

## Introducing the Role of Metals in Biology to High School Students

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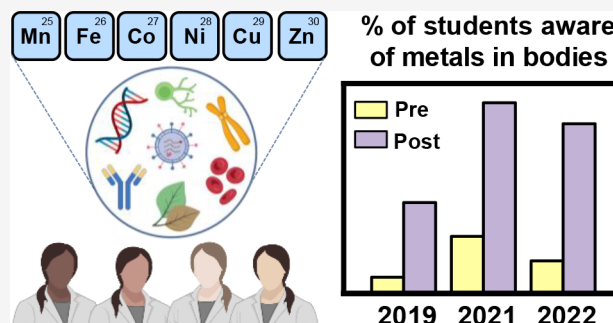
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**ABSTRACT:** Metals enable numerous physiological processes ranging from respiration to nitrogen fixation. However, the role of metals in biology and biocatalysis is not appreciated by the general public. This lack of knowledge around biological metals can lead to misinformation, especially regarding vaccines and health products. Here, we present a series of easy-to-implement experiments and demonstrations that can be incorporated in the high school curriculum to introduce students to the role of metals in biology. Our results from running these experiments/demonstrations in virtual ( $N = 6–10$ ) and in-person ( $N = 22$ ;  $N = 9–12$ ) formats reveal that only 9–30% of high school students are aware of the presence of metals in humans. These statistics can be changed to 48–100% by incorporating proposed experiments and content in the curriculum.

**KEYWORDS:** High School/Introductory Chemistry, Demonstrations, Laboratory Instruction, Public Understanding/Outreach, Hands-On Learning/Manipulatives, Bioinorganic Chemistry, Enzymes, Metals, Proteins/Peptides, Reactions, Transition Elements, Women in Chemistry



## INTRODUCTION

Metals catalyze numerous biological processes and serve as cofactors for several key enzymes.<sup>1–4</sup> In fact, it has been estimated that nearly half of all enzymes utilize metals to maintain their structural integrity and functional roles.<sup>5–8</sup> For instance, the heme-copper complex in cytochrome *c* oxidase binds oxygen and facilitates reduction of oxygen to water during respiration<sup>9–13</sup> (Figure 1A, top panel), and the heme-iron cofactor in myoglobin enables storage of oxygen (Figure 1A, bottom panel).<sup>14–16</sup> Similarly, plants use calcium and manganese cofactors to catalyze the oxidation of water during photosynthesis (Figure 1B, top panel)<sup>17,18</sup> while symbiotic nitrogen-fixing bacteria in legumes use iron and molybdenum cofactors to convert nitrogen to ammonia (Figure 1B, bottom panel).<sup>19</sup> While it is clear that metals are involved in some of the most crucial biological processes, the knowledge of their existence in biology and humans is low among general public.<sup>20</sup> Additionally, low awareness surrounding biological metals can lead to public misinformation, especially concerning vaccines and health products.<sup>21–23</sup> This rationale is supported by recent misinformation regarding the presence of metals in the COVID-19 vaccine which was misunderstood to make humans magnetic.<sup>24–28</sup> We believe introducing students at the high school level to the role of metals in biology will help alleviate misinformation in public and, simultaneously, engage students in science, technology, engineering, and mathematics (STEM) to build interest in science-related careers. Although there is significant research interest in the topic of metals in biology,<sup>29,30</sup> incorporation of such a topic in secondary-level

education curriculum is still lacking.<sup>31</sup> To overcome this gap in knowledge and education, here, we present a series of experiments and demonstrations that utilize inexpensive and accessible reagents, and simple protocols that can be easily implemented in a high school setting. Our results from running these experiments/demonstrations in virtual and in-person formats revealed that only 9–30% of high school students were aware of the presence of metals in humans. These statistics can be increased to 48–100% by incorporating proposed experiments and content in the curriculum.

## PEDAGOGICAL GOALS

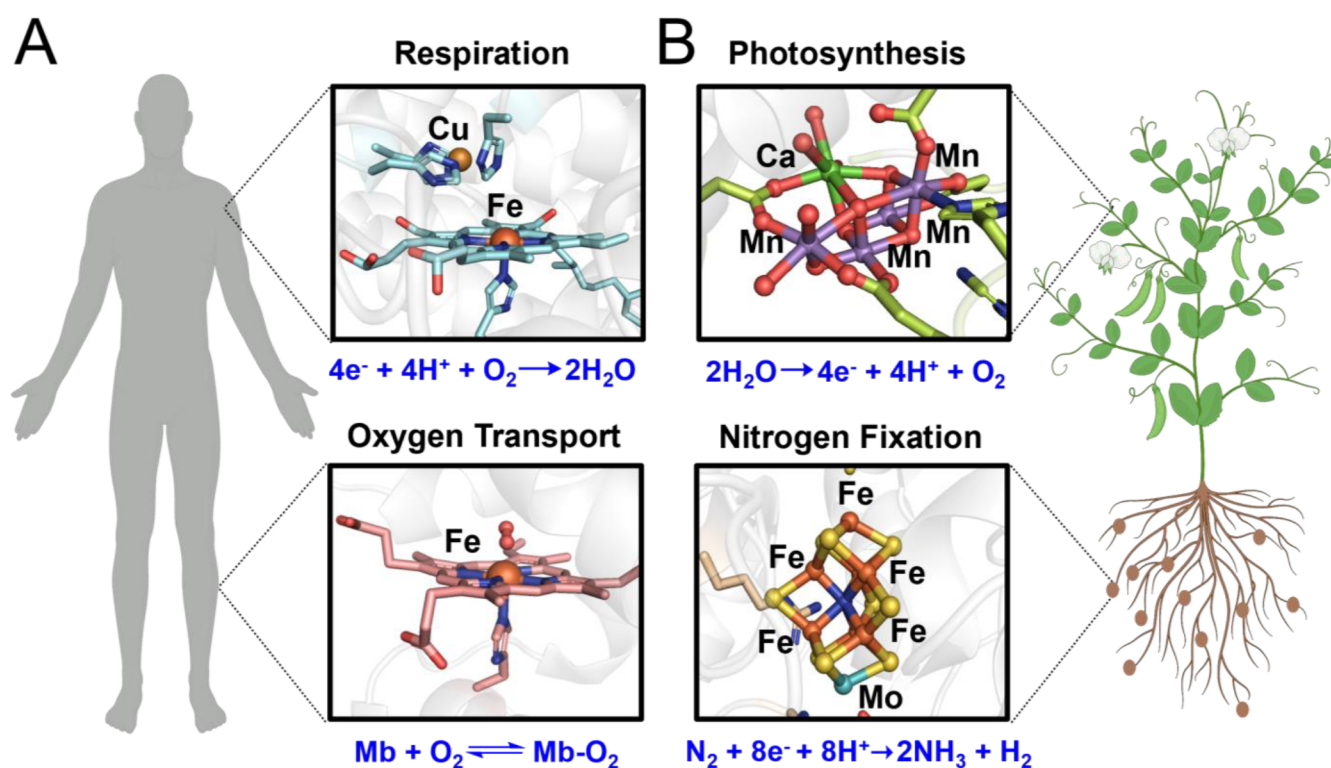
To increase knowledge of metals in biology among high school students, we adapted a series of four experiments/demonstrations focused on this topic. The impact of these experiments/demonstrations on enhancing the awareness was evaluated via the “Metals in Biology” outreach sessions conducted in collaboration with Girls Inc. Eureka!, a five-year summer program at the University of Minnesota. The Eureka! outreach program focuses on female students from Minneapolis and St. Paul school districts entering high school

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**Figure 1.** Representative examples of metals in biology. (A) Metal cofactors present in humans that drive crucial physiological processes. Structure of the heme-iron copper center in heme-copper oxidase (PDB: 7DEG) which drives oxygen reduction during respiration is shown at the top. Structure of the heme-iron center in myoglobin (Mb) (PDB: 2VLY) that enables oxygen storage in muscles is shown at the bottom. (B) Metal cofactors critical in biological processes in plants. Calcium–manganese cluster of photosystem (II) (PDB: 4UB6) which is involved in light-dependent photosynthesis is shown at the top, while the iron–molybdenum cluster in nitrogenase (PDB: 1M1N) responsible for reduction of nitrogen to ammonia is shown at the bottom. Chemical equations corresponding to each biological process are also shown at the bottom of each panel.

(eighth and ninth grade) and is aimed at supporting the professional interests of these students in STEM.<sup>32</sup> All participants in the program are underrepresented minorities in STEM, including first-generation college applicants and students of color. Given that students engage more proactively with personally relevant content,<sup>33</sup> our adapted experiments focused on the ubiquity of metals in biological systems and their importance in organisms, especially humans. Each experiment was structured around a series of active-learning discussion questions to engage students and develop their critical thinking and comprehension skills. The primary goals of this outreach were (1) educating students on the presence and importance of metals in biology and (2) assessing if the incorporation of such content in the high school curriculum would be feasible and beneficial. Our long-term goal with the outreach is to enhance students' interest in STEM fields and their ability to apply science not only in classrooms, but also in real-world scenarios.

## DESIGN AND IMPLEMENTATION

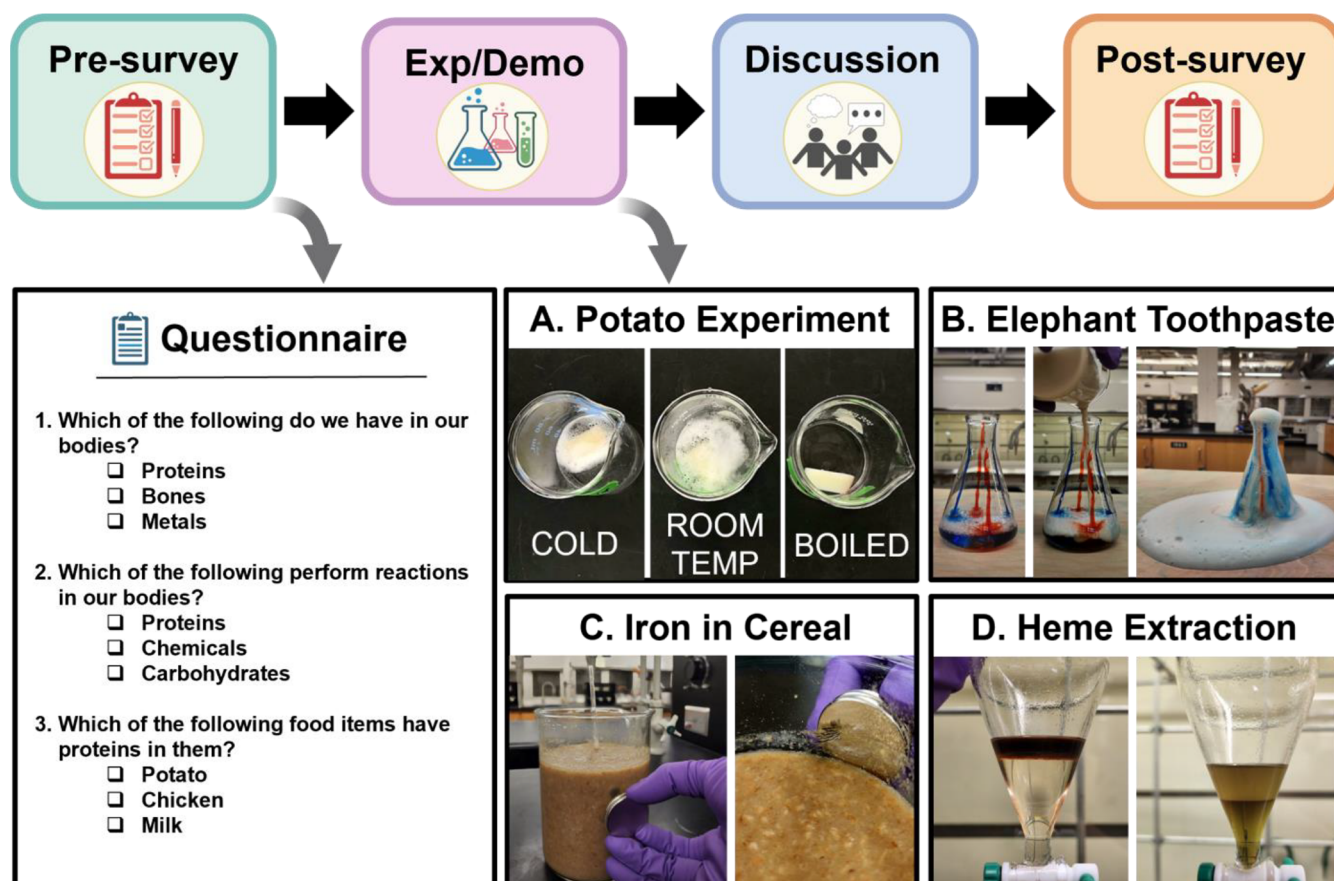
"Metals in Biology" sessions were conducted in-person in 2019, virtually due to pandemic restrictions in 2020–2021, and in-person again in 2022. While the in-person sessions involved students performing hands-on experiments, the virtual sessions involved experimental demonstrations for students online. Each session lasted approximately an hour. At the beginning of the session, students took a presurvey questionnaire (Figure 2), followed by an introduction to enzymes and their roles in biocatalysis. After this initial discussion, students were grouped

in teams of 2–3 for in-person sessions, and each team was provided a protocol for experiments to be conducted (see Supporting Information for detailed protocol, agenda, timetable, and slides used). For the virtual sessions, instructors performed the demonstrations while students were encouraged to make hypotheses, observe, and take notes. The 2019–20 sessions were standalone and consisted of the potato experiment (Experiment 1) and elephant toothpaste (Experiment 2) only (Figure 2A,B). In contrast, the 2021–2022 sessions were iterative with Experiments 1–2 conducted on Day 1 and two additional experiments ("iron in cereal" and "heme extraction", Figure 2C and D) conducted on Day 2.

After each experiment/demonstration, the experimental results were briefly discussed to educate students on the science behind the demonstrations and address any misconceptions. At the end of the outreach, students were asked to fill out the survey with the same questions to assess if there was any increase in knowledge after experiments/demonstrations. Below, we describe the experimental procedures, expected observations, and scientific explanations of the four experiments/demonstrations performed during the outreach.

### Experiment 1: Iron-Containing Catalase in Potatoes (~30–40 min; Figure 2A)

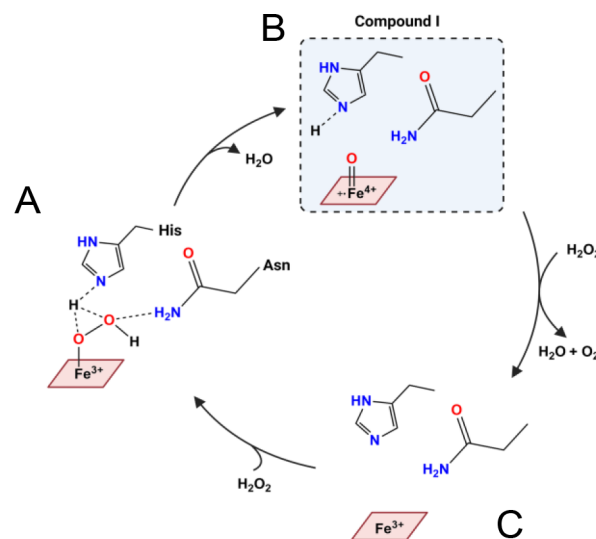
Numerous pedagogical studies have proposed catalase-based experiments at high school and undergraduate levels to educate students about biological catalysis and enzymology.<sup>34–36</sup> For instance, Kimbrough et al. and Latourelle et al. utilized catalase extracted from various sources in freshman biology laboratories



**Figure 2.** Design of “Metals in Biology” outreach sessions. Students were asked to take the presurvey questionnaire before being introduced to 4 different experiments, specifically potato experiment (A), elephant toothpaste (B), iron in cereal (C), and heme extraction (D). After a brief discussion to engage the students and correct any misconceptions, the outreach was concluded with students filling out the postsurvey, consisting of the same questions as the presurvey.

to investigate the effects of temperature and/or pH on enzymatic activity using UV–vis spectroscopy and visual cues.<sup>34,35</sup> Trujillo et al. demonstrated the use of catalase found in baker’s yeast as a safer alternative to potassium iodide catalyst for the elephant toothpaste demonstration to high school audience.<sup>36</sup> In this work, we use catalase as a representative metalloenzyme and discuss its reaction with the students from a bioinorganic perspective. Catalase is a heme-containing enzyme that reacts with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and performs a disproportionation reaction to produce oxygen ( $\text{O}_2$ ) and water ( $\text{H}_2\text{O}$ ). The ferric form ( $\text{Fe}^{3+}$  oxidation state) of heme binds to  $\text{H}_2\text{O}_2$  and activates the molecule via hydrogen-bonding interactions with protein-derived amino acids asparagine (Asn) and histidine (His) (Figure 3A). These hydrogen-bonding interactions enable O–O bond cleavage, releasing a water molecule, and forming the highly reactive compound I with iron in  $\text{Fe}^{4+}$  oxidation state (Figure 3B). A second molecule of  $\text{H}_2\text{O}_2$  then reacts with compound I, and a water molecule along with  $\text{O}_2$  are released as products, resetting the enzyme for further turnovers (Figure 3C).<sup>37</sup>

After introducing enzymes and their role in biology, we began Experiment 1 by using store-bought potatoes. The potatoes were prepared in advance by chopping them into 3 roughly equal portions, freezing one portion in a freezer, boiling one portion, and leaving one portion at room temperature.<sup>38,39</sup> Just before the experiment/demonstration, potatoes were taken out of the freezer or hot water bath. Each



**Figure 3.** Catalase catalytic cycle. The catalytic cycle of catalase proceeds through 3 simplified steps starting with hydrogen peroxide entering the binding site, forming two hydrogen-bonding interactions with protein-derived amino acids asparagine (Asn) and histidine (His). (A) The histidine acts as an acid–base catalyst, assisting in the formation of compound I (B) with the release of water. An additional  $\text{H}_2\text{O}_2$  molecule is utilized to reduce compound I, producing water and molecular oxygen, and re-establishing ferric heme (C).



type of potato was added to 3 different beakers containing ~10 mL of 3–12% hydrogen peroxide. Catalase in the potatoes reacted with  $\text{H}_2\text{O}_2$  to produce  $\text{O}_2$  which fizzed and was observable by the naked eye (Figure 2A). As expected, the enzymatic reaction was the fastest at room temperature (22 °C), and students observed maximum fizz, while catalase in the frozen potatoes was slowed and exhibited lesser fizz. On the other hand, boiled potatoes contained unfolded catalase enzyme that was no longer functional and produced no fizz. After this easy-to-implement experiment, we discussed the following: (1) the presence of iron in potato's catalase which facilitates this reaction, and (2) the role of temperature in maintaining protein structure and function. Overall, this experiment introduces the concepts of metals in biology, biocatalysis, and protein structure–function relationships.

#### Experiment 2: Elephant Toothpaste Generation via Catalase in Yeast (~10–20 min; Figure 2B)

While elephant toothpaste is a popular demonstration,<sup>40–45</sup> the role of the metalloenzyme, catalase, in enabling this reaction is typically not explained. We emphasized the role of catalase as a biocatalyst by performing an elephant toothpaste experiment which utilizes catalase found in yeast. The catalase in yeast is 22-fold more active than that in potatoes.<sup>46,47</sup> When used in conjunction with dish soap that provides surface tension and catches the released oxygen, the yeast catalase creates an even more vigorous reaction when mixed with hydrogen peroxide.

For this experiment/demonstration, 1 teaspoon of active dry yeast (store-bought) was dissolved in half a cup of warm water in a beaker and allowed to rest for at least 5 min. In an Erlenmeyer flask, a small amount of dish soap, 10 mL of 3–12% hydrogen peroxide, and food coloring of choice were added. Finally, the yeast solution was poured into the Erlenmeyer flask to initiate the reaction (Figure 2B). In discussions that followed, we explained that the catalase responsible for this experiment is the same type found in potatoes in Experiment 1. Finally, we discussed how catalase also clears out highly reactive  $\text{H}_2\text{O}_2$  and protects cells in humans and animals. Overall, through the two catalase-based experiments, the presence of metals in the enzymes of potatoes (plants), yeast (fungi), and humans (animals) was discussed.

#### Experiment 3: Iron in Cereal (~10 min; Figure 2C)

“Iron in Cereal” experiment introduced that metals (specifically iron) are acquired from our daily diet.<sup>48,49</sup> Solid iron ( $\text{Fe}^0$ ), with the electron configuration of  $[\text{Ar}]3\text{d}^64\text{s}^2$ , possesses magnetic properties due to one unpaired electron in its 3d atomic orbital. Owing to its ferromagnetism, the iron present in cereal can be collected with a strong magnet when dissolved and is a perfect example of how essential metal cofactors are commonly found in foods.<sup>50</sup> For this demonstration, 1 cup of iron-enriched cereal in a resealable bag was crushed into small pieces. To this, warm water was added, and the mixture was stirred using a plastic utensil. While stirring, the student held a strong magnet (like neodymium) to the side of the beaker for the iron particles to gather (Figure 2C). The cereal experiment (when coupled with Experiment 4) builds the connection between the requirement of iron in one's diet and its function in our bodies.

#### Experiment 4: Heme Extraction from Myoglobin Protein (~10–20 min; Figure 2D)

Proteins are made of hydrophilic and hydrophobic amino acids which form bonds with metal cofactors. Oxygen-storing

protein, myoglobin, utilizes a histidine amino acid to bind to the iron center of heme, a hydrophobic molecule easily detectable as red in color. In acidic conditions, heme-ligated histidine gets protonated, which disrupts its coordination bond with iron and releases the heme. This free heme can then be separated from the unfolded protein using the organic solvent, 2-butanone, which is immiscible with water and forms 2 layers when combined with aqueous, acidified myoglobin (Figure 2D). The hydrophobic heme moves to the organic butanone layer on top and colors the layer red while the apoprotein remains in the colorless aqueous layer at the bottom. Addition of a base can hydroxylate the heme, which moves to the aqueous layer and turns it green.

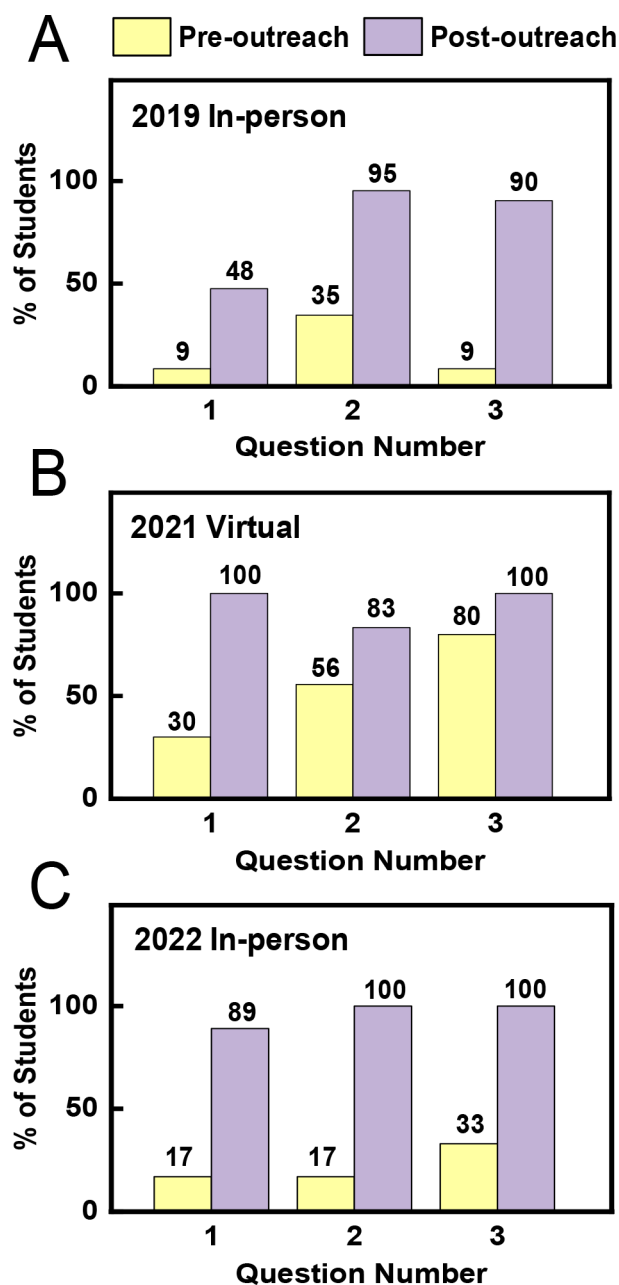
For this demonstration, 10 mL of 50 mM equine heart myoglobin was prepared using deionized water. The protein solution was transferred to a separatory funnel, and 100  $\mu\text{L}$  2 M HCl was added and mixed. After adding 10 mL water-saturated 2-butanone and mixing gently by inversion with occasional venting, the solution was allowed to settle before draining the bottom aqueous layer. A 1 mL portion of 5 M NaOH was added dropwise to the remaining layer to observe the formation of the green solution. This solution was mixed further by inverting the separatory funnel several times to obtain a green aqueous layer at the bottom and a colorless butanone layer on top (Figure 2D, right panel). Through Experiment 4, we demonstrated the chromogenic property of heme, principles of acid/base chemistry, and methodologies of an organic extraction.<sup>51</sup>

### SAFETY INFORMATION

Heme extraction from myoglobin involves chemicals that require careful handling and therefore should be demonstrated to the high school students. 2-Butanone is a colorless, slightly volatile liquid with a pungent odor. Although stable under normal conditions, it is highly flammable and can irritate one's nose, eyes, and throat.<sup>52</sup> Hydrochloric acid is a colorless liquid with a pungent odor and should be handled with extreme caution since it can cause severe skin burns and eye damage.<sup>53</sup> Sodium hydroxide is also corrosive and appears as white, odorless crystals in its solid form and a clear, colorless liquid in solution.<sup>54</sup> Although both hydrochloric acid and sodium hydroxide are corrosive, only a small amount of the chemicals (maximum of 1 mL) are used in heme extraction and, therefore, are relatively safe. Regardless, proper personal protective equipment (PPE) such as gloves and safety goggles should always be worn when handling these chemicals.

### PEDAGOGICAL OUTCOMES

To evaluate the impact of the outreach sessions, students were asked to take anonymous pre- and postsurveys either on a physical copy (in 2019 and 2022) or in Google Forms (in 2021). Quantitative assessment of the impact was not performed in 2020. The questions in the survey (available in SI) focused on assessing the short-term recall of 8th–9th graders about the role of metals in biology before and after the workshop. Our presurvey results showed that only 9–30% of students were aware of the presence of metals in our bodies (Figure 4: Q1). The discussions, experiments, and demonstrations described above increased this knowledge by 3- to 5-fold. Before experiments, only 17–56% of students knew that proteins perform reactions (Figure 4: Q2). After these experiments, this knowledge increased to 83–100%. Finally,



**Figure 4.** Survey results showing the % of students that correctly knew that (Q1) metals are present in our bodies, (Q2) proteins perform reactions, and (Q3) potatoes have proteins pre and post the experiments/demo and discussions. (A) Results from in-person sessions conducted in 2019 where the students ( $N = 22$ ) performed experiments. (B) Results from virtual sessions conducted in 2021 where the students ( $N = 6$ – $10$ ) were demonstrated these experiments. Ten students took the survey before the demo, and 6 students took the survey after the demo. (C) Results from 2022 in-person sessions where students ( $N = 9$ – $12$ ) performed hands-on experiments. Twelve students participated in the survey before the experiments, and 9 students took the survey after the session. “ $N$ ” is the total number of students who took the survey before/after each year.

the knowledge about the presence of proteins in potatoes was variable between groups (Figure 4: Q3). Only 9% and 33% of 2019 and 2022 group, respectively, knew that potatoes contained proteins, while 80% of the 2021 group already had this knowledge. Nonetheless, these experiments educated

students about the structure and function of proteins, and 90–100% of students knew that potatoes contain proteins after the experiments/demonstrations. Overall, the survey results allude to the success of these experiments, demonstrations, and discussions in educating students about the presence and role of metals in biology and biocatalysis. To further gauge student learning and their applied knowledge, we incorporated two open-ended questions in the 2022 postsurvey: “Is iron bad for you? Why or why not?” and “What was your favorite thing you learned? What were you surprised to find out?” While students varied in their reasonings, every student taking the postsurvey identified that iron is not inherently bad for their bodies. When asked of their favorite takeaways or surprises, several students noted that they were surprised to learn about the presence of metal (specifically iron) in their bodies and food, suggesting that our educational endeavor involving a compilation of experiments/demonstrations was effective (Table 1).

### INSTRUCTOR REFLECTION

The role of metals in biology and biocatalysis is not explicitly discussed in the high school curriculum, and the experiments/demonstrations and discussions described herein can help educate students easily in a 1–2 h time frame. To evaluate the outreach impact, the pre- and postsurvey results were compared. The 2019 and 2022 in-person sessions showed a higher percentage increase in acquired knowledge than in 2021, suggesting that hands-on experiments and in-person discussions are more effective in disseminating these concepts. Nonetheless, the experiments/demonstrations can be easily performed in both in-person (as in 2019 and 2022) and virtual (as in 2020–21) formats. In future endeavors, it would be interesting to probe for long-term retention of the knowledge gained in a larger sample size to assess if incorporating this type of curriculum will enhance students’ understanding of the role of metals in biological processes.

Experiments 1 and 2 introduce the role of metals in biology by demonstrating the prevalence of metalloenzyme catalase in potatoes and yeast. During Experiment 1, some students correctly predicted that the room temperature potato would react most vigorously creating the most fizz. After the experiment, we discussed how catalase in the potatoes stayed properly folded at room temperature and, therefore, was most active. This rather simple experiment introduced the concept of protein activity being dependent on temperature and enabled the students to appreciate the relationship between protein structure and function. The elephant toothpaste reaction (Experiment 2) was exciting and fun for the students since they chose the food coloring and observed the cloud of bubbles vigorously escaping the flask. We discussed how the reaction was caused by the same enzyme that is in potatoes (catalase), and how this enzyme is also found in human bodies to act as an antioxidant agent. To lay the foundation of further discussions in Experiments 3 and 4, it was emphasized that iron is essential for proper functioning of not only catalase, but also several other metal-containing proteins.

Through Experiment 3, we introduced how we acquire iron through food intake and metabolize it for use in biological processes such as oxygen transport. Students appreciated how we also intake other metals like calcium, potassium, and magnesium through our diets and that metals are essential for life-sustaining metabolism. In Experiment 4, students enjoyed color changes observed during the heme extraction of myoglobin. The discussion that followed reconnected to how

Table 1. Answers to Open-Response Questions Surveyed in 2022

| Q1: Is iron bad for you? Why or why not?                                                                                         | Q2: What was your favorite thing you learned? What were you surprised to find out?                              |
|----------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|
| Iron would be bad for you maybe if you have too much, but most of the time it is good for you because if you are low, it is bad. | Everything I loved.                                                                                             |
| No, iron is good for you because it makes your blood red.                                                                        | N/A                                                                                                             |
| No, it is not bad for you because it helps playing a part to make your body function.                                            | About iron and other metals. That we have it in our bodies.                                                     |
| No, because we eat it. If it was bad, we would not eat it.                                                                       | How much iron is in cereal.                                                                                     |
| No, it is not, because it makes our blood red.                                                                                   | I was surprised that there were little iron needles in cereal.                                                  |
| No, because you need to get energy from food.                                                                                    | The crushing up the cereal. I was surprised that the bag broke.                                                 |
| No, iron is basically energy and it also keeps your body balanced.                                                               | That there is iron in most of the food we eat. I just thought that iron that was in our bodies was good enough. |
| No, it carries oxygen through your blood so you do not die.                                                                      | I liked learning about the different colors iron can be.                                                        |
| No, it helps you not get a certain type of disease.                                                                              | 1. The elephant toothpaste. 2. Elephant toothpaste is kinda slow to rise.                                       |

the iron obtained from common foods gets processed in the body and eventually serves as a critical cofactor for metalloproteins such as catalase mentioned extensively in Experiments 1–2, myoglobin used in Experiment 4, and hemoglobin found in blood. Additionally, students were engaged with how different metals are responsible for different blood colors in various species. For example, humans bleed red due to heme-iron in hemoglobin, while some arthropods and mollusks bleed intense blue due to copper-based hemocyanin in their blood.<sup>55</sup> The outreach was concluded with a summary of how metals (specifically iron in our experiments/demonstrations) are present in organisms and essential for several biological processes to maintain homeostasis. Overall, we hope the experiments/demonstrations discussed herein will increase the level of knowledge acquisition with an experimental approach to metals in biology while also boosting the students', especially women's, interest in pursuing careers in the STEM field.

While our outreach efforts have focused on high school students, some of these experiments can be easily incorporated in the undergraduate nonscience curriculum, especially in remedial or primarily allied health courses, to foster awareness and alleviate misconceptions centering on biological metals. While we envision the described experiments and discussions being integrated in a one-credit lab course focusing on life sciences, these experiments require optimization to suit the undergraduate level. For instance, Experiment 1 can be adapted (as illustrated in Latourelle et al.)<sup>35</sup> to demonstrate chemical rate laws, investigate reactant concentration effects on kinetics, and illustrate the implications of H<sub>2</sub>O<sub>2</sub> in cells and the role of iron in catalase to maintain homeostatic concentrations of hydrogen peroxide. By applying such an interdisciplinary didactic pathway for undergraduate students, we expect to enhance the appreciation of metals in biology and, overall, cultivate a better connection between chemistry and other natural sciences.

## ■ ASSOCIATED CONTENT

### SI Supporting Information

The Supporting Information is available at <https://pubs.acs.org/doi/10.1021/acs.jchemed.2c00533>.

Agenda and timetable suggested for an outreach, list of materials required, hazards, experiment handouts and discussion questions, survey questions, and slides used during the outreach; PowerPoint slides utilized during the outreach (PDF)

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### Notes

The authors declare no competing financial interest.

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