not scrutinised in this investigation. Thus a conclusive elucidation could not be determined for the surprising increase in zinc concentrations in batches #4, #5 and #6.

Despite OSs averaging a higher resulting pH and a lower zinc concentration, the batches containing MCs were able to absorb a higher percentage of the AMD. That is, batches containing OSs and MCs averaged to absorb 0% and 12.5% respectively. This was another anomaly, as it was hypothesised that the amount of AMD absorbed would be higher if the resulting pH was higher and the resulting zinc concentration was lower. The results, however, observe these two values to not correlate. Additionally, the porous texture of the OSs was thought to be more absorbent than the MCs. The reason for this anomaly is likely due to human error. This is because Batch #9, which contained purely AMD and no other substrate, carried 3.75% less AMD on day 6 compared to day 0 (see Fig. 7). It is unknown whether this amount had leaked, or evaporated, but seeing that all batches were placed in the same room, and that batches #1, #2 and #3 contained the same amount of AMD on day 0 and day 6, the former seems more likely.

The two other controls - Batch #7 containing OSs and TW and Batch #8 containing MCs and TW - demonstrated similar and predictable results. The original pH and resulting pH equalling 7 for both batches is explained by the fact that TW does not react with the calcium carbonate in OSs and MCs (35). Calcium carbonate is nearly insoluble in TW (35). It is compelling that the OSs from Batch #7 had absorbed 6.25% of the TW, compared to category one, which absorbed 0% across all its batches. This is likely explained through human error. Interestingly though, this difference is likely not because of Batch #7 containing OSs with a higher surface area. As shown in Fig. 6 g), the OSs, especially those at the top of the cylindrical

separating funnel, are much larger compared to any OSs in Fig 6 a), b) and c). Therefore, it is most likely that Batch #7 had leaked.

Relevance to society

The research gap encompassing exactly how alkaline natural waste products such as OSs interact with AMD in a hybrid passive treatment is further elucidated through the findings of this investigation. OSs being increasingly used in future remediation projects involving AMD is substantiated by its original way to recycle and thus limit environmental burdens and provide economic value (21). Employing CWs using foundations similar to that used in this investigation also includes many benefits such as the low cost, simple maintenance, effectiveness and high biodiversity value (21). These benefits also further conform with the Australian Water Quality Guidelines for Fresh and Marine Waters - which establishes pH values and measures for a variety of heavy metals (16). These quantities should be the standard in every Australian water resource (16).

The pursuit for sustained improvement including water use, biodiversity conservation and mine rehabilitation is emphasised in this study (4). AMD generates changes towards the solubility of nutrients and metals, having compromised the availability of crucial elements of aquatic life for thousands of years (10). In fact, AMD can reform natural flow patterns, further affecting aquatic ecosystems and vegetation, and over long-term periods, human health may be at risk when they consume these contaminated organisms (10). The universal need for sustainable substrates in rehabilitation for AMD whilst considering financial, social and environmental costs is highly asserted.

OSs can serve this greater purpose rather than being disposed of. This method provides consistent treatment in the span of six days and is sustainable in its recycling properties. Additionally, implementing the Australian Water Quality Guidelines for Fresh and Marine Waters as a reference tool, would aid communities to embed specific management goals tailored to individual issues (15). Within the mining industry, remediation projects can take place using OSs to most effectively treat water bodies, allowing vegetation to flourish and aquatic life to be adequately nourished. Additional water resources can be used to progressively supply clean water to a variety of communities. Overall, the favourable benefits of OSs as a substrate in treating AMD appeals to Australia’s National Water Quality Management Strategy and can become universally applicable.

Limitations and suggestions for future studies

It is recommended that studies using a hybrid passive treatment system with a similar set up consistently check to see the nature of their materials. Whether or not the CWs can leak should be known, perhaps via a time-lapse recording the CWs across the days the treatment is in place. Additionally, due to the varying and busy schedules of participants of this study, the experiment was limited to six days, whereas the paper which underpinned this experiment had a

treatment period of seven months. That paper contained few unexpected results while this study presented significant anomalies, showing how acid mine drainage remediation is more effective when extended to long periods.

The increased precision of test kits is also another recommendation. The Quantofix zinc test kit used in this study had a colour comparison chart which was hard to distinguish. This chart showed large values in increasing intervals, meaning it was especially hard to discern how much the zinc concentration was for every batch. Therefore, comparing these zinc concentrations to the Australian Water Quality Guidelines for Freshwater, which stated that 0.008 mg/L of zinc was safe, was less accurate. However, due to the budget of this project, purchasing extremely precise test strips was not cost-effective. It is recommended that studies with higher funding, purchase zinc test strips which start from 0 and increase at small and even intervals. Additionally, removing heavy metals before using a dithizone test or using another test method is recommended for accurate results. The most precise method would be with an inductively coupled plasma (ICP) spectrometry which uses a hot argon plasma to detect and measure heavy metals (36). This tool is very expensive, priced anywhere from $50,000 - $500,000 and should thus be reserved for heavily endowed investigations (37).

Another suggestion would be to do with the substrates (i.e. OSs and MCs). Heavily researching the sources for OSs and MCs would greatly decrease the chance of unknown substances reacting with AMD. Anomalies would be more conclusively justified. Future studies could heavily focus their research on MCs, since they are such an uncommon commercial calcium carbonate material. They could concentrate on finding the most effective methods for reducing the amount of marble being lost during their quarrying and refining process. This marble can be recycled for other purposes, such as agriculture and chemical applications, because whilst marble is less sustainable than OSs, they are still 100% recyclable. The only caveat is with the polluting process of extracting and transporting this metamorphic rock. Therefore, further research could also be dedicated to discovering environmentally conscious methods of hauling and transferring marble. Afterall, this investigation broadly speaking seeks to find conservation-minded approaches towards direct and indirect environmental threats within the mining industry. Thus, this study does not limit its suggestions to be purely towards how OSs act in AMD, because that addresses only a minor fraction of an industry with a substantial environmental footprint.

CONCLUSION

In conclusion, the use of oyster shells as a substrate in a hybrid passive treatment proved to be more effective and efficient than marble chips. The inconclusive anomalies related to the increase in zinc concentrations using MCs identifies avenues for future experimentation. The auspicious, consistent and predictable results with the OSs, accomplishes the aim to find a more sustainable calcium carbonate for AMD treatment. OSs can be increasingly used for remediation projects of similar methods discussed in this investigation within mining industries. Ultimately, it supports the National Water Quality Management Strategy endeavouring to increase the quality of Australia's water resources while prolonging economic and social progression. Further applications of provided suggestions can mark the transition of environmental change from a regional to a global scale.

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