Design Brief

Bioremediation of metal-contaminated water using genetically modified *Escherichia coli*[*]

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In regions across the developing world, heavy metal contamination in drinking water poses a tremendous threat to under-monitored public health systems. While conventional remediation technologies exist, they remain costly and are restricted to certain communities. This project aims to utilize efficient and cost-effective technology to resolve the rampant issue of heavy metal water pollution in developing countries. We attempt to bioremediate water with particular attention to copper and mercury due to their prevalence in water bodies worldwide. In our lab, we plan to use the gene copA in tandem with copC and copD to allow Escherichia coli (E. coli) DH5-Alpha (DH5α) to diffuse extracellular Cu2+ ions into the cytoplasm. copA functions as a copper resistance gene, which provides increased defense against the toxic, heavy copper influx, while copC and copD code for metallochaperones and sequestration proteins. This strain will be grown on an agar plate, and then colonies will be placed in contaminated water. Once the E. coli takes in the Cu2+ ions, microfiltration techniques supported by material with smaller pore sizes will filter out E. coli and, thus, the heavy metals with it. Lambert GA-2 will use a colorimetric assay reliant on bathocuproine before and after to measure relative effectiveness.

Keywords: *CopA, CopC, CopD*, bioremediation, copper, pCusC promoter

Heavy metal contamination in water sources, particularly groundwater and surface water, has several harmful impacts on the human community (see fig. 1). The growth of urban landscapes, industrial development, and chemical fertilizer use in agriculture has resulted in an uptake of toxic metal contaminants in aquatic ecosystems through wastewater, drainage networks, and runoff management systems (Zhang et. al., 2023). Around 14-17% of the world's croplands have been recorded to be contaminated with heavy metals (Maitra, 2022). The heavy metal contamination present in the water decreases the quality of the water. Approximately 3,800 to 4,000 homes on St. Croix, U.S. Virgin Islands, experienced lead and copper contamination in their tap water, leading to a public health state of emergency (The Virgin Islands Consortium). Having relatively minimal sources of freshwater, these islands rely on purified rain and groundwater for agriculture, drinking, and cooking purposes. Developing a solution to efficient purification and bioremediation of water helps prevent public health crises in areas such as the Virgin Islands that lack a stable source of freshwater.

[*] *The authors were mentored by Catherine Sharer from Lambert High School and Dr. Meghdad from California State University. Please direct correspondence to: {Email Addresses}. This is an Open Access article, which was copyrighted by the authors and published by BioTreks in 2025. It is distributed under the terms of the* Creative Commons Attribution License*, which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited.*

Without sufficient purification, metal-contaminated water poses a severe threat to human health by inducing kidney damage and liver failure. Heavy metals can also result in carcinogenic effects (Guardian News and Media). Copper contamination of water is most prevalent and results in the aforementioned problems. To try and combat the effects, the EPA has set the action level for copper in drinking water at 1.3 parts per

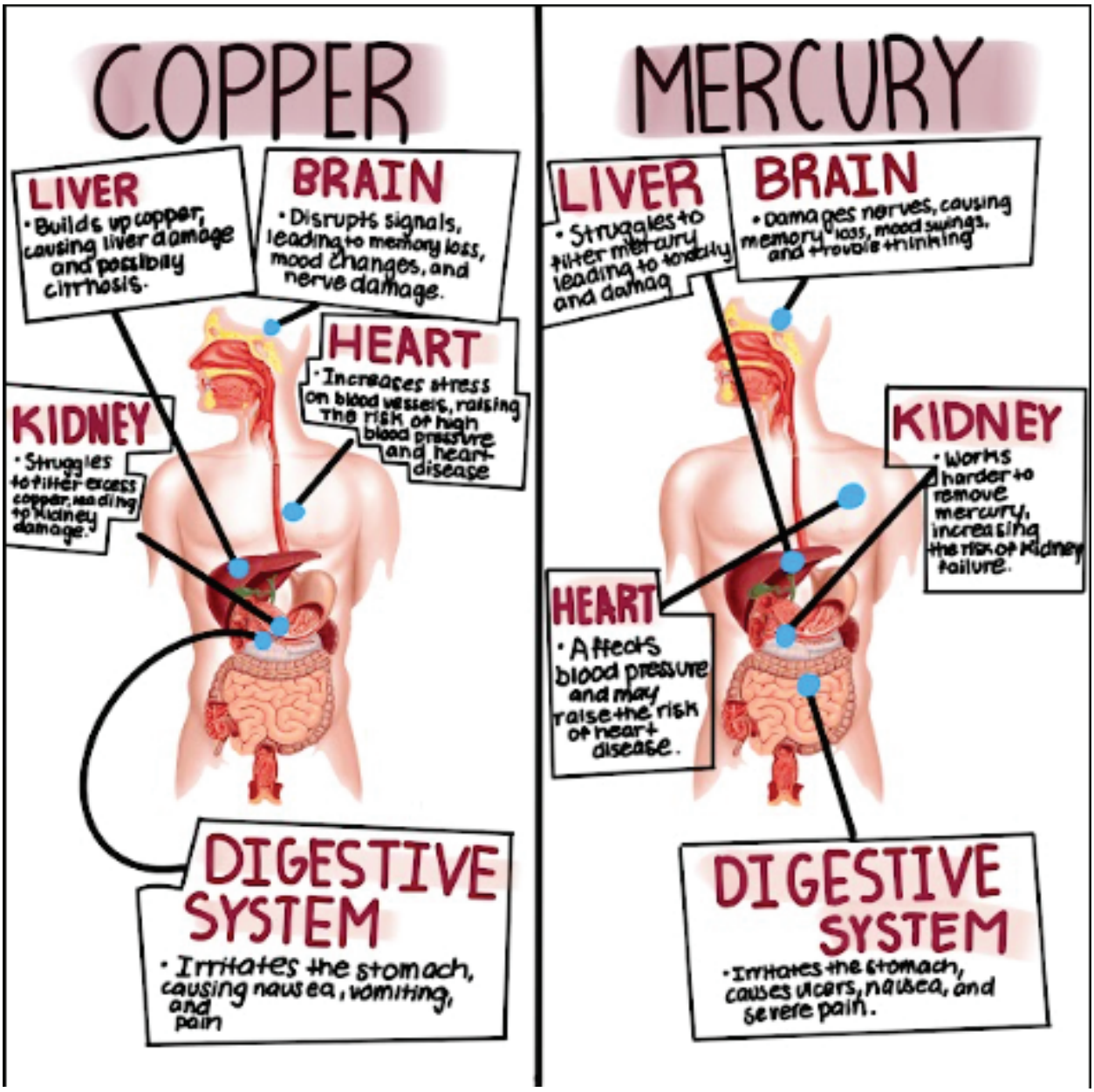


Figure 1. Diagram comparing the effects of elevated copper (left) and mercury (right) levels on major organ systems. This diagram illustrates the impact of elevated copper and mercury levels on key organ systems. It is divided into two bodies to visually compare the effects of copper (left) and mercury (right). Text boxes pointing to key organs are drawn to provide information relevant to the corresponding organ. Both metals disrupt brain function, leading to memory loss, mood changes, and nerve damage. The liver and kidneys struggle to filter these toxins, resulting in organ stress and potential failure. Cardiovascular health is affected through increased blood pressure and heightened risk of heart disease. Additionally, both metals irritate the digestive system, causing symptoms such as nausea, vomiting, and abdominal pain. These effects highlight the urgency of effective bioremediation strategies.

million (ppm). If more than 10% of tap water samples exceed this level, water systems are required to take corrective actions ("About Lead and Copper").

Systems level

This engineered system is designed to support the use of genetically modified bacteria in the removal of heavy metals from water, offering an environmentally sustainable and cost-effective solution. The genes, naturally occurring in bacteria, central to this project are *copC, copD* (Lawton et al., 2016). The strain of *Escherichia coli* DH5α acts as a chassis for these genes to create a genetic circuit. DH5α is chosen for its widely recorded use in experimentation and high transformation efficiency (ThermoFisher). This circuit enables the strain to internalize copper ions, facilitate bioremediation efforts, and detoxify aquatic environments (Lawton et al., 2016).

A thorough understanding of *CopA's* function, in its native context, is essential for optimizing its application in engineered systems for environmental remediation (Alquethamy et al., 2019). The gene is typically involved in the copper homeostasis of alphaproteobacteria, acting as a gene encoding a regulatory mechanism of the system. The *CopC* and *CopD* genes allow the genetically engineered system to produce proteins that allow for the binding and transport of copper ions into the cytoplasm (Lawton et al., 2016). By improving the bacteria's ability to survive and function in copper-contaminated environments, *CopA* plays a central role in supporting our system's effectiveness in bioremediation applications (Padilla-Benavides et al., 2014). The *CopA* gene serves to enhance copper resistance in heavy metal environments, reducing oxidative stress by sequestering Cu (I) in the periplasm. After the *E. coli* has bound to the metals in a solution, the solution will be run through microfiltration material with pores of 0.1 micrometers. The *E. coli* and its bound metals will be left at the top of the now filtered water.

Device level

The Cop gene family codes for creating copper-binding proteins, allowing for increased longevity of the modified system in question. *CopC* has binding sites for Cu(II) and regulates homeostasis response to high copper toxicity (Lawton et al., 2016). The protein's structure allows for high-affinity binding to Cu(II) ions, preventing it from exerting its toxic effects on the intracellular environment. Incorporating *CopC* allows the engineered strain to sequester elevated concentrations of copper (Lawton et al., 2016). *CopD*, on the other hand, serves as an inner membrane protein to transport copper as needed into the cytoplasm (Lawton et al., 2016). Working in tandem, *CopC* binds to free-floating copper in the periplasm, sequestering it until *CopD* requires it, while *CopD* transports it to the cytoplasm to be utilized by the cell for protein assembly and other metabolic functions (Lawton et al., 2016). Alongside *CopC*, *CopA* also aids in copper homeostasis. *CopA* is a membrane-bound P-type ATPase that exports monovalent copper ions (Cu^{+}) from the bacterial cytoplasm to the periplasmic space, helping maintain metal homeostasis and prevent toxicity by acting as a periplasmic multicopper oxidase. *CopD* and *CopA* work in tandem to regulate intracellular copper levels, maintaining an appropriate level of Copper in the periplasm and cytoplasm (Lawton et al., 2016). This oxidation step significantly reduces copper-induced oxidative stress and complements the roles of *CopA* and *CopD* in regulating copper levels (Lawton et al., 2016). *CopD* and *CopA* work in tandem to regulate intracellular copper levels, maintaining an appropriate level of copper in the periplasm and cytoplasm.*CopA* can act as a periplasmic copper oxidase that binds intracellular Cu^{+} through conserved metal-binding domains and uses energy from ATP hydrolysis to undergo conformational changes that actively transport the ions across the inner membrane into the bacteria (Lawton et al., 2016). This mechanism is crucial for detoxification, as excess intracellular copper can generate reactive oxygen species, damage iron-sulfur clusters, and disrupt essential proteins. (Lawton et al., 2016) The

plasmid containing the target genes will utilize the pCus promoter to regulate the level of expression of the genes. pCusc is a copper-responsive promoter that upregulates *CopC* and *CopD* genes in higher concentrations of copper, allowing a greater amount of *CopC* and *CopD* proteins to be transcribed as a response towards maintaining homeostasis. This dynamic regulation ensures that the E. coli strain minimizes overproduction of the aforementioned proteins, helping save intracellular resources and energy.

Parts level

Gene modification can be performed using recombinant DNA to achieve an engineered copper-absorption system. The genes *CopC*, *CopD*, and *CopA* can be cloned and added to a plasmid, which was then administered to the *E. coli* using the heat-shock transformation method. This technique relies on making the bacterial cells chemically competent, typically by treatment with calcium chloride ($CaCl_2$), which neutralizes the negative charges on both the plasmid DNA and the bacterial cell membrane, facilitating close associations between them.

During the transformation procedure, the plasmid DNA is mixed with chemically competent DH5α cells and incubated on ice to stabilize the cell membrane. A rapid increase in temperature occurs through a hot water bath, commonly at 42°C, and the plasmid DNA alongside the DH5α cells are left in it for about 30 to 60 seconds (Kaplan et al, 2013). This heat shock induces a transient increase in membrane fluidity and permeability, leading to the formation of pores that enable the plasmid to enter the bacteria. The cells are immediately returned to ice after the 30-60 second heat shock to stabilize the microtears in the membrane and allow recovery. Subsequently, the cells are incubated in a non-selective growth medium to grow mature colonies of transformed DH5α.

The plasmid used typically has a marker such as an antibiotic resistance gene to ensure stable maintenance within DH5α, such as Ampicillin resistance (Lawton et al., 2016). These features make DH5α a preferred host for plasmid propagation and genetic manipulation. Heat shock transformation into DH5α thus provides an efficient means of incorporating plasmids containing genes like *CopC*, *CopD*, and *CopA* to genetically engineer the bacteria for enhanced copper uptake and bioremediation applications.

Safety

To ensure the safety of our project, we are testing the genetically engineered *E. coli* (DH5α strain) under strict laboratory containment conditions such as PPE (Personal Protective Equipment), waste disposal, and environmental safety to prevent the release of GMOs into the environment. To further ensure the safety of our testing, the inserted genes *CopC*, *CopD*, and *CopA* are non-toxic and function only in the presence of copper, minimizing environmental risks (Mah et al., 2001). All testing is done without pathogenic organisms and uses established biosafety procedures like recombinant plasmid insertion via heat shock and controlled copper exposure. We aim to prevent all possible harm to the developers and the environment by using a non-virulent *E. coli* strain, preventing bacterial release outside controlled environments, and following biosafety level 1 (BSL-1). This system contributes to eco-friendly copper cleanup while prioritizing and ensuring bacterial containment and safe genetic engineering procedures.

Discussions

Heavy metal contamination in water systems has recently evolved into a widespread global issue. This global call to remediate bodies of water created the need for accessible and affordable technology that can be implemented in financially constrained regions. This challenges the ethicality and viability of possibly implementing this project on a global scale. Another limitation that exists is the requirement for further research and experimentation beyond the scope of the laboratory setup accessible to students. Experimentation using samples such as mercury could yield crucial information for our project due to its

abundance in local water sources. Mercury is more prevalent in water bodies in proximity to the experimentation site; thus, the project is more easily scaled when dealing with mercury, as there are water bodies present for large-scale implementation. However, it poses significant safety risks that exceed our laboratory's intended scope and safety capacity.

Despite promising advances in the synthetic biology field, various challenges and limitations remain prevalent when considering modern-day heavy metal bioremediation. Heavy metal contamination is a complex issue, involving various challenges when aiming to bioremediate water bodies. Issues include the dependency of bioremediation systems on detecting limited to single/ few metals at a time, when the source of heavy metal contamination has typically been recorded to consist of several mixtures of metals (Thai, Lim, & Na, 2023). This can result in differing concentrations, which could, as a result, also interfere with the specificity and efficiency of synthetically engineered biological responses. This results in bioremediation becoming dependent on certain environmental conditions such as pH and temperature. These conditions significantly affect the stability and activity

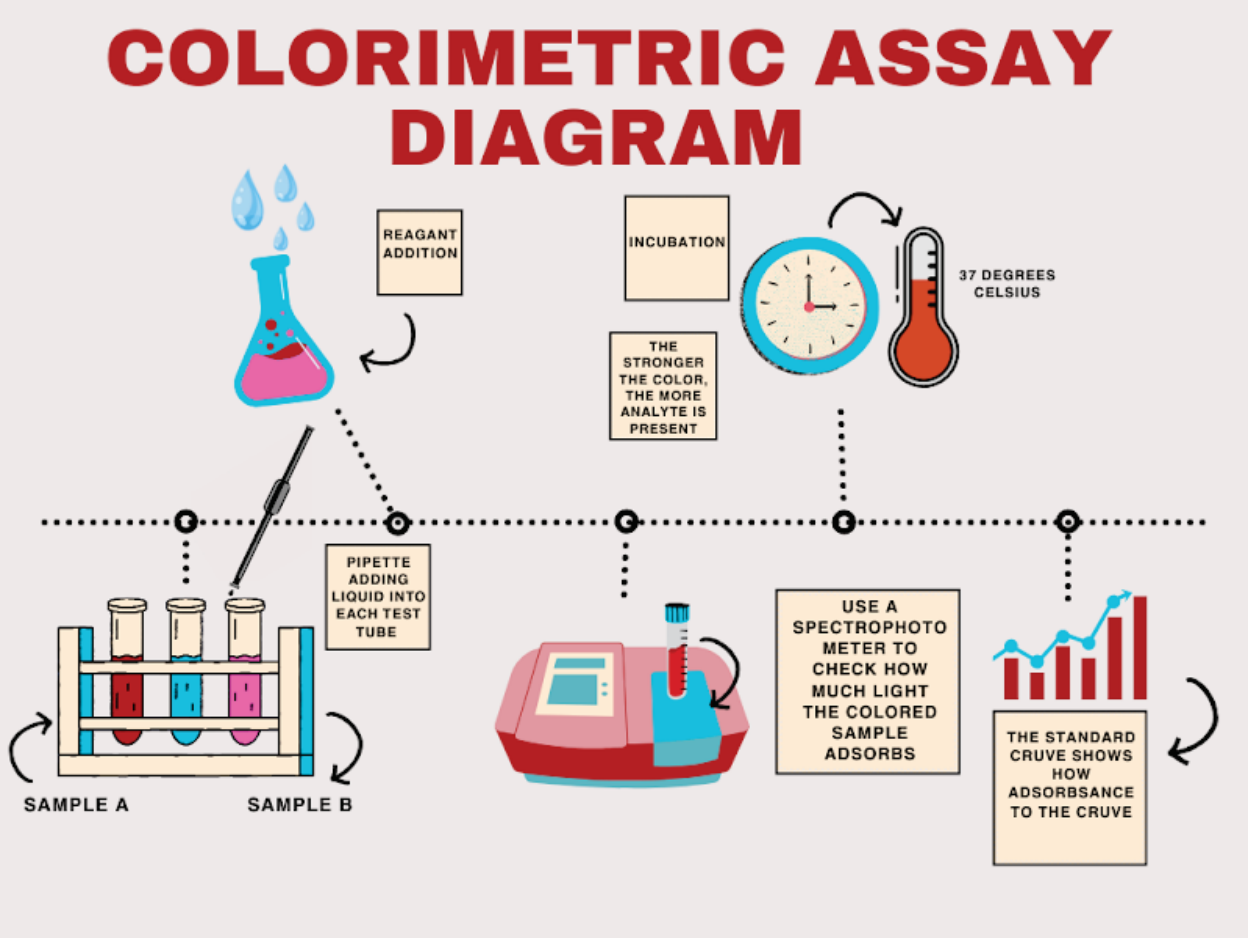


Figure 2. Step-by-step workflow of the bathocuproine-based colorimetric assay for copper detection. This diagram presents a step-by-step representation of a colorimetric assay: a method used to quantify substances based on color intensity. The process begins with adding samples to test tubes, followed by reagent addition. After mixing, the samples are incubated at 37℃ to allow the reaction to develop. The resulting color change indicates the presence and concentration of the analyte - the stronger the color, the more analyte is present. A spectrophotometer then measures how much light each sample absorbs. This data is compared against a standard curve, which relates absorbance to known concentrations. This assay provides a simple, rapid, and visual method to assess bacterial heavy metal uptake, demonstrating the effectiveness of engineered microbes for sustainable water purification.

of bioremediation systems, as the prime pH levels for most bacteria range between 5.5 and 6.5, as well as the optimum temperatures ranging from 25°C to 35°C (MDPI, 2025). The presence of multiple toxic ions can also interfere with or inhibit the transport and binding functions of metal-specific proteins used such as *CopC* or MerP (Hamlett et al., 1992).

Next steps

We will ensure the successful transformation of the genetically modified system through the usage of three trials, a fixed DNA to chemically competent *E. coli* ratio, and the arrangement of experimentation under optimized conditions for DH5α transformation. Then, a colorimetric assay containing Bathocuproine will be used to test the experimental accuracy of our *E. coli*. Bathocuproine is a chemical compound that can form a complex with copper ions. In colorimetric assays (Figure 2), bathocuproine is used to detect copper levels. The bathocuproine forms a complex with Cu(II) ions, producing a color change that can be quantified using a spectrophotometer. The intensity of the color is proportional to the concentration of copper, allowing for its measurement in biological or environmental samples.

Our next objective is to expand the current copper-focused system to include the remediation of mercury through additional recombinant DNA work. Using the same transformation techniques (Figure 3) applied in our copper system, we plan to clone MerP and MerT into a new plasmid for a new DH5 *E. coli* colony.

Once transformed, the modified strain will express MerP, a periplasmic protein that binds mercury ions, and MerT, an inner membrane transporter that facilitates the movement of Hg^{2+} into the cytoplasm. Together, these proteins function to enhance mercury uptake and resistance within the cell. Utilized in tandem with our original proposed solution, the two plasmids can control for both mercury and copper concentrations. Prior research indicates that both genes are necessary for efficient mercury detoxification, with MerP significantly increasing periplasmic capture and delivery efficiency, and MerT enabling internalization across the membrane (Hamlett et al., 1992). Once the genes are expressed, we will test the modified strain's ability to remove mercury from a simulated water sample using a colorimetric assay to quantify Hg^{2+} before and after exposure. These results will help determine the efficacy of our dual-metal bioremediation approach. Following treatment, we will use microfiltration

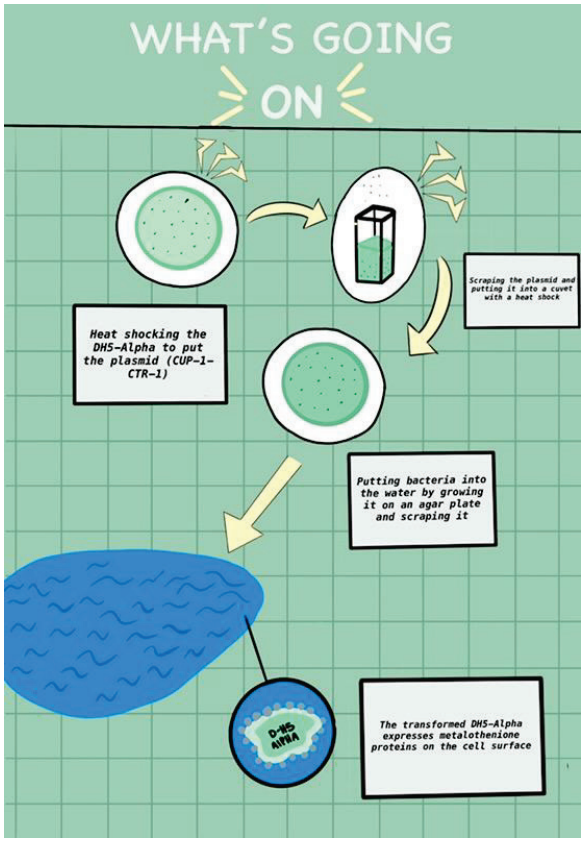
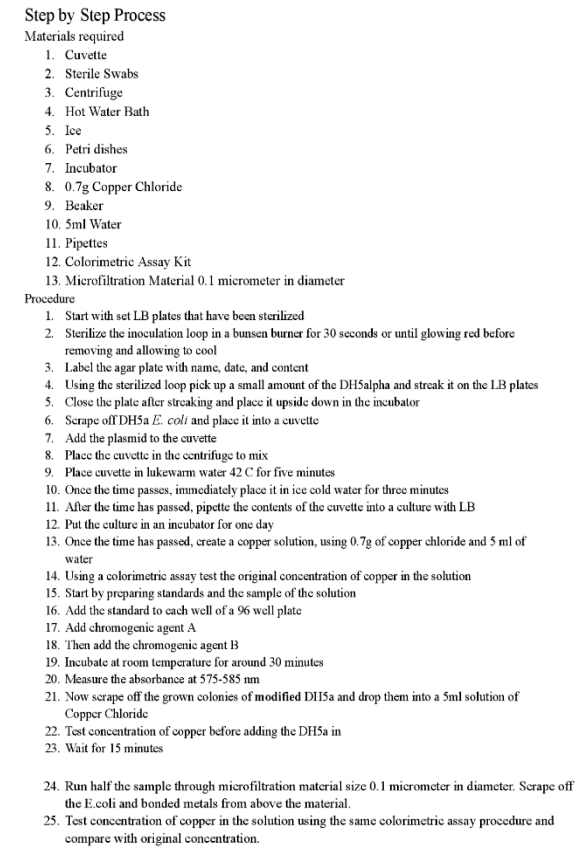


Figure 3. Genetic transformation of E. coli DH5-Alpha for heavy metal bioremediation. This figure illustrates the genetic transformation of E. coli DH5-Alpha to express metallothionein proteins for heavy metal bioremediation. Firstly, the plasmid containing the genes copA, copC, and copD is introduced to a colony of DH5-Alpha cells in a cuvette. Next, through heat shock, the bacterial cells undergo transformation and absorb the plasmid. Finally, the transformed bacterial colonies that exhibit the desired genetic properties are cultured on an Agar plate. When introduced to metal-contaminated water, these bacterial cells absorb and sequester metal ions from the extracellular environment.

methods to remove the bacteria from the solution, thereby extracting the heavy metals. This development would enable our system to target both copper and mercury contaminants, advancing toward a scalable, low-cost bioremediation solution. Similar to the water treatment's reuse of microfiltration material, this project utilizes microfiltration material that can be reused. For this reason, smaller lengths of this material are required; the costs of the desired material range from 0.5 dollars per millimeter. On a larger scale, this is relatively cheaper than current filtration technology, due to the system's reuse capabilities. Post-literature review in March, our team developed an experimental setup that could be accomplished as seen in Figure 4. Provided with more time, the team plans to follow the steps as listed below and record results to determine the efficacy of the recombinant-DNA solution.

This process enhances the bacteria's ability to clean and detoxify contaminated environments through targeted metal capture.



Step by Step Process

Materials required

1. Cuvette
2. Sterile Swabs
3. Centrifuge
4. Hot Water Bath
5. Ice
6. Petri dishes
7. Incubator
8. 0.7g Copper Chloride
9. Beaker
10. 5ml Water
11. Pipettes
12. Colorimetric Assay Kit
13. Microfiltration Material 0.1 micrometer in diameter

Procedure

1. Start with set LB plates that have been sterilized
2. Sterilize the inoculation loop in a bunsen burner for 30 seconds or until glowing red before removing and allowing to cool
3. Label the agar plate with name, date, and content
4. Using the sterilized loop pick up a small amount of the DH5alpha and streak it on the LB plates
5. Close the plate after streaking and place it upside down in the incubator
6. Scrape off DH5a *E. coli* and place it into a cuvette
7. Add the plasmid to the cuvette
8. Place the cuvette in the centrifuge to mix
9. Place cuvette in lukewarm water 42 C for five minutes
10. Once the time passes, immediately place it in ice cold water for three minutes
11. After the time has passed, pipette the contents of the cuvette into a culture with LB
12. Put the culture in an incubator for one day
13. Once the time has passed, create a copper solution, using 0.7g of copper chloride and 5 ml of water
14. Using a colorimetric assay test the original concentration of copper in the solution
15. Start by preparing standards and the sample of the solution
16. Add the standard to each well of a 96 well plate
17. Add chromogenic agent A
18. Then add the chromogenic agent B
19. Incubate at room temperature for around 30 minutes
20. Measure the absorbance at 575-585 nm
21. Now scrape off the grown colonies of **modified** DH5a and drop them into a 5ml solution of Copper Chloride
22. Test concentration of copper before adding the DH5a in
23. Wait for 15 minutes
24. Run half the sample through microfiltration material size 0.1 micrometer in diameter. Scrape off the E.coli and bonded metals from above the material.
25. Test concentration of copper in the solution using the same colorimetric assay procedure and compare with original concentration.

Figure 4. Experimental workflow for testing copper bioremediation by transformed E. coli. This figure details the step-by-step procedure for transforming E. coli DH5-Alpha with metallothionein-expressing plasmids and testing copper bioremediation. The process begins with streaking the bacteria on LB agar plates, followed by scraping colonies into a cuvette with plasmid DNA. After mixing via centrifugation, the cells undergo heat shock at 42℃, then rapid cooling on ice. Transformed bacteria are cultured overnight, then exposed to a copper chloride solution. Copper concentration is measured before and after treatment using a colorimetric assay and microfiltration to separate bacteria-bound metals. This method quantifies the ability of genetically modified E. coli to reduce copper levels in contaminated water.

Author contributions

S.G. and D.K. researched gene compatibility with the strain. R.D. and Ay. D. researched the methodology of the process. H.A. co-authored Abstract, Discussions, Figure 2, and edited references. P.B. co-authored Abstract, Background, and Discussions. Ay. D. co-authored Abstract, Systems Level, Device Level, Author Contributions, and Parts Level. Al. D co-authored Parts Level. R.D. co-authored Systems, Device Level, Parts Level, and Next Steps. S.G. co-authored Systems Level and Device Level, organized references, and edited Next Steps and Parts Level. P.K. co-authored Next Steps and Keywords and edited Safety. D.K. co-authored Systems Level, Device Level, Next Steps, and edited Parts Level. A.P. co-authored Figures 1,2,3, and 4. A.S. authored Safety.

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