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Fall sunset

The sunset was taken from the Bay Bridge in Bay St Louis, MS on November 15, 2025 at 4:54 pm by Michelle Tucci. I phone 14 pro was used to capture the image- iso 64 162 mm f 1.78 1/2070 s

Journal of the Mississippi Academy of Sciences

Volume 70

October

Number 4



TABLE OF CONTENTS

Editor

Michelle Tucci
University of Mississippi Medical Center

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Hamed Benghuzzi
Jackson State University

Olga McDaniel
University of Mississippi Medical Center

Editorial Board

Paul Tseng
Mississippi State University

Ibrahim O. Farah
Jackson State University

Robin Rockhold
University of Mississippi Medical Center

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Michelle Tucci
University of Mississippi Medical Center

Kenneth Butler
University of Mississippi Medical Center

Research Articles

- 265 **Effect of an Immersive Environmental Science Program on Learning by Rural Elementary School Students**
Leslie M. Burger and Jessica L. Tegt
- 276 **Detection of Antibodies to SARS-CoV-2 Antigens in Serum of COVID-19 Infected and Vaccinated Patients by Immunoblot Analysis**
Shivum Desai, Virginia Gammon, Christian Hollis, Abby Sartin, Suzanna Horn, and Wayne L. Gray
- 284 **Wildfire and Other Environmental Hazard Detection Capabilities of a Low-Cost System**
Samuel Swanner, Kaitlyn Hebig, Alondra Arreola-Espino, Angela Carraway

Departments

- 287 **Call for Abstracts**
- 288 **Author guidelines**
- 291 **Advertisement**

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Effect of an Immersive Environmental Science Program on Learning by Rural Elementary School Students

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ABSTRACT

We evaluated an immersive, environmental education program designed to address natural science knowledge deficiencies in Mississippi public school students. Fourth and fifth grade students ($n = 570$ and $n = 608$, respectively) from a rural school district participated for five consecutive days (30-hours) during 2011 - 2013. A pretest-posttest design detected posttest knowledge scores were higher than pretest scores. Significantly higher posttest scores were observed in students who were female, non-Black, or from higher economic status. The pedagogical approach, as measured by knowledge gains, was equally effective at educating students from diverse socioeconomic and demographic groups.

KEY WORDS: elementary science, achievement gap, science immersion

INTRODUCTION

For decades, those concerned about environmental and natural resources have attempted to engage and educate a generation of future citizens and leaders. Educational approaches range from classroom enhancement modules (e.g., Project WILD) to youth summer camps and outdoor field experiences to environmental magnet schools. The research described in this document was conducted to determine the efficacy of another approach--science immersion within a traditional elementary school setting.

Science instruction in schools is often passive, conducted via teacher lectures, textbook readings, and worksheets. However, experiential learning theory proposes an active, exploratory approach to education is more efficacious for promoting knowledge gains (Kolb & Fry, 1975; Jacobson, McDuff, & Monroe, 2006, National Research Council, 2000). Youth Environmental Science (YES) is a natural science program that immerses upper elementary students in experiential learning. Its pedagogy stresses hands-on learning through inquiry and experimental activities with goals for providing measurable academic achievement in science and promoting environmentally literate youth.

YES addresses the need to connect children with

nature (Louv, 2005) and improve science literacy, especially among demographic subgroups that lag behind their peers (Duschl, Schweingruber, & Shouse, 2007). Science achievement disparities among student demographic groups are well-recognized phenomena. Females often exhibit lower success on science assessments than their male counterparts (e.g., Gonzales et al., 2004; Lee & Burkam, 1996; Weinburgh, 1995). White and Asian students generally perform better than Hispanic and Black students (ACT, Inc., 2012; Lee, 2002; National Center for Educational Statistics [NCES], 2012), and students from low-income homes score considerably lower on science assessments than peers from higher-income situations (NCES, 2012; Sirin, 2005). The YES model could be especially relevant in schools which serve students with cultural, access or financial barriers to science enrichment opportunities. However, because evaluation of environmental education (EE) programs is often overlooked (Fien, Scott, & Tilbury, 2001; Lucko, Disinger, & Roth, 1982), our research purpose was to evaluate the efficacy of YES to provide measurable academic improvement in natural science achievement for all students.

Research on the effectiveness of in-school, elementary EE programs is limited. A literature review of classroom EE interventions by Leeming et

al. (1993) reported only five evaluated programs in elementary school settings (Armstrong & Impara, 1991; Asch & Shore, 1975; Fennessey, Livingston, Edwards, Kidder, & Nafziger, 1974; Jaus, 1982; Jaus, 1984). Lieberman and Hoody (1988) surveyed EE programs in 40 U.S. schools, 15 of which were in elementary schools. Many classroom-based interventions are short-duration, such as field trips (Farmer & Wott, 1995; Farmer, Knapp, & Benton, 2007; Knapp & Poff, 2001) and enrichment units (Adams, Charles, Greene, & Swan, 1985; Higginbotham, 1997; Morgan & Gramann, 1989; Powell 1996; Randler & Bogner, 2002). Nevertheless, positive effects on students' environmental and multi-disciplinary content knowledge were attributed to the interventions and their pedagogy (National Environmental Education & Training Foundation (NEETF), 2000).

Evaluated EE programs that spanned multiple days were usually affiliated with camp programs (Bogner, 1998; Burrus-Bammel & Bammel, 1986; Knox, Moynihan, & Markowitz, 2003; Larson, Castelberry, & Green, 2010). However, economic and cultural barriers may restrict access to only those with the means to attend. Research is needed to determine whether a longer-duration, school-based EE program can produce enhanced science achievement for students, particularly in schools with achievement gaps associated with gender, race/ethnicity, or economic status.

Our objective was to assess the efficacy of an experiential, in-school EE program on students' knowledge of natural science, as mediated by race/ethnicity, gender, and economic status. Two focal questions were:

1. Does Youth Environmental Science result in gains in environmental science knowledge in elementary school participants?
2. Does YES close achievement gaps in a diverse student population?

MATERIAL AND METHODS

Participants

Participants were fourth and fifth grade students from a small-town, public school in rural Mississippi. Teachers were invited to voluntarily enroll their class

in YES for one week. Students participated in YES for six hours on each of five consecutive days using on-campus classrooms and outdoor spaces; a field trip to a local environmental learning center provided additional outdoor learning experience. By operating during the normal academic day, economic, and/or cultural barriers to science enrichment opportunities were removed. Curricula were comprised of lessons that met standards established by the North American Association for Environmental Education (2004) and were correlated to the Mississippi Science Framework. Lessons were linked by daily themes and were unique to each grade. During the study, the authors taught the same lessons to every class to minimize instructor effects on student responses on the evaluation instruments.

Classroom populations were predominately Black (64%), with smaller numbers of White (31%), Asian (2%), Hispanic (1%), and other races/ethnicities (2%). Nearly equal numbers of males (51%) and females (49%) were present. Sixty-eight percent of the students were eligible for the National School Lunch Program (NSLP) (NCES, 2013a; NCES, 2013b). Only students from low-income homes are eligible for free or reduced-price school lunches; therefore, eligibility for the NSLP was used an indicator of economic status. Proficiency or better rankings on the 2012 state science test were achieved by approximately two-thirds of the school's White and Asian fifth grade students; only 20% of Black students achieved this ranking. Thirty-eight percent of female fifth grade students performed at proficiency or above as compared to 29% of male students. Only 14% of economically disadvantaged students achieved proficiency or above in science (Mississippi Department of Education, 2013).

Instrument Development and Administration

We developed a multiple-choice survey instrument for each grade to measure changes in YES subject matter knowledge. The surveys contained 17 questions in 2011-2012 and 20 questions in 2012-2013 selected from grade-appropriate, science tests available online through state departments of education. Survey instruments and permission forms were approved by the Mississippi State University Institutional Review Board (Protocol #11-234). We assigned unique identification codes to each student

to protect identities. Student gender, race/ethnicity (White, Black, Asian, Hispanic, or Other), and NSLP enrollment eligibility were obtained from students, YES enrollment records, and school administration. Students voluntarily completed the test instrument at the beginning of their week in the YES program and at the end of the week's instruction. Tests were administered in home classrooms to maintain students' familiarity with the testing environment.

Data analysis

We excluded data from students who had fewer than 24 of the 30 possible hours of YES participation (caused by school absence, in-school suspension or remedial interventions), incomplete tests (defined as failure to complete one or more entire test pages) or missing pretests or posttests. We also excluded data from fifth grade students who had attended YES in fourth grade, since this prior experience may have influenced their test scores (Kruse & Card, 2004). Test responses were double-entered by identification code and graded using a series of if-then statements. Percent correct was calculated for each student's pretest and posttest. Demographic (gender, race/ethnicity, economic status) and academic (grade, school year, class) data were coded and assigned to each respective student code. Because sample sizes were small for the Asian, Hispanic, and other demographic groups, these categories were combined, and three levels of race/ethnicity (White, Black, and Other) were used in the analysis.

We tested the null hypothesis that students' pretest and posttest scores would not differ using a generalized linear mixed model. Pretest and posttest scores (i.e., time) served as a repeated measure response variable. The main effect of time tested for changes in test score (learning gains) over the five-day instructional period. Main effects of academic year, grade, race/ethnicity, gender, and economic status and their interactions with time were included

as fixed factors in the analysis to test hypotheses about these sources of potential variation in academic performance (main effects) and moderating effects on learning gains (interactions with time). Two-way interactions among race/ethnicity, economic status, and gender were also included in the model. Classroom was included as a random effect because students within a class were not independent (Leeming, Dwyer, Porter, & Cobern, 1993). The generalized linear mixed models used a normal distribution with an identity link, Type III sums of squares, restricted maximum likelihood estimation, and compound symmetry covariance structure (as indicated by the smallest AIC (Akaike's Information Criterion) value returned after analysis of models using different candidate covariance structures [e.g., Gutzwiller & Riffell, 2007]). An alpha level of .05 was chosen a priori. Residuals were normal, and variances were homogeneous. All statistical analyses were conducted using IBM® Statistical Package for the Social Sciences (SPSS)® Version 21.

RESULTS AND DISCUSSION

During 2011-2012, 11 (n = 224 students) of the 15 fourth grade classes and 14 (n = 295 students) of the 15 fifth grade classes in the school enrolled. In 2012-2013, all fourth grade (n = 16 classes, 346 students) and fifth grade (n = 15 classes, 313 students) classes enrolled. We analyzed pretest and posttest scores from 702 students: 141 from fourth grade and 183 from fifth grade in 2011-2012; 274 from fourth grade and 104 from fifth grade in 2012-2013 (Table 1). Although male students slightly outnumbered females in the student body (51% males), female students were more prevalent in the analyzed data set (52%) because a higher proportion of incomplete tests or missing pretests/posttests was associated with male students. For similar reasons, survey data showed a lower representation of Black students (61%) than in the school population (64%).

Table 1. Descriptive statistics from pretest-posttest analysis of YES participant knowledge.

	Number of Students	Pretest		Posttest	
		<i>M</i> ¹	<i>SE</i>	<i>M</i>	<i>SE</i>
All students	702	55.67	1.63	72.18	1.79
2011-2012	324	53.08	1.97	68.82	2.14
2012-2013	378	58.17	1.82	75.65	1.99
Male	337	53.16	2.09	70.18	2.24
Female	365	58.18	1.93	74.17	2.09
4th	415	56.27	1.85	72.00	2.01
5th	287	54.97	1.94	72.47	2.12
Black	429	42.94	.74	58.43	.92
White	226	57.84	1.10	75.68	1.09
Other	47	61.34	2.29	78.99	2.07
Hispanic	13	52.64	4.57	67.82	5.13
Asian	16	63.60	4.03	77.04	4.50
Additional	18	60.70	3.80	79.81	4.25
NSLP eligible ²	465	51.80	2.11	67.09	2.26
NSLP ineligible	217	59.53	1.96	77.26	2.13

¹ *M* is least squares mean

² National School Lunch Program (NSLP) eligibility status was unknown for 40 students

The generalized linear mixed model analysis indicated the main effects of time (pre-YES vs. post-YES), academic year, race/ethnicity, gender, and economic status were significant factors (Table 2). Least square means estimated from the model are reported. Posttest scores ($M = 70.72$, $SE = 1.28$) for all YES participants were significantly higher than pretest scores ($M = 53.76$, $SE = 1.19$), suggesting content area knowledge was gained by students during their

five days in the program (Figure 1). Mean test scores were higher in 2012-2013 ($M = 65.13$, $SE = 1.28$) than in 2011-2012 ($M = 59.36$, $SE = 1.47$). No observed difference in test scores between grades implies lessons and instructional methods employed in the curriculum were grade-appropriate. No time interactions were significant, indicating test score gains were not mediated by covariates.

Table 2. The main fixed effects used in the generalized linear mixed model to analyze YES pretest and posttest scores.

Source	<i>F</i>	<i>df_{num, dem}</i>	<i>p</i> -value
Model	52.09	15, 1348	.000
Time (pre-post)	168.23	1, 1348	.000
Year	10.38	1, 1348	.001
Grade	0.10	1, 1348	.752
Race/Ethnicity	53.55	2, 1348	.000
Economic Status ¹	43.42	1, 1348	.000
Gender	10.10	1, 1348	.002
Race/Ethnicity*Economic Status	0.20	2, 1348	.820
Race/Ethnicity* Gender	6.27	2, 1348	.002
Time*Race/Ethnicity	0.20	2, 1348	.820
Time*Economic Status	1.17	1, 1348	.279
Time*Gender	0.32	1, 1348	.574

Students from higher income situations had higher test scores ($M = 66.93$, $SE = 1.27$) than those from low-income homes ($M = 57.55$, $SE = 1.27$); however, gains were comparable between income levels (15.76% and 15.18%, respectively;

Figure 1). Although economic status and race/ethnicity were significant main effects in the model, their interaction was not ($p = .291$), indicating there is an effect of economic status that transcends racial/ethnic boundaries.

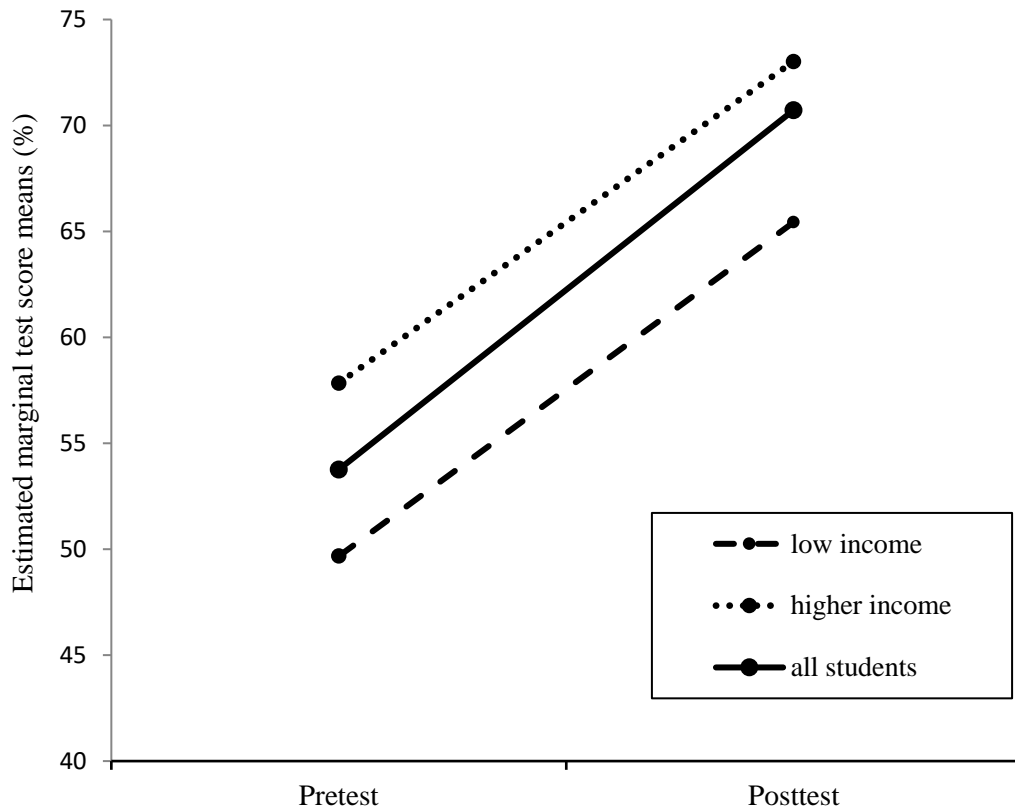


Figure 1. Mean test scores for all YES participants and by economic status (as indicated by eligibility for the National School Lunch Program: low income = eligible; higher income = ineligible).

The interaction of race and gender was significant (Figure 2) and indicates gender-based performance was not uniform across racial/ethnic groups. Post hoc, multiple-comparison tests showed that scores from White and Other male participants were similar ($p = .687$), whereas those of Black male participants were 20% and 21% lower, respectively ($p < .001$). A similar pattern was observed in female participants. Black female students scored an average of 8 to 14 percentage points lower than non-Black females ($p < .0001$); in comparison, female White and female Other participants performed similarly ($p = .194$). Within-ethnicity/race

comparisons of male and female achievement showed gender differences in Black students ($F = 18.31, p < .001$). Scores from Black male students were an average of 7% lower ($M = 50.18, SE = 1.32$) than those from Black female students ($M = 57.17, SE = 1.29$). In contrast, no significant gender differences in test performance were detected in White and Other students. No significant interactions between time and demographic factors (gender, economic status, or race/ethnicity) were detected, indicating that the effect sizes across levels were similar; i.e., no demographic group appeared to benefit from YES more than others.

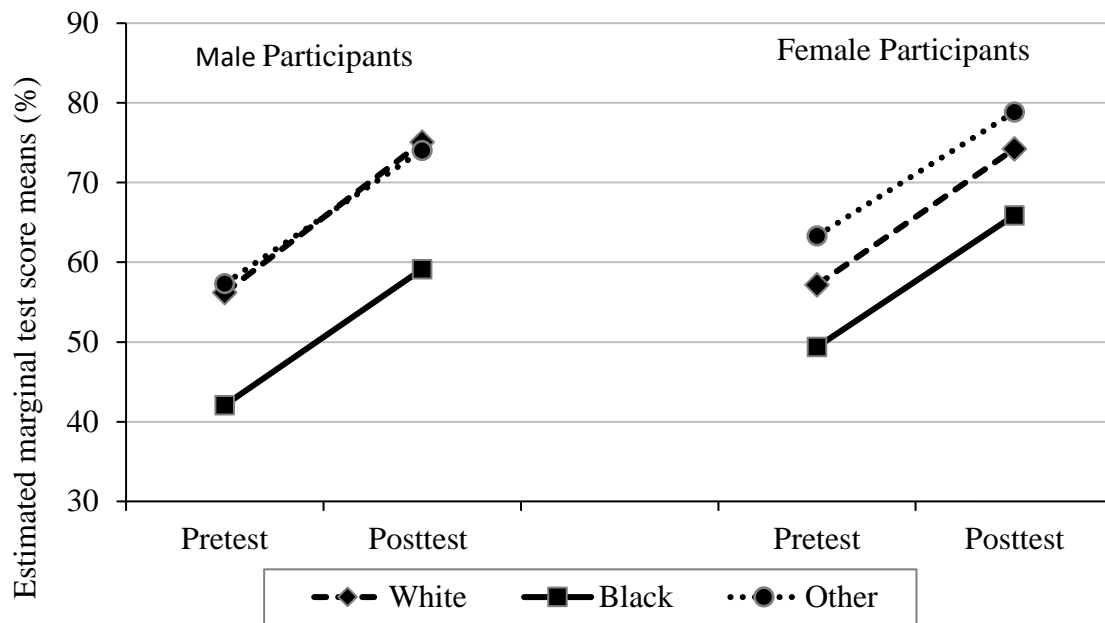


Figure 2. Mean test scores for YES participants by race/ethnicity and gender

DISCUSSION

The experiential learning pedagogy was effective at generating an average of 23% increase in program content knowledge in participants; thus, the results support our first hypothesis that students' posttest scores would improve from pretest levels in response to YES participation. Culen & Volk (2000) documented significant increases of 8-15% in posttest scores following a multi-week EE program for junior-high science classes. Similarly, Powell & Wells (2002) noted significant improvements of 18-24% on fifth grade posttests after 14 days of classroom-based, experiential EE. Handler & Duncan (2006), Kruse & Card (2004), and Markowitz (2004) reported self-perceived increases in student knowledge resulting from longer-term EE interventions.

Non-formal educational settings such as summer camps have the curriculum flexibility to offer in-depth coverage of environmental topics. They may also contribute to cognitive development in participants (Burrus-Bammel & Bammel, 1986;

Larson, Castleberry and Green, 2010). However, assessments may be biased toward positive outcomes since participants are often self-selected with preexisting interest in EE topics (Bogner, 1998; Lisowski & Disinger, 1991); they may not be representative of the larger youth population. Benefits of camp learning may be limited if transference of the acquired knowledge to classroom situations is impeded by lack of correlation to state standards (Randler, Ilg, & Kern, 2005). Additionally, economic and cultural barriers may restrict access to those with the means and motivation to attend, thereby exacerbating achievement gaps (Oakes, Ormseth, Bell, & Camp, 1990).

Few studies have examined the impact of demographic factors on learning through EE. Larson, Castleberry, & Green (2010) reported significantly lower baseline environmental knowledge scores among Black children than White children but did not specifically test the effect of race on EE outcomes. We observed comparable performance on YES pretests and posttests by White and Other (non-White

or unknown) female and male students; scores from Black female students were lower, and those from Black males were the lowest. Behavioral problems during YES participation caused black males to be disproportionately removed from the program, resulting in missing posttests and likely biasing performance estimates. Also, data for non-White and non-Black students were combined because of small sample sizes, potentially masking further differences in performance.

Research on the relationship between economic status and EE is limited. The correlation between race/ethnicity and economic status may result in effects of economic status being inadvertently subsumed into race/ethnicity effects (e.g., Hobbs & Stoops, 2002). However, research suggests student economic status may be a major factor impacting science education. Fisman (2005) documented a significant positive relationship between environmental knowledge and neighborhood income level. Standardized test scores in an urban, “disadvantaged”, North Carolina elementary school increased after integrating outdoor nature activities (NEETF, 2000). Five Louisiana K-8 schools with student populations similar demographically to our study population experienced improvements in their state test scores concurrent with implementation of EE programs (Emekauwa, 2004). These studies, in concurrence with our research outcomes, indicate the impact of economic status on EE should be examined further and separately from race/ethnicity.

Perhaps most importantly, we observed similar rates of improvement in posttest scores in all demographic groups. This result implies YES content, rigor, and instructional methods created equal learning opportunities for all demographic groups, and assessment instruments were appropriate for all cultural and economic backgrounds represented at the study school (Haycock, 2001; Lee, 2002). Educational researchers suggest challenging curricula taught in a safe and positive classroom environment by teachers possessing a strong content foundation can close achievement gaps (Becker & Luthar, 2002; Haycock, 2001; Kober, 2001). These elements were provided in YES, and students appeared to have responded correspondingly. These outcomes support

the YES pedagogy as a mechanism for closing achievement gaps and thus answer our second research question. Further research is needed to identify the length of time YES content knowledge is retained (e.g., Kuhar, Bettinger, Lehnhardt, Tracy, & Cox, 2010; Lisowski & Disinger, 1991; Randler & Bogner, 2002), particularly as mediated by race/ethnicity and economic status.

During the second year of our program evaluation, we did observe higher average pretest and posttest scores than during first year. Although YES instructors were the same throughout the assessment period, this pattern could be the result of improvements over time in content delivery, program implementation, and/or classroom management. However, it is also likely that the higher scores resulted from a broader, general improvement in student achievement rather than anything inherently associated with YES. This is supported by concurrent increases in the district’s scores on the fifth grade state standardized science assessment (56% of students were ranked proficient or advanced in 2013 vs 34% in 2012 [Kieffer, 2013]).

CONCLUSION

Environmental education that provides science-based knowledge, increases environmental awareness, improves critical-thinking skills, and promotes environmental stewardship is needed, particularly among underrepresented groups who are often unreached and negatively impacted. Yet EE remains an uncommon occurrence in formal educational settings, often inserted during brief windows of opportunity rather than intentionally incorporated. The immersion approach used in YES provides students with greater exposure to environmental science content and methods than generally found in classroom enhancement lessons without incurring the access barriers associated with extracurricular experiences nor the financial and facility requirements of environmental schools. The educational experience in YES resulted in positive knowledge gains of 15-17%--the equivalent of 1.5 letter grades--across gender, racial/ethnic, and economic groups, suggesting this pedagogy may be effective at closing achievement gaps. Wider adoption of experiential EE curricula taught by well-prepared educators may provide students--regardless

of race/ethnicities, economic levels, or gender--with the opportunity to succeed academically and address current environmental issues.

Conclusions of this study can only be applied to those students who attended YES. Students with behavior or learning issues that resulted in low enrollment hours or missing tests (often because the students were removed from YES) were not included in the evaluation. Our study population may be biased toward students who were capable of handling the new learning environment typified by hands-on and active lessons, often in teams or outdoors, and generally without homeroom teacher supervision.

Secondly, we assumed changes in test scores could be ascribed to the treatment rather than to familiarity caused by the pretest. In an evaluation of an EE program with similar target group and duration, Smith-Sebasto and Semrau (2004) concluded students who took a pretest were not sensitized to the instrument. However, since this was not tested in our study, we acknowledge the pretest could have influenced how students responded on the posttest and confounded assignment of treatment effects.

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Detection of Antibodies to SARS-CoV-2 Antigens in Serum of COVID-19 Infected and Vaccinated Patients by Immunoblot Analysis

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ABSTRACT

A COVID-19 immunoblot assay was developed to confirm the presence of antibodies to the SARS-CoV-2 spike (S) glycoprotein and nucleocapsid (N) antigens in patient serum. The S1 component of the SARS-CoV-2 S and the N antigen were expressed in *E. coli*, purified, fractionated by SDS-PAGE gel electrophoresis, and transferred to nylon membranes. Purification of the viral S1 and N proteins was confirmed by immunoblot analysis using rabbit polyclonal antiserum against the SARS-CoV-2 S and N antigens, respectively. Using this SARS-CoV-2 immunoblot assay, the presence of antibodies to the S1 and N antigens was confirmed in sera derived from SARS-CoV-2 positive patients, but not in sera derived from SARS-CoV-2 negative patients. In addition, antibodies to the S1 were detected in sera of COVID-19 vaccinated individuals. The results verify immunoblot assay as a reliable approach to confirm antibodies in serum of SARS-CoV-2 infected and vaccinated patients, providing evidence of immunity to COVID-19.

KEYWORDS: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), COVID-19, immunoblot

INTRODUCTION

The COVID-19 pandemic has had a detrimental impact on Mississippi's healthcare, economic, and educational systems. The disease has caused over one million confirmed infections with over 16,000 deaths ranking Mississippi among the top U.S. states in COVID-19 morbidity and mortality per capita (Mississippi Department of Health). While a vaccine has helped control the pandemic, infections and deaths persist, although at a lower rate, requiring continued diagnosis and treatment.

COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a positive-polarity RNA virus with helical capsid symmetry surrounded by an envelope. In addition to several non-structural proteins, the 29.9 kilobase (kb) RNA genome encodes four structural proteins, including the viral spike (S) and nucleocapsid (N) proteins (Mariano et al., 2020). The envelope S protein plays a critical role in binding to host cell receptors, permitting entry into cells (Walls et al., 2020). The S protein is composed of two subunits, S1 and S2. The S1 component includes the receptor binding domain (RBD) which directly binds to the host cell angiotensin-converting enzyme 2 (ACE2) receptor. The S2 subunit is involved with virus-host membrane fusion and entry. The highly basic

422 amino acid N protein binds to and packages the viral nucleic acid into a ribonucleocapsid and plays a role in viral assembly (Wu et al., 2023).

The development of diagnostic tools has been vital for controlling the COVID-19 pandemic (Yuce et al., 2021). A reverse-transcriptase polymerase chain reaction assay (RT-PCR) accurately detects viral RNA within an infected patient nasopharyngeal swab while a rapid antigen assay detects viral proteins in a patient specimen. While these are effective approaches to confirm SARS-CoV-2 infection during the acute stage of COVID-19 disease, they may not detect viral RNA or antigen during the convalescent stage of disease after viral load is cleared from the body.

Serological assays are valuable to confirm SARS-CoV-2 infection during the acute stage of disease and in patients that have recovered from the infection (Kontou et al., 2020). Serological assays are an indirect diagnostic approach and do not directly detect viral components. However, detection of anti-viral antibodies in patient serum can confirm recent or past infection and verify effective vaccination. Enzyme linked immunosorbent assay (ELISA) and lateral flow assays are most commonly employed to screen patient serum for the presence of antibodies

against SARS-CoV-2 antigens. While these serological assays are generally reliable, false positive results may occur when antibodies react with non-specific cross-reacting antigens (Peterick, 2020; Ye et al., 2021). Such a result suggests that a person may not truly have protective antibodies and immunity and thus may be susceptible to SARS-CoV-2 infection. In this study, a COVID-19 immunoblot assay was employed to provide a reliable means to confirm the presence of antibodies to the SARS-CoV-2 S1 and N antigens in sera of COVID-19 infected and vaccinated patients.

MATERIALS AND METHODS

Expression and purification of SARS-CoV-2 S1 and N proteins

SARS-CoV-2 genes (Wuhan strain) encoding the S1 component of the S gene and the N gene were chemically synthesized by Integrated DNA Technologies (IDT). The S1 DNA sequence encodes 588 amino acids including amino acids 686 through 1273 of the S protein with a predicted molecular size of 75 kilodaltons (kD). The N gene includes the 1,260 bp open reading frame (ORF) encoding 420 amino acids with a predicted size of 45 kD. The S1 and N DNA sequences were PCR amplified using high fidelity Pfu polymerase and then inserted into the cloning site of the Invitrogen Champion pET100/D-Topo expression vector such that the carboxy end of the expressed protein included a 6X histidine tag (Figure 1A).

Expression vectors containing the S1 and N genes were transformed into chemically competent *E. coli* BL21 cells by heat-shock at 42°C for 30 seconds and incubated overnight at 37°C in 10 mls Luria Broth (LB) media including carbenicillin (50 µg/ml). The following day, 150 mls of culture was incubated for two hours at 37°C to an optical density 0.6 followed by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG, 100 mM) to induce expression of the S1 and N genes under control of the T7 promoter and lac operator. After four hours, cells were pelleted by low-speed centrifugation and frozen overnight at -80°C. Cell lysates were prepared by resuspension in guanidinium buffer, three freeze-thaw cycles, and

sonication, followed by centrifugation (3000g for 15 minutes).

The his-tagged S1 and N proteins were purified on His-Pur-Nickel-NTA resin (Thermo Scientific) columns using the Invitrogen ProBond Purification System. Briefly, the cell lysates were added to the resin column, washed with denaturing binding buffer, and the purified S1 and N proteins were released in elution buffer (pH 4.0). Following overnight dialysis in 10 mM Tris, pH 8.0, to remove urea, the preps were concentrated to 0.5 ml using Pierce Protein Concentrators (30 kD molecular weight cut-off) and frozen at -80°C.

Immunoblot analysis

The purified S1 and N proteins were solubilized in Lammelli sample buffer (0.1 M Tris base, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.002% bromophenol blue, pH 6.8) and fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) at constant 100 volts for 75 minutes. The S1 and N proteins were transferred to Novex PVDF membrane filters by the Western transfer method for 90 minutes at constant 20 volts. Protein molecular size standards were included on each gel (Thermoscientific Spectra Multicolor Broad Range Protein Ladder). Filters were incubated in blocking buffer with 5% nonfat protein (Blotto) prior to immunoblot assay.

The immobilized S1 and N proteins were reacted with rabbit anti-SARS-CoV-2 S antibody or anti-SARS-CoV-2 N antibody (Sino Biological), respectively, or with patient serum samples (1/500 dilution in blocking buffer) for two hours at 25°C. After washing, goat anti-rabbit or anti-human IgG horseradish peroxidase antibody conjugate (Invitrogen) was added (1 to 2000 dilution in blocking buffer) for one hour at 25°C, followed by extensive washing. Antibodies to the S1 and N antigens were detected by chemiluminescence using Pierce Enhanced Chemiluminescence (ECL) blotting substrate and chemiluminescent imaging (Azure Biosystems).

Patient serum samples

Twenty patient serum samples derived from COVID-19 patients were purchased from Ray Biotech (COVID-19 patient serum set #1,

Peachtree Corners, GA.) (Table 1). Patients were confirmed to be SARS-CoV-2 positive by ELISA and by RT-PCR assay. COVID-19 ELISA titers for patients are available from Ray Biotech. COVID-19 negative serum samples were collected from individuals prior to the COVID-19 outbreak in the U.S. and obtained from Ray Biotech. This nonidentified patient cohort included sera derived from males and females

ages 18 to 85. Most sera were collected during the convalescent stage of disease after a positive COVID-19 RT-PCR or ELISA test. Serum samples from nonidentified individuals vaccinated with the Moderna mRNA vaccine were purchased from Ray Biotech. Blood samples were collected from subjects with informed consent as approved by Ray Biotech IRB (ID# 8291).

Table 1. Detection of antibodies to SARS-CoV-2 S1 and N antigens in sera of infected and vaccinated patients

A. COVID-19 Patients

Patient ID	Sex-Age	Delay (days) ¹	Test	Anti S1 ²	Anti-N ²
PS 302	F - 67	33	RT-PCR	+	+
PS 303	M - 76	33	RT-PCR	+	+
PS 304	M - 85	33	RT-PCR	+	+
PS 305	F - 76	33	RT-PCR	+	+
PS 308	M - 50	34	RT-PCR	+	+
PS 310	F - 76	34	RT-PCR	+	+
PS 313	F - 69	34	RT-PCR	+	+
PS 314	F - 64	33	RT-PCR	+	+
PS 333	M - 54	34	RT-PCR	+	+
PS 346	F - 80	34	RT-PCR	+	+
PS 347	F - 83	8	RT-PCR	+	+
PS 348	F - 76	1	RT-PCR	+	+
PS 351	M - 67	4	RT-PCR	+	+
PS 357	F - 74	33	RT-PCR	+	+
PS 358	F - 63	33	RT-PCR	+	+
PS 359	M - 61	33	RT-PCR	+	+
PS 375	ND	ND	RT-PCR	+	+
PS 602	F - 30	9	RT-PCR	+	+
PS 603	F - 21	30	ELISA	+	+
PS 616	F - 59	35	RT-PCR	+	+

B. COVID-19 Negative Individuals

Patient ID	Sex-Age	Anti S1 ²	Anti-N ²
SN 204	M - 46	-	-
SN 205	F - 22	-	-
SN 206	M - 18	-	-
SN 207	M - 47	-	-
SN 208	F - 25	-	-
SN 209	M - 65	-	-
SN 210	M - 28	-	-
SN 211	M - 63	-	-
SN 212	F - 38	-	-
SN 213	M - 36	-	-

C. Vaccinated Individuals

Vaccinee ID	Anti S1 ²	Anti N ²
V 730	+	-
V 731	+	-
V 732	+	-
V 736	+	-
V 800	+	-

¹ days between date of positive COVID-19 test and collection of patient serum

² positive or negative detection of antibodies to the viral S1 or N proteins by immunoblot analysis

RESULTS

Expression and purification of the SARS-CoV-2 S1 and N proteins

The SARS-CoV-2 S1 and N genes were chemically synthesized, inserted into an expression vector (Figure 1A), transformed into *E. coli*, and induced for expression. His-tagged S1 and N proteins were purified by affinity chromatography, fractionated by SDS-PAGE,

and transferred to PVDF membranes. The S1 and N proteins on the blots were detected using rabbit polyclonal antibodies to the SARS-CoV-2 S and N proteins, respectively, followed by reaction with HRP-conjugated goat anti-rabbit IgG antibody and chemiluminescence assay (Figure 1B). The results confirmed purification of the 75 kD S1 and the 45 kD N proteins for use as antigens for detection of antibodies to the SARS-CoV-2 proteins in patient serum.

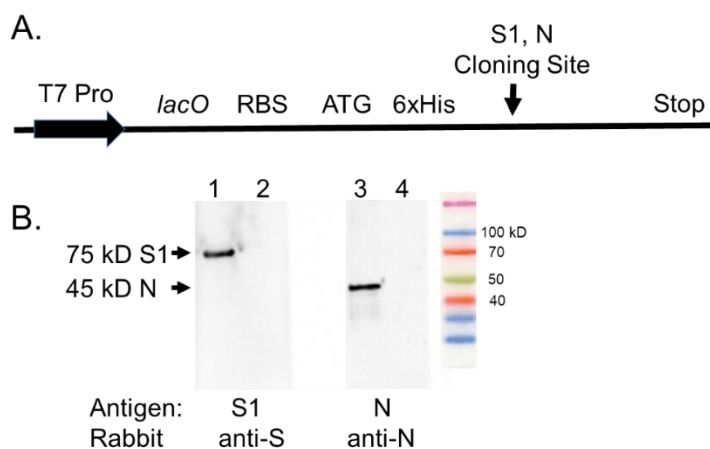


Fig. 1- Expression and purification of His-

tagged SARS-CoV-2 S1 and N proteins in *E. coli* by column chromatography. (A) The S1 and N genes were inserted into the cloning site of the pET100/Topo expression vector under control of a T7 promoter. The vector includes a *lac* operator, ribosome binding site (RBS), ATG start site, a polyhistidine tag (6X His), and a stop site. (B) Detection of purified S1 and N proteins by immunoblot analysis using primary rabbit anti-S and anti-N antibodies, respectively, secondary goat anti-rabbit IgG-HRP labeled antibody, and chemiluminescent imaging. Protein molecular weight standards are included.

Detection of antibodies to the S1 and N proteins in COVID-19 patient sera

Antibodies to the SARS-CoV-2 S1 and N proteins in patient sera were detected by immunoblot analysis. Sera derived from 20 U.S. patients confirmed to have COVID-19 infection by RT-PCR and/or ELISA were analyzed for the presence of antibodies to the viral S1 and N proteins. Sera was also derived from ten individuals prior to the COVID-19 outbreak in the U.S. The results demonstrated that all 20 COVID-19 positive patients had antibodies

against both the SARS-CoV-2 S1 antigen (representative results in Figure 2A, Table 1A) and the N antigen (representative results in Figure 2B, Table 1A). The assay's specificity was indicated by the inability of patient antibodies to react with protein molecular weight standards in adjacent lanes. In contrast, antibodies to the S1 or N proteins were not detected in COVID-19 negative patient sera collected prior to the U.S. SARS-CoV-2 outbreak (Figure 2A, 2B, and Table 1B). The results confirmed detection of antibody responses to the S1 and N proteins in patients following SARS-CoV-2 infection.

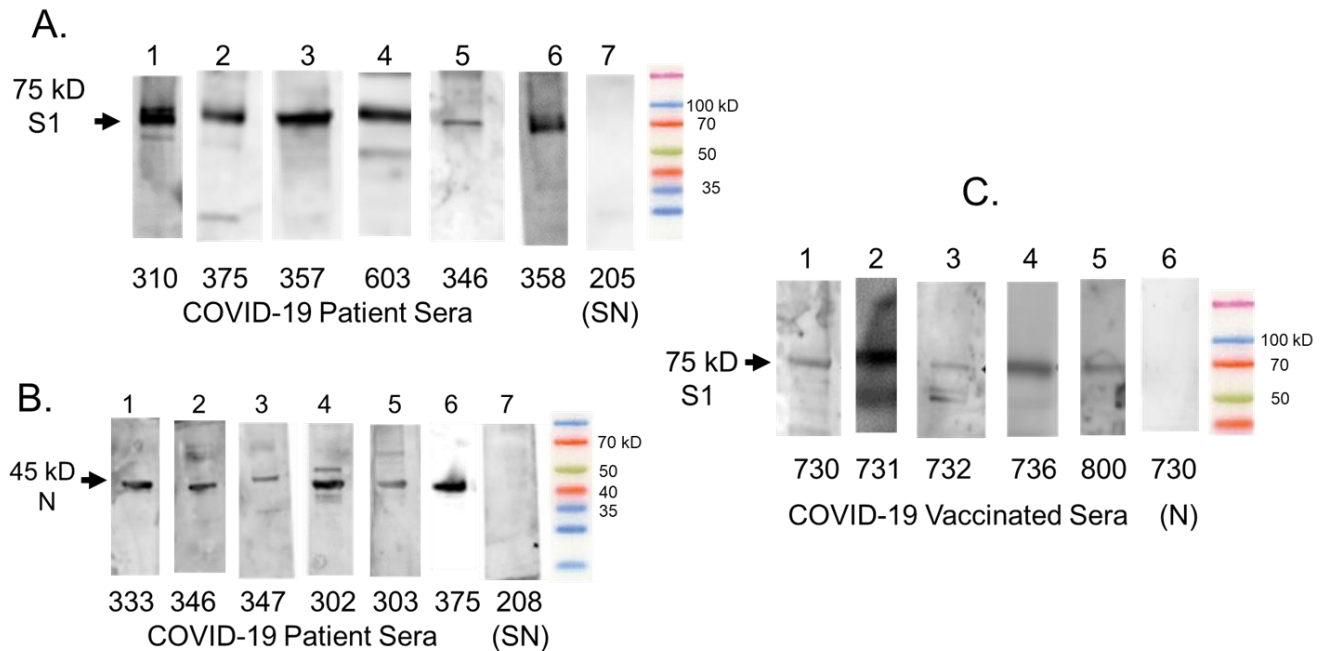


Figure 2- Detection of antibodies to the SARS-CoV-2 S1 and N antigens in sera of infected and vaccinated patients by immunoblot analysis using secondary HRP-tagged anti-human IgG antibody and chemiluminescent imaging. (A) Detection of antibody to the SARS-CoV-2 S1 antigen in sera of six COVID-19 patients (lanes 1-6). Serum from an uninfected individual (lane 7). (B) Detection of antibody to the SARS-CoV-2 N antigen in sera of six COVID-19 patients (lanes 1- 6). Serum from an uninfected individual (lane 7) . (C) Detection of antibody to the SARS-CoV-2 S1 antigen in sera of five vaccinated individuals (lanes 1- 5). Lack of detection of antibody to the SARS-CoV-2 N antigen in serum of a vaccinated individual (lanes 6). Protein molecular weight standards are included.

Detection of antibodies to the S1 protein in COVID-19 vaccinated individuals

Sera derived from five individuals immunized with the COVID-19 Moderna mRNA vaccine, which expresses the SARS-CoV-2 S protein, were analyzed for immunoblot detection of antibodies to the SARS-CoV-2 S1 and N proteins. The results demonstrated that all five COVID-19 vaccinated individuals had antibodies against the S1 protein (Figure 2C, Table 1C). In contrast, none of the five vaccinated individuals had antibodies to the N antigen indicating lack of prior exposure to SARS-CoV-2. The detection of antibody responses to the viral S1 protein confirmed immunization of these patients with the COVID-19 Moderna vaccine.

DISCUSSION

Serological assays are valuable to confirm a diagnosis of acute COVID-19 infection or to make a retrospective diagnosis in patients that have recovered from infection but are no longer positive as determined by RT-PCR or antigen based assay. In addition, serological assays are important for epidemiological studies to determine the proportion of exposed individuals and overall immunity in the population.

The accuracy of COVID-19 antibody tests depends on a high degree of sensitivity and specificity (Kontou et al., 2020). Sensitivity refers to the ability of a serological test to

correctly recognize patients that actually have had COVID-19 infection. Specificity indicates the ability of a test to rightly identify individuals that have truly not been infected. A false positive antibody test result indicates a person that tests positive for COVID-19, but has not actually been infected and does not have immunity. A false negative COVID-19 antibody test may occur when the test is performed on serum taken during the early stage of infection, prior to the person developing antibodies against the virus.

ELISA and lateral flow assays are the most common means to determine the COVID-19 immune status of an individual. The accuracy of these COVID-19 antibody tests was evaluated in a systematic meta-analysis including 38 studies involving 7848 individuals (Kontou et al., 2020). ELISA based methods had an overall sensitivity of 93.5% ranging from 90.0% to 97.1% with a specificity ranging from 96.1 to 99.5%. Lateral flow based tests had a lower overall sensitivity of 80% (range 66.3 to 93.5%) with a specificity of 98.4% (range 96.9 to 99.9%).

The results of this COVID-19 immunoblot assay to detect anti-viral antibodies demonstrated a high sensitivity with all 20 of the RT-PCR or ELISA confirmed patients testing positive for antibodies against both the SARS-CoV-2 S1 and N antigens. The specificity of the immunoblot assay was 100% with each of the ten patient sera derived prior to the U.S. COVID-19 outbreak testing negative for antibodies to the S1 and N antigens.

COVID-19 antibody tests detecting IgG antibodies to the virus in patient serum perform with a higher sensitivity than tests detecting IgM antibodies (Kontou et al., 2020). IgM antibodies generally arise early during acute SARS-CoV-2 infection, but are transient and decline during convalescence, although IgM responses can be variable (Bauer, 2020; Kaduskar et al., 2022). A stronger IgG response is induced during acute infection and persists through convalescence and recovery. The immunoblot assay developed in this study focused on detection of IgG antibodies to SARS-CoV-2 antigens since most of the patient sera was collected during the convalescent stage of the disease.

The SARS-CoV-2 S1 and N antigens were chosen as targets for the immunoblot assay as these viral proteins are prominent immunogenic coronavirus antigens (Liu et al., 2020). Antibody tests employing the S1 protein as target antigen have greater sensitivity than antibody tests using the S2 protein target (Okba et al., 2020) likely due to a greater number of antigenic epitopes within the S1 compared to the number of antigenic sites within the S2 (Maache et al., 2006; Shafqat et al., 2022). In addition, the S1 is demonstrated to be a more specific antigen compared to the S2 which has a higher degree of cross-reactivity with other coronaviruses (Okba et al., 2020). Likewise, antibody assays based on the N antigen have been shown to have high sensitivity, but a lower specificity compared to S based assays due to conservation between the SARS-CoV-2 N protein with other coronaviruses (Kontou et al., 2020)

This COVID-19 immunoblot study utilized both the S1 and N antigens for detection of SARS-CoV-2 antibodies in patient serum, rather than using a single protein. The use of multiple SARS-CoV-2 antigen targets enhances the sensitivity and specificity of immunoblot assays and reduces the chances of false-positive and false negative results (Shah et al. 2021). This immunoblot assay utilized purified SARS-CoV-2 S1 and N targets that were expressed in *E. coli* rather than protein lysates of SARS-CoV-2 infected cells. The use of recombinant antigens expressed in *E. coli* has advantages including less stringent biosafety requirements, improved assay standardization, and cost-effectiveness.

This study also demonstrates the utility of an immunoblot assay to confirm antibodies to SARS-CoV-2 S glycoprotein in serum of COVID-19 vaccinated patients. Antibodies to the SARS-CoV-2 S1 were detected in serum derived from five individuals vaccinated with the Moderna mRNA vaccine which expresses the SARS-CoV-2 S protein. Antibodies to the N protein were not detected in sera derived from these vaccinees indicating that these individuals had not been previously infected with SARS-CoV-2.

ELISA and lateral flow-based assays provide rapid, sensitive, and possibly quantitative approaches to detect SARS-CoV-2 antibodies in patient serum. In comparison, the immunoblot blot approach is limited in that it is not quantitative and is more labor-intensive requiring SDS-PAGE and transfer of proteins to membranes in addition to antibody detection by chemiluminescent analysis. Immunoblot analysis, while not a primary screening tool, does provide a specific and sensitive approach to confirm detection of antibodies to SARS-CoV-2 antigens in infected and vaccinated individuals. The assay reduces the chance of false positive results, especially in persons at high risk of severe COVID-19. This traditional immunoblot analysis may be adapted to detect multiple SARS-CoV-2 antigens by multiplex assay and automated to save time and enable a less exhaustive protocol (Shah, et al. 2001, Edouard et al. 2021).

Demonstration of antibodies to SARS-CoV-2 antigens by antibody binding assays, including ELISA and immunoblot tests, provides evidence of immunity and may correlate with immune protection against subsequent SARS-CoV-2 infection. Seroconversion is usually followed by a steady decline in SARS-CoV-2 viral load (Wolfel, 2020). In addition, the presence of antibodies to the SARS-CoV-2 S and N antigens is associated with a reduced risk of reinfection for at least six months (Harvey et al., 2021; Lumley et al., 2020). Viral neutralizing antibody titers are optimal indicators of immune protection, but serum neutralization assays involve infectious virus and biosafety issues. The COVID-19 immunoblot assay described here provides a highly sensitive and specific approach to confirm SARS-CoV-2 antibodies and possibly immune protection, especially for susceptible individuals at risk of severe COVID-19 infection.

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AUTHOR DISCLOSURES

The authors have not stated any conflicts of interest.

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Wildfire and Other Environmental Hazard Detection Capabilities of a Low-Cost System

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ABSTRACT

Early detection of environmental hazards such as wildfires and harmful algal blooms is often limited by the high cost and restricted accessibility of satellite and aerial monitoring systems. This study evaluates the feasibility of a low-cost, portable imaging system constructed from modified Raspberry Pi cameras and narrowband optical filters for hazard detection. The system targets two key signatures: potassium emission near 770 nm associated with burning vegetation and spectral reflectance differences between plants and algae in the 710–750 nm range. Spectral measurements confirmed a distinct potassium emission peak in burning vegetation that was absent in non-biological materials, enabling discrimination of wildfire sources. Reflectance analysis revealed a measurable divergence between plant and algal spectra, allowing the development of a simple index (I_a) that reliably differentiates between the two. Results demonstrate that inexpensive, readily available components can produce meaningful environmental sensing data with potential applications in early hazard detection. This approach offers a scalable and accessible supplement to conventional monitoring technologies, with implications for improving environmental response in resource-limited settings.

KEYWORDS: Wildfire detection; Harmful algal blooms; Low-cost sensing; Multispectral imaging; Near-infrared spectroscopy; Environmental monitoring

INTRODUCTION

Dengue fever, caused by the dengue virus (This study addresses the cost and accessibility limits of satellite monitoring of environmental hazards with a proposal for a low-cost and highly portable imaging system. The goal is to demonstrate its feasibility for the detection of these hazards. Specifically, it focuses on wildfire and algae detection—both of which can lead to irreversible damage, with harmful algae blooms posing a severe threat to Mississippi. This approach uses inexpensive Raspberry Pi cameras with their infrared filters removed, combined with narrow bandwidth optical filters, to detect characteristic features of burning vegetation and algae. The system reliably detected the 770 nm potassium spike that is characteristic of vegetation and was able to distinguish algae from plants using a reflectance index (I_a) between 710–750 nm. By offering a more accessible and scalable monitoring solution, this technology could supplement the high-cost monitoring technologies on drones and spacecraft, ultimately improving early detection and response times.

MATERIALS AND METHODS

Two systems were used in this study—a camera and a small spectrometer. There were two phenomena studied—emission from fires and reflectance from plants and algae. For the sake of brevity, this paper presents only spectrometer measurements of fire emission and only camera measurements of plant/algae reflectance.

The camera was a Raspberry Pi HQ camera that had the IR filter and color mask removed. An f/2, 25 mm focal length lens was used. A set of Thorlabs optical filters, 10 nm full width half maximum, with center wavelengths ranging from 650 – 780 nm, was used to isolate specific wavelength bands. The spectral response of the Raspberry Pi camera plus filters was measured by Innovative Imaging and Research (<https://www.i2rcorp.com/>) at the NASA Stennis Space Center. The spectrometer was an uncalibrated Vernier Go Direct Spectrovis Plus with a flexible probe attached.

Images of plants and algae were captured using a NorbEVERYDAY-SUN light directly illuminating the samples. This light has a visible spectrum similar to the sun. For each image of algae and plants, a white reference piece of paper was placed beside the sample. The ratio of the average corrected pixel values for the densest portion of the sample to those for the reference paper was the reflectance. The pixel values of all images were corrected for the response curves and analyzed using Mathcad Prime to generate spectral plots. The tests described here were done repeatedly over a 10-week period. The data presented are representative of the large amount of data acquired.

RESULTS

The spectral data from burning pine needles show a clear spike at 770 nm, whereas those from burning strips of plastic bags do not. This spike, clearly visible in Figure 1, corresponds to emission from hot potassium, an element present in most vegetation but generally not in plastic.

Reflectance measurements of algae and plants reveal a noticeable difference in the 710–750 nm range. As shown in Figure 2, plant reflectance increases in this range, while algae reflectance stays flat or declines.

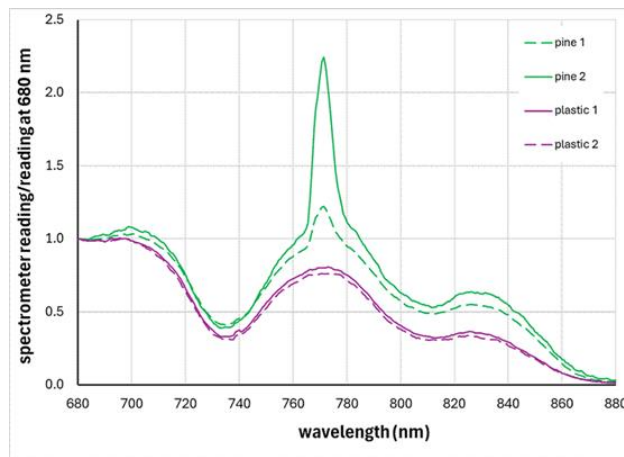


Figure 1 Near infrared spectra of burning pine needles and plastic shopping bags showing potassium spike at 770 nm characteristic of burning vegetation.

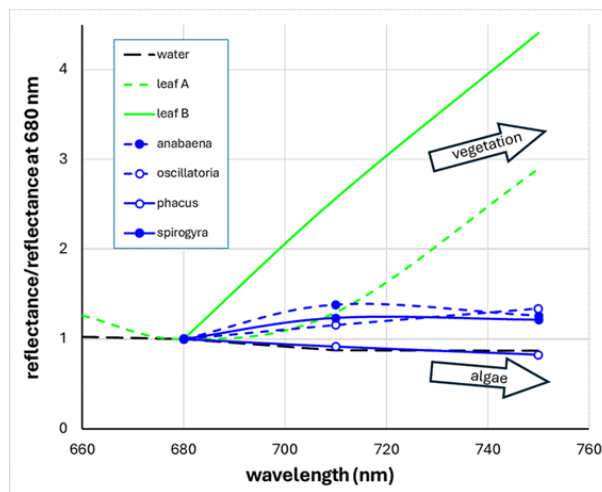


Figure 2 Reflectance of leaf and algae samples in the far red visible region. Equation 1: $I_a = (R_{750} - R_{710}) / R_{750}$

DISCUSSION

With respect to identifying wildfires, literature data support potassium spikes at “766.5 and 769.9 nm” (Amici et al., 2011). Our data confirm a potassium spike around 770 nm. This spike is characteristic of burning vegetation and can be used to detect fires sooner than traditional methods, which typically rely on thermal radiation. The method studied could be used to identify smoldering brush and therefore prevent wildfire spread earlier.

Literature findings indicate a difference between the reflectance of algae and that of plants at wavelengths beyond 680 nm (Fernandez-Figueroa et al. 2021). Our data supports these findings. While plant reflectance is increasing between 710-750 nm, algae reflectance is flat or decreasing. The difference in the two reflectance ranges allow the creation of an algae index I_a that can reliably distinguish plant matter from algae where $I_a = (R_{750} - R_{710}) / R_{750}$ with R_{750} and R_{710} being the reflectances at 750 nm and 710 nm, respectively. For the data in Figure 2, the I_a values for the leaves are 0.42 and 0.55. The algae and water indices are in the range of \square 0.11 to 0.14, with all but *Oscillatoria* being negative.

There is a distinct deviation in reflectance measurements between 710 – 750 nm. The slope for plants noticeably trends upwards beyond 710 nm (the characteristic “Red Edge” of vegetation), while the slope for algae trends downwards around 710 nm. As a result, the index will be positive for plants and negative or nearly zero for algae.

The tests and results presented here were done in a chemistry lab room with general room lighting that was not controlled. The influence of this light needs study. The distance between the samples and the sensors was kept constant. A study varying the distance should be explored.

A companion effort at Mississippi State University has developed two multispectral systems using similar cameras and computers. Integration with a drone and testing are currently

underway.

Meaningful hazard detection does not require expensive satellites or specialized infrastructure. A system built from accessible components – affordable cameras, interchangeable filters, and easily accessible spectrometers – can provide reliable, early indicators of both wildfire activity and algal presence. By making advanced sensing achievable for schools, community groups, and regions with limited resources, this approach offers a scalable way to enhance environmental safety.

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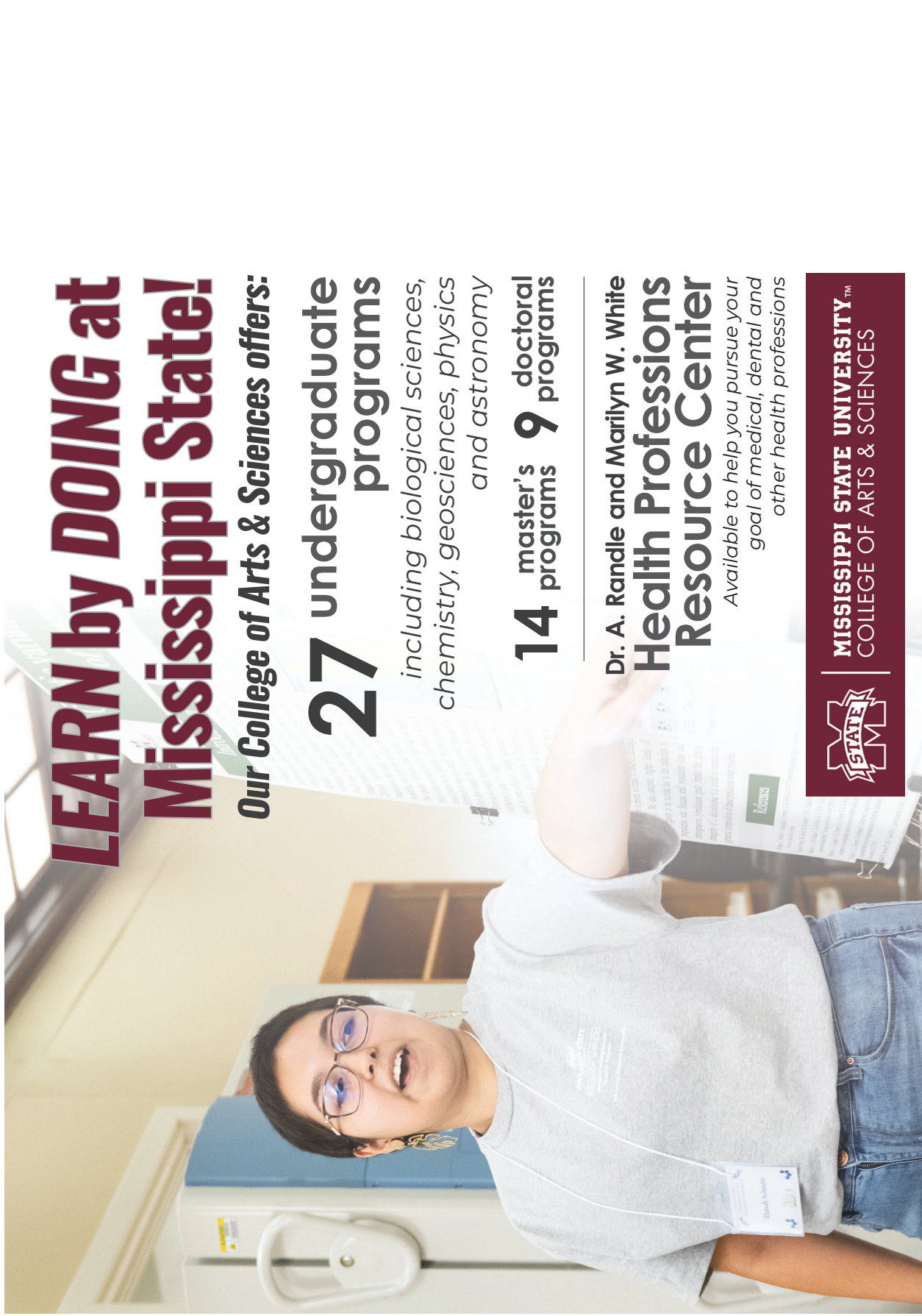
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