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THIN LAYER CHROMATOGRAPHIC ANALYSIS OF STABILITY OF CURCUMIN AND CURCUMIN IN TURMERIC AFTER REFLUXING IN WATER AND USING DOUBLE MOBILE PHASE AFTER HEATING IN EDIBLE OILS

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ABSTRACT

Turmeric is a popular curry spice widely used in cooking ethnic foods around the world. The active ingredient of turmeric is curcumin which has strong antioxidant and anti-inflammatory properties. These properties make curcumin a candidate for therapeutic investigations for many diseases including cancer and Alzheimer's. The objective of this study is to study the effect of heat on reference curcumin and curcumin in turmeric in both water and edible oil, as well as isolated curcumin in water. This investigation will help determine the stability of curcumin in turmeric in domestic cooking. Typically, curcumin dissolved in oil cannot be analyzed successfully using thin layer chromatography (TLC) due to interference from the oil. A new TLC method was developed using two different mobile phases: hexane followed by a mixture of dichloromethane (98.5%) and methanol (1.5%). The TLC plates were then scanned with a standard desktop scanner and used for quantification via Quantity One 1-D analysis software (Bio-Rad Laboratories). It was found that when refluxed in water, reference curcumin and isolated curcumin were fairly stable, but curcumin in turmeric degraded by as much as 47% in 60 minutes. It was also found that at increasing temperatures from ambient temperature to 250°C, by 250°C, reference curcumin dissolved in oil had degraded by 25%, but curcumin in turmeric had degraded by 70%. Finally, it was found that when samples dissolved in oil were held at a maintained temperature for 60 minutes, all samples remained relatively stable until 225°C, at which point about 45% degradation was observed in both reference curcumin and curcumin in turmeric.

Keywords: Thin Layer Chromatography, Curcumin, Turmeric, Double mobile phases, Heat stability, water, Edible oils.

INTRODUCTION

Turmeric is a popular curry spice widely used in the Indian sub-continent, South East Asia, Middle East and Africa. This yellow powder has been used for centuries for various ailments in the Indian Ayurvedic and Chinese traditional systems of medicine. The USFDA classifies turmeric as 'Generally Recognized as Safe.' The active compound in turmeric is curcumin, which has strong antioxidant and antiinflammatory properties. Because of these properties, curcumin has become a candidate for research in manifold directions. A recent review describes the latest situation of research on curcumin (1). Recent research has shown that curcumin could be used to prevent, as well as to treat, various maladies including HIV, cancer and Alzheimer's disease (2). A large number of turmeric/curcumin related patents granted in the USA (more than 120 total patents granted since 1976) indicate the potential of curcumin as a future drug of choice for some diseases. For example, turmeric has been found more effective in inhibiting formation of protein fragments than many other drugs being tested as Alzheimer's treatment (3). Similarly, curcumin hybridized with melatonin has been showing promising results as an Alzheimer's treatment as well, stopping cell death more effectively than either molecule by itself (4). Countries in which turmeric is used as a daily dietary spice have significantly reduced cases of Alzheimer's disease and many forms of cancer when compared to the incidences seen in the USA (5, 6, and 7). Turmeric is a curry staple in India, where Alzheimer's disease rates are reportedly among the world's lowest. More specifically, the prevalence of Alzheimer's disease among 70- to 79-year-olds in India is 4.4 times lower than that of the United States (5). Preliminary results suggest that could be because of the consumption of turmeric cooked into food. Knowing the effects that cooking has on the stability of curcumin would thus be very useful.

The stability of curcumin under various pH conditions and in physiological matrices has already been established (8). It has been reported that curcumin in aqueous media is highly stable at room temperature at a pH below 7 (9). However, there is one report (10), which indicated the loss of over 85% of curcumin between 15 and 30 minutes of cooking.

Curcumin is not typically soluble in water. However, it is slightly soluble (0.02-0.04%) in edible oils such as peanut, olive and vegetable oils (11). Much of Indian cooking, especially curries with spices including turmeric, is heated in edible oil.

We have chosen to study curcumin and turmeric in both water and edible oil (peanut or vegetable oil). In addition to dissolving curcumin, edible oil also allows for heating to much higher temperatures. The standard TLC analysis of curcumin using a normal TLC plate with a mobile phase of 97% dichloromethane, 3% methanol works well (12). However, when the same mobile phase was used for the oil-based solution of curcumin, the spots were malformed due to the interference of the oil. The reverse phase TLC plate produced no better results. Attempts were made to use double mobile phases.

The objective of this study is to find the effect of heat, at temperatures up to simulated cooking temperatures, on the stability of reference curcumin and curcumin in turmeric.

MATERIALS AND METHODS

Materials. The reference sample, curcumin used in this investigation was bought from Sigma-Aldrich and is a mixture of curcuminoids. Curcumin is the major component, making up about 77% of the sample, while its related compounds are present to а lesser extent. Demethoxycurcumin makes up about 17% and bisdemethoxycurcumin makes up about 3% of the reference sample (12). Commercially available turmeric (Radhuni brand bought from Indian grocery shop), peanut oil (LouAna brand), and vegetable oil (Great Value brand) were used. Normal phase silica-gel HPTLC plates, plastic or glass supported were purchased from Fisher Scientific. Other chemicals used, including dichloromethane and methanol, were analytical grade. All heating was carried out in a regulated heating mantle.

Isolation of Curcumin from Turmeric. Curcumin was isolated using dichloromethane according to the procedure of Anderson et al. (12) from turmeric. A sample of 5.0 g turmeric in 30 mL dichloromethane was prepared in a 50 mL round-bottom flask with a magnetic stirrer. The sample was refluxed for 60 minutes. Normal filtration was done, leaving behind the insoluble compounds. The filtrate was collected in a beaker. A volume of 20 mL hexane was added to the filtrate, precipitating the curcuminoids. The rest of the solution, an oily mixture, was decanted off the top into a separate beaker. All three resulting samples, the curcuminoids, oily mixture, and insoluble compounds, were dried in the hood. TLC was done between isolated curcumin and reference curcumin for comparison.

Double Mobile Phase TLC. Each sample dissolved in oil was analyzed using double mobile phase TLC. A plate was prepared and run normally using hexane as the first mobile phase. The plate was then dried, and run again with a second mobile phase of 98.5% dichloromethane, 1.5% methanol.

Quantification of Curcumin using Quantity One 1-D Software. For all three experiments, the quantification of curcumin and related compounds was done by scanning the chromatographic plates. Scanning was carried out with a Canon Pixma MG2220 standard desktop scanner. Scanned images were converted to TIFF format using Photoshop and then subjected to Quantity One 1-D analysis software (13)

Reflux of Sample in Aqueous Phase. Three samples of 10 mg curcumin in 25 mL water were prepared in 50 mL round-bottom flasks with magnetic stirrers. One sample was stirred for 30 minutes with no heating. The other two samples were refluxed for 30 and 60 minutes respectively. The curcumin in water was then extracted using two extractions of 15 mL dichloromethane and a separatory funnel. Once the samples were all collected, they were measured and additional dichloromethane was added as needed to reach a volume of 30 mL per sample. TLC was done with 4 μ L of each solution and using a mobile phase of 98.5% dichloromethane, 1.5% methanol. This procedure was then repeated using samples of 200 mg turmeric in 25 mL water, and again using 10 mg of curcumin isolated from turmeric in 25 mL water.

Continuous Heating of Samples in Edible Oil. A sample of 7 mg curcumin was dissolved in 30 mL oil in a 50 mL round-bottom flask with magnetic stirrer and heated to 250° C. As the temperature increased, samples of 1 mL

solution at various temperatures were collected. Double mobile phase TLC was done with 2 μ L of each sample. This procedure was then repeated using 180 mg turmeric in 30 mL oil.

Heating of Samples at Different Maintained Temperatures. A volume of 30 mL oil was put in a 50 mL round-bottom flask with a magnetic stirrer and heated to 100° C. The temperature was maintained by adjusting the dial on the regulated heating mantle to prevent the temperature from deviating too far from the desired 100° C. When the temperature was felt to be stable, 7 mg curcumin were dissolved in the oil. The temperature was then maintained at 100° C for 60 minutes while samples were collected at 10 minute intervals. Double mobile phase TLC was done with 2 µL of each. This procedure was then repeated at various temperatures, and repeated again at various temperatures using 200 mg turmeric.

For all trials, the temperature was maintained within two degrees Celsius of the target temperature. Reference curcumin was tested at $100\pm1^{\circ}$ C, $200\pm2^{\circ}$ C, and $225\pm2^{\circ}$ C. Turmeric was tested at $100\pm1^{\circ}$ C, $150\pm1^{\circ}$ C, $200\pm2^{\circ}$ C, and $225\pm2^{\circ}$ C.

RESULTS AND DISCUSSION

Isolation of Curcumin from Turmeric. A sample of 0.143 g curcuminoids was obtained from the isolation, making up 2.86% of the original sample. However, some

of the curcuminoids remained in the oily sample as well, which came out to 0.225 g. The comparison of isolated curcumin to reference curcumin by TLC showed them to have similar composition.

Double Mobile Phase TLC. The TLC plate of curcuminoid samples from the heating in edible oil obtained by using a mixture of dichloromethane and methanol (97:3) v/v as a mobile phase composition mentioned by Anderson et al. (12) is shown in Figure 1.

Figure 1 indicated the disruption of curcumin spot (top one) because of the interference with oil. Different compositions of mobile phase of dichloromethane and methanol (see Table 1) have been tried but didn't produce any better result. As can be seen from Figure 1 & 3, the oil spots in TLC did not show any particular pattern and unless oil is removed from the vicinity of the spots of our interest, it is impossible to identify and quantify the curcuminoid's compounds.

It is to be noted that in the isolation of curcumin from turmeric, dichloromethane is used, where curcumin is soluble. The curcuminoid is then precipitated by adding hexane. We have thus chosen hexane, a nonpolar solvent as the first mobile phase eluted to separate edible oil from curcumin. In fact, elution of nonpolar hexane moves only the nonpolar oil sample, leaving behind the curcuminoids at their origin (see Figure 2). After drying the plate in the fume hood for couple of minutes, the second mobile phase consisting of 97% $CH_2Cl_2 - 3\%$ MeOH was used.



Figure 1. A typical TLC of Curcumin and curcumin in turmeric samples in edible oil using a mixture of CH_2Cl_2 & CH_3OH (97:3) v/v as mobile phase.



Figure 2. A typical TLC analysis of heat-treated curcumin and turmeric samples in vegetable oil, seen under with 365 nm UV light, shows that using hexane as a mobile phase pushes the oil out of the way, leaving the curcumin behind for further analysis.

Expt	Mobile Phase	Rf Value of Curcumin	Rf Value for Demethoxycurcumin (related curcumin)	Rf Value for Bis- Demethoxycurcumin (related curcumin)	Comment
1	97% CH ₂ Cl ₂ - 3% CH ₃ OH	0.85	0.46	0.28	Curcumin spots were disrupted by oil
2	98% CH ₂ Cl ₂ - 2% CH ₃ OH	0.50	0.41	0.23	Curcumin spots were disrupted by oil
3	99% CH ₂ Cl ₂ - 1% CH ₃ OH	0.29	0.11	0.03	Rf value of demethoxycurcumin was too low
4	98.5% CH ₂ Cl ₂ – 1.5% CH ₃ OH	0.46	0.22	0.11	Best separation achieved

Table 1. Mobile Phase Composition and Separation of Curcuminoids

This mobile phase is relatively polar compared to hexane and a separation of curcuminoids occurred, however the resultant TLC plate appeared not to be the best for quantification. Thus a series of TLC experiments were done by changing the composition of the mixture of second mobile phase. The result is given in Table 1. The best composition of mobile phase was then determined (#4 in Table-1) as a mixture of 98.5% dichloromethane & 1.5% methanol and used for our project works. Using this TLC method thus developed, the samples of both curcumin and curcumin in turmeric treated in heated edible oil were successfully separated and used for quantification purposes. The spots with variable concentrations can be easily viewed with naked eyes. A typical TLC plate is provided in Figure- 3.

Quantification of curcumin in different temperatures. The volume in intensity/mm2 data plotted from Quantity One analysis software produced a linear curve using standard

curcumin solution in dichloromethane in different concentration.

All the heat treated samples either in water or edible oils were analyzed using the similar way by Quantity One software as that shown in calibration curve in Figure 4.



Figure 3. A typical TLC plate using two mobile phases eluted, hexane first followed by a mixture of dichloromethane – methanol (98.5: 1.5) v/v.



Figure 4 Calibration curve using standard curcumin solution in dichloromethane in concentration ranging 1-5 mg/mL.

Reflux of Sample in Aqueous Phase. Both the reference curcumin and isolated curcumin were relatively stable, but the curcumin in turmeric degraded much more quickly. Curcumin in turmeric reached as much as 47% degradation over the course of 60 minutes (Figure 5).

It appears that the curcumin in turmeric degrades more, possibly because of other compounds in turmeric.

However, nobody uses the process of heating turmeric in water for cooking purposes.

Continuous Heating of Samples in Edible Oil. Reference curcumin degraded slightly, reaching 25% degradation at 250°C, but again, curcumin in turmeric degraded more significantly, reaching 70% degradation at 250°C (Figure 6).

Similar degradation data were observed while heating in edible oil. The curcumin in turmeric degrades more in edible oil compared to reference curcumin. However, it is

♦ Reference Curcumin

Curcumin in Turmeric

30

0

RESIDUAL ADJUSTED VOLUME (%)

to be noted Heating of Samples at Different Maintained Temperatures. Reference curcumin and curcumin in turmeric both showed similar results. At 100°C, rather than degradation, there was an increase in concentration suggesting an increase in solubility. At 150°C, at which only turmeric was tested, the sample showed slight At 200°C, there was slightly more degradation. degradation than at 150°C. However, at 225°C the degradation in both samples is significant at 46% and 44% for reference curcumin and curcumin in turmeric respectively (Figure 7).



Figure 5. Stability of curcumin after refluxing in water at different time

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Figure 7. Stability of curcumin at maintained temperatures in edible oil.

The above conditions are more close to domestic Indian cooking. The oil was first heated and then cooking staffs such as vegetables, chicken or meat were added to hot edible oil and mixed thoroughly. In general, the cooking materials mixed with turmeric and other spices are not kept at high temperature for a long time. Our experiments showed no significant degradation of curcumin or curcumin in turmeric occurs at 150°C, however, they do degrade if kept for a longer time, which is more unlikely in normal cooking practices that it takes one and half hours to reach 250°C in edible oil. In domestic cooking, none cooks for that long time at a continuous increasing of temperature.

CONCLUSIONS

The experiments in determining the stability of reference curcumin or curcumin in turmeric provided the following important information:

- a) A new TLC method using two mobile phases of different polarity has been developed to separate, identify and quantify curcuminoids in edible oil.
- b) Role of polarity of mobile phase is clearly exemplified in this paper.
- c) Nonpolar solvent, hexane, when eluted first pushes only edible oil leaving curcumin and related curcumin compounds at their origin.
- d) This method has been found suitable in quantifying curcumin in edible oil using TLC,
- e) The reference curcumin and curcumin in turmeric do degrade in water or edible oil and curcumin in turmeric does degrade more compared to reference curcumin.
- f) Heating samples of curcumin or turmeric at different maintained temperatures, it showed significant degradation if kept at longer time, e.g. one hour at 225°C.
- g) Our experiences with domestic cooking with turmeric spices when compared to these experiments, we can reasonably expect some degradations of curcumin, however there is always intake of curcumin from everyday domestic cooking.

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CONNECTING SECONDARY EDUCATION TO THE JOB MARKET IN MISSISSIPPI: ESTABLISHING TRUE MEASURES OF COLLEGE AND CAREER READINESS

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ABSTRACT

In this project, researchers have employed the Mississippi statewide longitudinal data system (SLDS) to address, 1) the current workforce demand and 2) the observed outcomes for students exiting secondary education. Understanding the current state of affairs linking Mississippi's secondary education system to the Mississippi workforce will allow policy makers to make informed decisions about both curricular objectives and measures of accountability. Data sampling started with the 2006 Mississippi Department of Education cohort to provide sufficient time for participants to exit both community college and four-year college programs.

The ideal career starting point for students leaving Mississippi's secondary education system is the middle-skill level. The current education system has failed to provide the required skills and training to enter the workforce at this level. College readiness has also fallen short with more than half of Mississippi's graduates requiring remedial coursework upon entry into post-secondary education. Comparatively, Mississippians are on par with the rest of the nation. Nationally, 58.24% of students entering two-year college degree programs require remedial coursework. In Mississippi are tied with the national average of 31% for those who need to take remedial coursework. This snapshot can serve as a baseline to compare the success of innovative programs currently underway in the state. Using Mississippi's SLDS, policymakers and educators will be able to establish true measures of college and career readiness and gauge the effectiveness of innovative secondary education programs across the state.

Key Words: middle-skill; college and career readiness; statewide longitudinal data system; secondary education; workforce

INTRODUCTION

For more than a decade, Mississippi has cultivated a cultural and political environment that supports and encourages data sharing between education and workforce entities. Mississippi sees the integration of education and workforce data as one of the most critical assets for the development and implementation of policies and initiatives aimed at improving workforce, education, and economic outcomes. The statewide longitudinal data system (SLDS) Grant Program through the Educational Technical Assistance Act of 2002 has been authorized to award competitive, cooperative agreement grants with the goal of helping state's to make better decisions based on information provided through these systems. Since November of 2005, the SLDS Grant Program has supported state efforts to

develop data sharing agreements between agencies to develop, implement, and expand K12 and P-20W (early learning through workforce) longitudinal data systems (National Center for Education Statistics, n.d.). Fortyseven states, the District of Columbia, Puerto Rico, and the Virgin Islands that have developed systems, but many are currently only integrating one or two state level data systems (National Center for Education Statistics, n.d.). Mississippi has built one of the most comprehensive and effective SLDS in the nation, and is now poised to reap the benefits (Mississippi Department of Employment Security, 2014).

A key area where the state sees promising use of the SLDS is in the growing challenge of filling middle-skill jobs to facilitate the development of Mississippi's

science, technology, engineering, and mathematics (STEM) based economy. The term "middle-skill" refers to jobs that require more than a high school diploma but less than a four-year college degree. Many of these jobs require highly skilled trade and technical workers, with preparation including a combination of coursework, apprenticeships, on- the-job training, and assessments to earn certifications. In our current education system, this training is offered at community colleges, but the origins of this training model are firmly rooted in secondary education. Recently there has been a push to include elements of career and technical education (CTE) into the secondary (academic) education environment, or at least to blur the lines between traditional academic courses and CTE classes. Unfortunately, efforts to retool, strengthen, and expand CTE programs are constrained by geographic location (bussing students to facilities), pre-existing stigma linking CTE programs to "blue collar" jobs, state mandated testing, and already stretched school resources.

Current workforce and education efforts in Mississippi offer researchers an opportunity to understand how the misalignment of student/school accountability has influenced the secondary education system's ability to meet the needs of their students. Furthermore, measuring outcomes with respect to college or career readiness, the research methodology used in this research, presents us with a solution to the unintended consequences of school accountability. The secondary education system should reconsider how we measure school accountability and how we assess college or career readiness. By rethinking graduation requirements, Mississippi has allowed secondary schools to retool their own curriculum to include more integrated STEM programs and/or harness the established capacity of the community college system to offer training and successfully transition graduates into college or career.

In this project, researchers have employed the Mississippi SLDS to address, 1) the current workforce demand and 2) the observed outcomes for students exiting secondary education. Understanding the current state of affairs linking Mississippi's secondary education system to the Mississippi workforce will allow policy makers to make informed decisions about both curricular objectives and measures of accountability.

BACKGROUND

High Stakes Testing in Mississippi.

The use of high-stakes tests in the United States has drastically shifted over the last few decades. High-stakes examinations have gone from being accurate indicators of student learning to the primary tools for education reform. The 2001 implementation of No Child Left Behind (NCLB) is largely to blame for this transference (Au, 2011).

Research indicates that there are several unintended consequences associated with high- stakes examinations. These include but are not limited to: extreme pressure to lift performance from education and government leaders, threats to teaching and other jobs in education if test scores don't improve, a shift in attention from low achieving students to high achieving students (Klenowski & Wyatt-Smith, 2011). In the context of school reform these outcomes appear manageable and somewhat on target, but the testing environment that creates these outcomes is are not aligned to the needs of the student.

Furthermore there are increased instances of cheating and an observed narrowing of curriculum. Narrowed curriculum as a result of high-stakes testing is often described as a reduction in the intended curriculum, which is defined as any knowledge or skill that teachers deem critical for instruction to take place (Yeh, 2005). This fundamental concept drives the notion that teachers teach to the test. Teaching to the test as it relates to curriculum narrowing can result in de-emphasizing or completely ignoring topics or skills that are not addressed in the test (Klenowski &Wyatt-Smith, 2011). This occurs most often when the state standards and the high-stakes assessments are aligned most closely to factual knowledge rather than thinking skills (Amrein & Berliner, 2002). The result is that students may not fully comprehend the underlying concepts associated with certain topics, leaving them with a superficial knowledge base. Reactionary content-based instruction aligns itself easily to multiple choice testing (i.e.: memorization, low level thinking, rote procedures, surface understanding). Thus, any improvement in student test scores as mandated by NCLB are reflections of skills specific to the test rather than reflecting gains in student learning (Yeh, 2005). The reduced curriculum limits students' understanding of potential career pathways and what those career pathways require academically (degrees, certifications, and compilation of coursework) and technically (skills and experience).

The nature of assessments have drastically shifted. Assessment items that cater to higher order thinking and inquiry like constructed response are too expensive for large scale scoring and are replaced by multiple choice items that are cheaper and easy to score (Au, 2011). Berliner demonstrates that the narrower a particular curriculum is during a student's youth, the less likely any associated background knowledge will be available in later years. Students taught under a restricted curriculum that focuses on test preparation are not able to fully develop the sound background knowledge base crucial for future learning. Evidence of this narrowed curriculum manifests itself in higher grades and later in life. Narrowing curriculum restricts student growth and a thorough understanding of what is being assessed as well as students' creative thought process and natural inquiry (Berliner, 2011).

In order for high school students to graduate in the state of Mississippi, they must demonstrate a mastery of the academic courses they have taken. This is measured by the Subject Area Testing Program (SATP2). Subject areas tested include Algebra I, Biology I, U.S. History, and English II. Reform has begun to alleviate the pressure of high stakes testing by providing students with multiple options to meet graduation requirements.

Students can choose both academic and CTE pathways, but they will still be required to take secondary SATP2 tests because the tests are still tied to federal measures of school accountability.

College and Career

Vocational training or career preparation is not a new idea in secondary education. The Cardinal Principles of Secondary Education, a report written in 1918 from the Commission on the Reorganization of Secondary Education, outlines key objectives to provide a solid foundation for secondary education. The goal was a comprehensive education system that could meet the needs of students with a variety of talents, backgrounds and abilities (Wraga, 2001). The curricular layout designed by the commission had three distinctive stages. Stage one stated that the early years should be devoted to teaching certain fundamental process, such as reading, writing, arithmetical computations, and the elements of oral and written expression (National Education Association of the United States, 1918). Stage two stated at about 12 or 13 years of age, students should begin a preliminary survey of the activities of adult life, and a survey of their aptitudes, focusing on a special field that students could potentially pursue as a career (National Education Association of the United States, 1918). Stage three asserted that the concept described in Stage Two is responsible for the division of secondary education into junior and senior periods. The junior section was the place for students to explore their aptitudes and make provisional choices about their future work. The senior section provided training in the students' chosen field, and additional special instruction for students who needed remedial classes. The philosophy of college or career readiness is at the heart of this system. For a student to choose a field or discipline specialization requires that he or she be provided a secondary education with: 1) a wide range of subjects, 2) exploration and guidance, 3) adaptation of content and methods, 4) flexibility of organization and administration, and 5) differentiated curriculum (National Education Association of the United States, 1918).

Current education reform is pushing standards that strive for college or career readiness, but there has been some difficulty aligning secondary school curricula to achieve both goals for all students. For example, only 6% of middle-skill careers require Algebra 2; but this course is a necessary requirement for the majority of college degrees (Iasevoli, 2015). High school graduates today require more remedial coursework when they get to college. Among traditional college students 58% of those attending two-year colleges enrolled in a remedial course as did 31% of students at nonselective four-year colleges (Attewell, Lavin, Domina, and Levey, 2006). Secondary education needs to insure that there are opportunities to develop professional skills, explore career options, and have a strong academic foundation to support those on a college pathway.

Mississippi Department of Education requires that all students develop a career inventory by the 8th grade but it is not clear the extent to which this requirement is fully implemented. Only 18% of all secondary education students in Mississippi enrolled in a career and technical education program, or CTE (LifeTracks, 2012). The CTE pathway is set up to expand students' knowledge of career options but the system does not reach all students. More recently, the state added career readiness competency skills across all K-16 education programs. Career readiness is a process of attaining and demonstrating requisite competencies, from K-16, to transition students into the workplace (State Workforce Investment Board, 2015). The core competency skills identified by Mississippi's State Workforce Investment Board (SWIB) Task Force include:

- Critical thinking/problem solving
- Oral/written communications
- Teamwork/collaboration
- Information technology application
- Leadership
- Professionalism/work ethic
- Career management

Although the inclusion of these competencies is another step in the state's ongoing efforts to prepare students for

college or career, student performance needs to be evaluated and schools still need to be held accountable. School systems are in the era of assessment (Atkin & 2003). Mississippi must assess career Black, competencies by using student-level data as evidence of success in college or career rather than arbitrary assessments selected from core areas linked to graduation requirements. The measures must be authentic and correspond to the goals of the curricula (Wiggins, 2005). Even with career readiness and skills at the center of the reform and the secondary education curriculum, assessment measures still need to look at the systems to place graduates in careers, and how well graduates are prepared to succeed in colleges and universities.

Middle-skill job market employment offers sustainable, livable wages, and should the target for preparation by the secondary education system. These jobs make up a significant portion of our growing STEM based Economy nationally (National Skills Coalition, n.d.). Entry-level middle skill jobs often allow for upward movement with employer training and/or gained experience, which is the definition of a career. Low-skill jobs have high turnover rates because they are not sustainable enough to meet the definition of a career. At the other hand, high-skill, or highly specialized, employment requires advanced degrees and a large investment of both time and resources. Individuals on this career path often fail to critically evaluate their chosen direction until they have passed the point of no return, where they have already invested much time and resources. Students may have accumulated a significant student loan debt, for a degree they will not use or is not very marketable. A paradigm shift is beginning - both colleges and students are realizing the value of workrelated or discipline specific experience. For colleges this means favoring candidates that have related experience or credentials. For students, it means exploring personal interests and a career before making a commitment.

Research Questions

- 1. What does the middle-skill job market look like in Mississippi?
- 2. To what extent is the current Secondary education System preparing Students for entry into college or career?

METHODOLOGY

NSPARC

The National Strategic Planning & Analysis Research Center (NSPARC) is a research center at Mississippi State University and has served as a partner to several state and national agencies to collect, manage, and explain administrative data. NSPARC is nationally recognized as an intellectual, interdisciplinary hub for advancing methods and analytical techniques to transform "big data" into "smart data," raising the theoretical and empirical understanding of how individuals and groups think and behave in society.

SLDS

Mississippi has a comprehensive education and workforce longitudinal data system built upon the clearinghouse model. Mississippi's data clearinghouse is at Mississippi State University (MSU), and is managed by NSPARC. The clearinghouse includes data from early childhood entities (e.g., Head Start); Mississippi Department of Education (MDE); all 15 community colleges and the community college board; all eight public universities and the Institutions of Higher Learning (IHL); Mississippi Department of Employment Security (MDES); Mississippi Department of Human Services (MDHS); Mississippi Department of Rehabilitation Services (MDRS); Mississippi Department of Corrections (MDOC); Mississippi Development Authority (MDA), and Mississippi State Department of Health (MSDH). It includes data from 2000 thru today. Data from entities are regularly transmitted and made available through a secure transfer according to SLDS Governing Board rules and regulations and via memorandums of understanding (Mississippi Lifetracks, 2013). The state data clearinghouse developed and manages Mississippi's online SLDS reporting system, LifeTracks (www.lifetracks.ms.gov). All data are stored in a stateof-the-art data center built for managing large databases and hosting mission critical systems. The SLDS has access to one of the most powerful high-performance computing systems in the country to use for highperformance computations for complex modeling. The SLDS provides technical and scientific expertise in data modeling and system development.

Personal Identifiers

All education and workforce data in the Mississippi SLDS have a 10-digit unique identification number (ID10) created at random using a hardware-based quantum physics true random number generator. This identifier replaces Social Security numbers, used only for assigning the ID10, and becomes the common link contributing data to SLDS. All de-identification procedures were implemented with protections to maximize security, minimize risk, and ensure regulatory compliance as prescribed by the SLDS Governing Board Rules and Regulations.

Data Quality Measures

The state data clearinghouse takes two steps to ensure data quality. The first step is to fully inventory data transferred to the clearinghouse. Data contributors submit data in accordance with state and federal law. Upon receipt of data, NSPARC verifies the list of data elements received within ten working days of receipt. Upon verification of the list of fields, tables, and relationships between tables by the data contributor, the data undergo a complete data inventory process. This process includes updating or creating data dictionaries and program data mapping documents. Data dictionaries include metadata such as meaning, relationship to other data, origin, usage, and format. The second step is data validation that includes: (1) Checking that all tables, records, and fields, and the full contents of each field, have been successfully transmitted and read; (2) Comparing record counts between the source data and the data transmitted to the third party contractor; and (3) Producing a report with basic summary statistics for validation by the data contributor.

Security Measures

Security is maintained on multiple levels, including SSL encryption, user-specific data access controlled via user accounts, and access control lists (ACLs) for all data.

NSPARC protects information from a variety of threats and stresses the importance of multi-layer protection. Through staff orientation, Institutional Review Board for the Protection of Human Subjects (IRB) certification, university information security certification, and regular staff meetings, all NSPARC researchers and staff are aware of and accountable for their roles in protecting sensitive information. System security features ensure secure access by only authorized personnel, and with reporting required by FERPA and state and federal laws. All sensitive data are transferred via a secure web server; uploaded data are encrypted and stored in a secure offline location, and a primary identifiers (e.g., names, street addresses, telephone numbers, and identification numbers) are stripped from datasets, once the ID10 has been assigned. Security policies and procedures are continually reviewed and updated in response to changing information security technologies, requirements, and threats.

Sample

Data sampling started with the 2006 Mississippi Department of Education cohort to provide sufficient time for participants to exit both community and fouryear college programs. All relevant data from the SLDS system was analyzed as part of ongoing research efforts to improve connections between education and workforce systems.

RESULTS AND DISCUSSION

MISSISSIPPI MIDDLE-SKILLS JOB MARKET

In Mississippi, middle-skill jobs make up almost 67% of the job market, but only 31% of the state's workforce has the required education and training to fill them (seeFigure 1). In contrast for jobs requiring college credentials and beyond, Mississippi has more workers than available jobs. For low-skill jobs requiring a high school diploma or less, the market is saturated with over two times the number of potential workers for available jobs. Closing the gap between the demand and supply of middle-skill workers is an issue faced not just Mississippi but in all states.



Source: Bureau of Labor Statistics 2014

Figure 1: Mississippi Jobs and Workers by Skill

In Mississippi, the urgency of addressing the middle-skill jobs gap is evident. More than 58,000 individuals with a high school diploma and/or some college sought additional noncredit hour training from a Mississippi community college in 2014 (see Figure 2). In 2014, more than 87,000 job seekers with a high school diploma and or some college sought employment assistance through services provided by MDES (see Figure 3). Mississippians who achieve any credential beyond high school improve their earnings outcomes (see Figure 4). By 2024, Mississippi is expected to have 70,227 additional middle-skill jobs and an equivalent number of low skill workers far in excess of the number of low-skill jobs. If Mississippi could transfer the surplus of low-skill workers to meet future demand for middle-skill jobs, the state could generate an additional \$74 million in tax revenue and improve the labor market for jobs requiring high skill levels and college credentials.



Source: Mississippi SLDS, Life Tracks, 2014 Figure 2: Level of Community College Trainee



Source: Mississippi SLDS, Life Tracks, 2014 Figure 3: Mississippi Job Seekers by Education Level



Source: Mississippi SLDS, Life Tracks, 2014 Figure 4: Five-Year Average Annual Earnings

Students Exiting the Secondary Education System into College and Career

To intercept Mississippians headed to the saturated lowskill job pathway, educators and the public must create an environment in the state's secondary education system that supports the development of middle-skills. This middle-skill pathway should include access points to reenter education and training for those individuals who were not initially served by the secondary education system, such as dropouts or those who were promoted without the prerequisite knowledge needed for success. Using archived data from the Mississippi SLDS system, Table 1 presents 2006 secondary education system cohort outcomes exiting the (N=27,899). Of this cohort, 7.543 (27%) individuals entered directly into the workforce with credentials for the low-skill level of employment. This includes both drop-outs (n=2,365) and high school graduates (n=5,178). MDHS offered 1,181 (4.2%) members of this cohort, adult services such as SNAP benefits, including both dropouts (n=634) and graduates (n=547).

Of those graduates who and went straight into a four year college (n=4,564), 1396 or 30.58% of them were required to take remedial classes. The percentage of those transitioning into a two year degree program (n=11,229) who were required to take remedial courses is 54.5% or 6,120 students. It is important to note that these students passed all SAPT2 exams, because new MDE graduation

options were not implemented until 2015. The secondary education system in Mississippi did not meet the college or career readiness goals of 58.3% (n=16,240) of its 2006 cohort clients.

Table 2 presents additional outcomes for the 2006 cohort of Mississippi graduates. Of those that were ready for college, 3,534 (42.7%) dropped out. This includes both four-year (n=1245) and two-year (n=2289) college programs. Of those that were not ready and were required to take remedial coursework, 4631 (61.6%) dropped out, as represented by 898 students from four-year institutions and 3,733 students from two-year college programs. One important note is that students in this sample exited secondary education in 2006; their entire secondary experience took place after the implementation of No Child Left Behind (2001). These students were part of the first post-NCLB cohort presented with a narrowed secondary education curriculum that focused on test scores rather than career interest, career exposure, and the development of career readiness competencies. While the secondary education system is not solely responsible for college graduation rates, a robust college readiness program allows students to explore the expectations and outcomes for degree pathway, increasing the likelihood of success.

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Of those who drop out of high school		4,101
% that enter workforce	57.67%	2,365
% that enter GED training	4.90%	201
% that begin receiving MDHS adult services (e.g., SNAP) and do not enter workforce	15.46%	634
% that are out of workforce because of disability	0.27%	11
Of those who graduate High School		23,798
% who are college ready and attend four-year school	13.31%	3,168
% who are college ready and attend two-year school	21.47%	5,109
% who are not college ready and attend four-year school	5.87%	1,396
% who are not college ready and attend two-year school	25.72%	6,120
% who enter workforce	21.76%	5,178
% who do not enter workforce and seek MDHS adult services	2.30%	547
% who do not enter workforce because of disability	0.02%	4

Table 1: Mississippians Exiting the Secondary Education System in 2006

Source: Mississippi SLDS, LifeTracks 2015

Table 2: Additional Outcomes for 2006 Cohort of Mississippi Graduates

Of those who are college ready and attend four year school				
% who finish college	60.70%	1,923		
% who drop out of college	39.30%	1,245		
Of those who are not college ready and attend four year school		1,396		
% who finish college	35.63%	497		
% who drop out of college	64.37%	898		
Of those who are college ready and attend two year school		5,109		
% who finish	37.84%	1,933		
% who drop out	44.80%	2,289		
Of those are not college ready and attend two year school		6,120		
% who finish	27.24%	1,667		
% who drop out	61.00%	3,733		

Source: Mississippi SLDS, IHL data 2008-2011

CONCLUSIONS

The ideal career starting point for students leaving Mississippi's secondary education system is the middleskill level. The current education system has failed to provide the required skills and training to enter the workforce at this level. College readiness has also fallen short with more than half of Mississippi's graduates requiring remedial coursework upon entry into postsecondary education. Comparatively, Mississippians are on par with the rest of the nation. Nationally, 58.24% of students entering two-year college degree programs require remedial coursework. In Mississippi that number is slightly lower at 54.5%. Students exiting secondary education into four-year colleges in Mississippi are tied with the national average of 31% for those who need to take remedial coursework.

Current national assessments by the National Assessment of Educational Progress (NAEP) place Mississippi at the bottom of the list. If the ultimate goal of the state's education system is college or career readiness, researchers do not believe this assessment is an accurate measure, a problem evident throughout the country. Mississippi has a distinctive advantage -- the comprehensive SLDS data allow for education reform at the state level to make policy decisions about school accountability.

Implications

Linking graduation requirements to standardized test scores has a narrowing effect on secondary education curriculum. The narrowed curriculum is a barrier to the original scope and focus of secondary education. Expanding course options and connecting secondary education students to community college coursework will help them explore career options and find their place in the Mississippi workforce ecosystem. With the shifts in education reform from school accountability to career or college readiness outcomes, it is important to implement true measures of success. Mississippi's SLDS links K-12, community college, intuitions of higher learning and workforce data, allowing researchers and policymakers to use this tool to analyze real-time data, which can be to adapted to better meet the needs of both individual students and the workforce.

This snapshot of the 2006 cohort can serve as a baseline to compare the success of innovative programs currently underway in the state. Using Mississippi's SLDS, policymakers and educators will be able to establish true measures of college or career readiness and gauge the effectiveness of innovative secondary education programs across the state.

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Society is always in the process of development; character of secondary school population undergoes modification; and the sciences of which the educational theory and practice depend constantly furnish new information. Secondary education, however... is conservative and tends to resist modification (p. 7). The Cardinal Principles of Secondary Education 1918.

PHILOSOPHICAL CONSIDERATIONS OF QUANTUM MECHANICS

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ABSTRACT

Quantum mechanics is a theory which helps explain the wave-particle duality of matter and energy. Apparently all objects obey the laws of quantum mechanics, even though there seems to be a disconnect between what happens in the microscopic world and the macroscopic one. There are various aspects of quantum mechanics which seem strange and mysterious – those leading to philosophical speculations about the role of the observer and even the mystery of consciousness itself. This paper uses a "maieutic" method of questioning, similar to a midwife assisting patients in giving birth to their own understanding, to probe and elucidate some of these enigmatic characteristics of quantum mechanics in the hopes that layers of false views will give way to truth, albeit a tentative, cautious, and perhaps even conflicted truth.

INTRODUCTION

Quantum mechanics is a mathematical theory for predicting the behavior of atomic and subatomic particles which helps explain the wave-particle duality of matter and energy. Some scientists consider quantum mechanics as a separate branch of physics (Liboff 2002, Griffiths 2004). Mathematically, the theory works extremely well, and has been verified repeatedly in numerous experiments. In fact, predictions resulting from quantum mechanics have been 100% correct, at least thus far. Apparently all objects, either microscopic or macroscopic, obey the laws of quantum mechanics, even though there seems to be a disconnect between what happens in the microscopic world and the macroscopic one. The real problem seems to be what the world is really like. Is it a logical common-sense world, as we perceive it to be at the macroscopic level, or a bizarre, unpredictable, nothing-can-really-bedetermined-for-sure world as described by quantum mechanics?

Electrons do not circulate around the nucleus like planets around the sun in a predictable pattern. Instead, they occur in an uncertain, non-deterministic, probabilistic wave function around the nucleus, or even through it. Actually, this is fortunate because if they did circulate in a planet-like manner, they would eventually collapse into the nucleus. The uncertainty principle in quantum mechanics states that both the position and momentum of a subatomic particle cannot be simultaneously measured, but observation fixes that particle's position or momentum. Under certain interpretations (but not all) of quantum mechanics, a system ceases being a superposition of states and becomes either one or another when an observation takes place, i.e., observation collapses the wave function. The most famous application of this phenomenon is the Schrödinger's cat thought experiment (Schroedinger 1935), in which a theoretical cat in a box is simultaneously both alive and dead. When one looks in the box (observes), then the cat is either alive or dead, but not both. This example illustrates some of the strangest aspects of quantum mechanics – those leading to philosophical speculations about the role of the observer (Albert 1994, Omnes and Sangali 2002) and even the mystery of consciousness (Rosenblum and Kuttner 2011).

In the days of the ancient Greeks, the Sophists were professional rhetoricians who specialized in training their followers to win arguments at all costs. Sophists were radical relativists. Socrates, on the other hand, valued truth-seeking over persuasion and believed that truth would ultimately be revealed through philosophic inquiry. Through his method of questioning, Socrates would gradually peel away layers of false views until the truth was revealed. Some have said Socrates's questioning was like a "maieutic" method, wherein a midwife essentially assisted patients in giving birth to their own understanding (Blackburn 2005). That is not exactly what we attempt in this paper, but close. Herein we strive to construct a series of carefully chosen questions to probe and elucidate some of the enigmatic characteristics of quantum mechanics in the hopes that layers of false views will give way to truth, albeit a tentative, cautious, and perhaps even conflicted truth.

ASSUMPTIONS

Some assumptions in this paper are implied, while others are stated and defined. They include:

- 1. There is a true reality. This is difficult to prove, but almost impossible not to accept.
- 2. Reality is knowable and we know it through our senses. However, this knowledge is limited because what our consciousness receives from the environment is "qualia," not direct or real perceptions. For example, the color red is actually a wave-length and sound is movement of a medium.
- 3. The ability of an observation to collapse a wave function applies to the macroscopic world and not just subatomic particles. This is controversial and not a widely held view. Further, we are assuming that the wave function only collapses when an actual, purposeful observation is performed. As Feyman said, when "Nature knows" the wave function collapses. Many other physicists believe the wave function collapses because of interaction with the environment (decoherence). For example, the fact that a particle hits the moon creates a record of the moon being there and collapses its wave function.

METHODS

The following questions were devised to test the logic of key elements of quantum mechanics. We let the published definitions and explanations of the theory be our guide to answering the questions, except where new or additional logical conclusions or inferences could be drawn.

- 1. If an observer is necessary to collapse an object out of its super-positional state, can the observer be inanimate?
- 2. If two different observers "look" at an entity, collapsing the wave-function, are the results the same? In other words, wouldn't the consistency of multiple observers seeing the same thing or getting the same result argue against the observer-makes-reality-theory?
- 3. Does observation of an entity in a superposition state, which collapses the wave function, create the object? If so, would not the act of observing create matter/energy ex nihlo? (This would violate the 1st Law of Conservation of Matter and Energy)
- 4. Granted that classical physics left little room for human free-will, does quantum mechanics allow for free-will? Perhaps even quantum mechanics only grants an illusion of free-will.

RESULTS

Application of Socratic reasoning to key elements of quantum mechanics led to several observations and inferences:

1. If an observer is necessary to collapse an object out of its super-positional state, can the observer be inanimate?

We concluded that good arguments can be made on both sides of this issue. First, we consider that the observer must be a conscious entity. Even a machine designed for observation is created, set up, and operated by human beings. The human that makes the machine builds into it decisionmaking capabilities. An a-priori assumption is that a human tells the machine what to assess. It follows then, that if a conscious observer is needed to collapse a wave function, then the corollary might be that ability to collapse a wave function can be a test for consciousness. On the other hand, conscious observation may not be necessary. Quantum computing is a good example. When building a quantum computer, care needs to be taken to isolate it from the environment because this is the main difficulty in developing one. Therefore, observation may not be needed, only interaction with the environment.

2. If two different observers "look" at an entity, collapsing the wave-function, are the results the same? In other words, wouldn't the consistency of multiple observers seeing the same thing or getting the same result argue against the observer-makes-reality-theory?

First of all, we need to clarify the term "look." In the context of this article, looking involves hitting an object with a photon or some other particle (This is actually more complicated. We can also determine the location of an object by knowing where it is not, i.e., finding out the location without actually hitting the particle.) Some researchers think of the uncertainty principle in these terms – in order to locate an object accurately you need to use a short wavelength photon. The shorter the wavelength, the higher the energy. If you want to know the position accurately, you have to hit the object very hard. This causes an enormous change in its momentum, which is therefore unknown.

Multiple observers seeing the same thing does not disprove quantum mechanics. Only the first observer fixes the object, then all subsequent observers see it that same way. This is a law of nature – all things have consistent histories. 3. Does observation of an entity in a superposition state, which collapses the wave function, create the object? If so, would not the act of observing create matter/energy ex nihlo? (This would violate the 1st Law of Conservation of Matter and Energy)

> Observation of an object, subatomic or even macroscopic, does not create it. The matter/energy was already present in some superpositional state. Observation of an electron cannot produce the electron. It had to already be there.

4. Granted that classical physics left little room for human free-will, does quantum mechanics allow for free-will? Perhaps even quantum mechanics only grants an illusion of free-will.

All human societies, past and present, generally hold a solid belief in free will. Our laws, our system of punishment and rewards, and almost all our activities, presume that when we do something we could have chosen to do something else instead. Any change in this fundamental assumption of freedom of choice could radically change society. This is so unsettling that some philosophers who do not believe in free will choose to keep that belief a secret. In a deterministic, clock-like, universe everything has a cause and any present event is produced by a previous one. Following that chain backward leads back to the big bang. This interpretation means all that is happening and will ever happen in the universe was pre-set at the beginning, and therefore, no free will is possible. This idea was very disturbing to Newton-era physicists.

While Newtonian physics was deterministic, essentially precluding free-will, quantum mechanics may allow for free-will. Quantum mechanics, and especially its ideas of randomness, greatly improve the possibility for free-will. Suddenly there is a set of all possible actions and interactions available. However, the problem of free-will is still not yet resolved. Yes, there are multiple possibilities and choices, but in quantum mechanics, the outcome is random. To many, this is still not satisfying. We do not have any explanation of who or what is selecting the desired outcome(s). If everything is truly random, then that may not be much better than everything being pre-set (determined).

CONCLUSIONS

As we can see from trying to answer these probing questions about quantum mechanics, the theory sometimes yields an unpredictable, contradictory view of the world. Many issues remain unresolved, especially that of free-will, which clearly has philosophical and religious implications. Humans would do well to try to better understand quantum mechanics to gain a clearer view of what the world is really like.

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ASSESSMENT OF WATER QUALITY OF TWO LOTIC BODIES OF WATER IN JEFFERSON COUNTY, MISSISSIPPI

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ABSTRACT

Water quality standards depend on the type and / or use of the water (e.g., potable water, water for industrial use, agricultural use, etc.). This study of Coles and Mud Island Creeks was conducted to assess their physical, chemical and biological profiles, the seasonal distribution of pollutants, to compare the water quality between the 2002-03 study and the 2012-13 study and to determine if they met the Mississippi Water Quality Criteria (MSWQC) / Environmental Protection Agency (EPA) standard. Water samples were collected in plastic water containers 50 meters apart at one week intervals from three sites in each of the two bodies of water, taken to the laboratory and then tested with LaMotte pollution test kits according to the manufacture's protocols and the results were tabulated and statistically analyzed. Both creeks were practically normal and had no significant water quality differences during the three study seasons. The physical test results showed that Mud Island Creek's turbidity (59.85 NTU / 50 NTU) and surface water temperature (25.6°C / 32.2°C) and Coles Creek's turbidity (63.73 NTU / 50 NTU) and surface water temperature (20.2°C / 32.2°C) met the MSWQC/EPA standard. The chemical test results showed that Mud Island Creek's alkalinity (129.06 ppm / 3.08 ppm), CO₂ (28.13 ppm / 10 ppm) and hardness (104.53 ppm / 50 ppm) and Coles Creek's alkalinity (73.06 ppm / 3.08 ppm), CO₂ (17.66 ppm / 10 ppm) and hardness (78.4 ppm / 50 ppm) exceeded the MSWQC / EPA standard. Carbon dioxide rose significantly higher (p < 0.05) in the 2012-13 study. The seasonal results revealed that Coles Creek's winter hardness (68 ppm) was highest. Mud Island Creek's winter alkalinity (140 ppm) was highest. The biological tests indicated the presence of *E.coli* and coliform bacteria in both creeks. Continued periodic study of these creeks is recommended.

Keywords: Water Quality Assessment, Lotic Waters, Coles Creek, Mud Island Creek, Mississippi

INTRODUCTION

The Natchez Trace Parkway is a federal parkway maintained by the National Park Service (see Figure 1). It is a scenic road that stretches 444 miles in length, 800 feet in width, and covers 52,289 acres through three states, from the south terminus in Natchez, MS through Cherokee, AL, to the north terminus in Linton, TN (Natchez Trace Parkway, 2013). People have picnics in a designated place or walk along a nature trail. With all the activities someone may throw trash or dump something in the water that may lead to pollution of the creeks. Testing of these two water bodies will detect any pollution problem that may be reported.

Water quality standard depends on its type and / or use, which includes but not limited to potable, recreational, agricultural and propagation of wildlife. Coles and Mud Island Creeks were studied (Figures 2-3). These creeks are lotic water bodies because they are flowing water. Velocity of flowing water varies from stream to stream and within the stream itself. Nutrients and wastes are carried to and away from aquatic organisms as water flows from upstream to downstream allowing nutrient cycling to take place along the stream (Renn, 1970).

Just as nutrients and wastes are found in the creeks, so are pollutants. Pollutants are found in the environment because of human activities. Some people overuse chemicals to kill pests and use them in agriculture. These chemicals can enter the streams and rivers through runoff. Road salts used on roadways during heavy snow can be found in streams. These examples are mentioned because they affect the Natchez Trace Parkway and are of concern. Along the Natchez Trace Parkway some people have private property on which farming is done. The chemicals used can runoff into the creeks. The people who travel in the Natchez Trace Parkway visit sites along the parkway and may throw things in the water without thinking about how they would harm the water or the land. The Natchez Trace Parkway is not immuned to contaminants within / outside its boundaries. The objectives of this study were: (a) to check the concentration of contaminants or pollutants during three seasons of the year (fall, winter and spring) in two lotic water bodies, namely, Coles Creek and Mud Island Creek (b) to compare the extent of contamination between the two creeks (c) to compare the results of this study with the previous one done in 2002-2003 (Hopkins and Acholonu, 2005) (d) to find

out if the two water bodies met the quality standard of freshwater bodies as set by the State of MS and the EPA

and (e) to check the biological profiles of the two creeks.



Figure 1: An entry sign to the Natchez Trace Parkway.



Figure 2: Study site Coles Creek located off of the Natchez Trace Parkway



Figure 3: Study site Mud Island Creek located off of the Natchez Trace Parkway

MATERIAL AND METHODS

Site Description

This study was done on two creeks which are lotic freshwater bodies, namely, Coles Creek (see Figure 2) and Mud Island Creek (see Figure 3). Mud Island Creek is located off the Natchez Trace Parkway in Jefferson County, MS. It is about 17 miles southwest of Alcorn State University campus. Coles Creek is about 29 miles southwest of Alcorn State University and 12 miles southwest of Mud Island Creek on the Natchez Trace Parkway. Both creeks have trees and other types of vegetation at their banks and have picnic areas. People in the surrounding communities travel on this route, visit the areas and domestic animals and wildlife use the two water bodies.

Procedures

During the period September 2012 to March 2013, water samples were collected from the creeks, then taken to the Alcorn State University biological laboratory where tests were performed. Water samples were collected in sterile plastic containers from three sites in each of the creeks, about 50 meters apart. In the laboratory, LaMotte pollution test kits (Carolina Biological Supply Inc.) were used to perform various chemical tests and the results were analyzed. The physical parameters, water temperature as well as dissolved oxygen (chemical), were measured in the field (in situ) with Dissolved Oxygen Meter (Carolina Biological Supply, Inc). Fourteen different chemical parameters were tested (see Table 1). The turbidity was measured using the Turbidimeter (Orbec Analytical Systems Inc.) The results were statistically analyzed using the paired t-test with the SigmaStat for windows version 3.0.1 (SPSS Inc., 2003) program. The biological profiles were

checked by using MacConkey agar, Nutrient agar and Lauryl Tryptose broth (Carolina Biological Supply, Inc.). The bacterial pollution tests checked for the indicator organisms for fecal contamination. The MacConkey agar supports gram- negative bacteria like E. coli, an indicator of fecal contamination. Lauryl Tryptose broth supports gram negative bacteria like coliform bacilli, another indicator of fecal contamination. "Nutrient agar is a general medium that supports the growth of a broad range of bacteria-both gram negative and gram positive bacteria." (Carolina Biological Supply Company, 2014). These tests were conducted according to the operational guidelines of the "Carolina Bacterial Pollution of water kit" (Carolina Biological Supply Company).

RESULTS AND DISCUSSION

After conducting the various tests, the results revealed that alkalinity, carbon dioxide, and hardness were above the MSWQC / EPA (see Table I). Chemical analysis of magnesium, phosphate, silica and ammonia-nitrogen showed that they met the MSWQC / EPA. MSWQC / EPA requires pH to be between 6.5 and 9.0 and dissolve oxygen to be at 4-5ppm. The dissolved oxygen (DO) and the pH for both creeks were within this range set by the MSWQC / EPA. With respect to the biological tests, the MacConkey agar, Nutrient agar, and the Lauryl Tryptose broth indicated the presence of coliform bacilli and E. coli in both creeks in each season as noted in Table I. The colonies on the Nutrient agar plates were both, gramnegative and gram-positive bacteria (see Figures 4-5). The MacConkey agar supports only gram-negative bacteria, which include E. coli (see Figures 4-5). Fermentation of sugars, which produces the gas bubble in the Lauryl Tryptose broth, also confirms the presence of coliform bacilli and E. coli (see Figures 6-8).

Table 1: Mud Island and Coles Creeks 2012-13 study. Chemical, physical and biological tests results averages comparedwith the MSWQC / EPA standard

Parameters	Mud Island Creek	Coles Creek	MSWQC/ EPA
Chemical ppm			
Alkalinity*	129.06	73.06	3.08
Ammonia-N	0.9	0.5	10.0
Calcium	64.53	49.86	200.0
Carbon dioxide*	28.13	17.66	10.0
Chloride	23.73	29.6	230.0
Hardness*	104.53	78.4	50.0
Iron	0.0	0.0	0.2
Magnesium	40.0	30.4	150.0
Phosphate	0.1	0.1	0.1
Silica p	20.0	20.0	75.0
Nitrate-N	0.25	0.266	10.0
Dissolved oxygen	7.42	8.93	5.0
Salinity	0.16	0.193	

Parameters	Mud Island Creek	Coles Creek	MSWQC/E PA
Physical			
Turbidity NTU	59.85	63.73	50.0
Odor	Odor None		None
Surface H ₂ O °C	25.6	20.2	32.2
pH	7.1	7.08	6.5-9.0

Biological Colonies/100 mL	Mud Island Creek	Coles Creek	MSWQC/ EPA Colonies/ mL
Lauryl Tryptose broth	coliform	coliform	200 - 400
Nutrient agar	coliform	coliform	200 - 400
MacConkey agar	coliform	coliform	200 - 400

<u>Note</u>: Asterisk = parameters above the MSWQC/EPA standard. Parts per million = ppm. Parts per trillion = ppt. Nephelometic Turbidity Unit = NTU. Dash = not found



Gas from col iform Bacteria



Figure 6: Mud Island Creek; fermentation in Lauryl Tryptose Broth

Water quality is a serious matter not only to the USA but, also other nations like African countries. Water serves several purposes including, but not limited to, being a potable water resource, place to obtain food, for industrial use and for recreational use. Okorie and Acholonu (2008) study of Nworie River in Owerri, Nigeria gave great examples of how important water quality is. There are human and industrial pollutants discharged into the Nworie River. The Nworie serves as a sewage disposal, recreational resource, and as a food resource. There are times in which the river serves as a drinking water source. This becomes a health concern to the people who are eating and drinking from the river. The chemical quality and the organic quality were examined to find out how polluted the river was. The results of the study showed that the river was "relatively unpolluted chemically". However, "the low dissolved oxygen concentration and high carbon dioxide concentrations imply that organic waste was the pollution problem" (loc. cit.).

This study was conducted for the same reason, namely, to examine the water quality of Mud Island and Coles Creeks. The physical, chemical and biological profiles of both creeks were assessed. Contaminants entering the water bodies in the Natchez Trace Parkway could be from point source (e.g., release of contaminants from upstream or downstream factories) and/ or non-point source (e.g., runoff from farmers application of fertilizers) if used in close proximity to the water bodies. Non-point source contaminants could also be from humans leaving trash in the area or from cars' exhaust emissions when traveling over low bridges.

Pollutants and human contamination were found in each of the creeks. Human contamination was evident from the trash found in the creeks. An empty beer bottle and a gas jug were found in Mud Island Creek and a flower pot was found in Coles Creek. Fecal contamination was evident from the positive bacterial pollution test results, which could be from humans and animals.

Eighty percent of the chemical parameters checked were below or within range of the MSWQC / EPA standards.



Gas from col iform Bacteria

Figure 7: Coles Creek; fermentation in Lauryl Tryptose Broth

For example, the MSWQC / EPA set safe water quality for ammonia-nitrogen (ammonia-N) at 10 ppm, the ammonia-N reading for Mud Island Creek was 0.9 ppm and Coles Creek was 0.5 ppm. Chloride safe water quality was set at 230 ppm, Mud Island Creek chloride was found to be 23.73 ppm and Coles Creek chloride was 29.6 ppm (see Table 1).

The average seasonal surface temperature of Coles Creek was 20^oC and Mud Island Creek was 25^oC, both did not exceed the 32^oC standard. The turbidity readings for Mud Island Creek and Coles Creek were 59.8 NTU and 63.7 NTU, respectively. MSWQC/EPA recommends no more than a 10% increase for water bodies of 50 NTU or less (State of MS, 2013) (see Table 1).

The chemical parameters above the MSWQC / EPA standards for both creeks were alkalinity (3.08 ppm), carbon dioxide (10.0 ppm) and hardness (50.0 ppm). Mud Island Creek's alkalinity was 129.06 ppm, 46 ppm higher (p < 0.05) than Coles Creek's result of 73.06 ppm. Mud Island Creek's hardness was 104.53 ppm, 26.13 ppm higher than Coles Creek hardness of 78.4 ppm, but not significantly higher (p > 0.05). Coles Creek's carbon dioxide was 17.66 ppm, 10.47 ppm lower than Mud Island Creek's result of 28.13 ppm, a significant difference (p < 0.05) (see Figure 8).

There were seasonal differences between the creeks. The alkalinity results for Mud Island Creek were significantly higher (p < 0.05) for all seasons. Hardness for Mud Island Creek (162ppm, see Table 2) was significantly higher than Coles Creek (81.3 ppm, see Table 3) during the spring (p < 0.05). The results for Mud Island Creek fall carbon dioxide (41.3 ppm vs 17.3 ppm) was significantly higher (p < 0.05) than Coles Creek's. Mud Island Creek's calcium (126.66 vs 45.0 ppm) spring result was also significantly higher (p < p0.05) than Coles Creek. Coles Creek's fall chloride (52.0 ppm vs 21.3 ppm) was significantly higher (p < 0.05) than Mud Island Creek's with a mean difference of 52. The dissolved oxygen for Coles Creek spring result was significantly higher (p < 0.05) than Mud Island Creek's result with a mean difference of 1.067. These variations

are to be expected irrespective of the season. Mud Island and Coles Creeks are two lotic bodies of water (not stagnant). So the concentration of the chemical pollutants may not be the same at any point in time with the water flowing continuously. The variation may also be caused by deferential non-point source pollutants from surface runoffs from livestock farms and animal and human wastes from use of the two creeks. As observed by Renn (1968), changes in water quality occur due to thermal layering. Also waters that contain living things show day-to-night changes in quality.

Biological Profiles

Water samples for biological tests were taken from the same sites as the chemical tests. The biological profiles of these two creeks were not different. E. coli was expected to be in the water because animals like deer are on the Natchez Trace Parkway. The colony growth on the MacConkey agar indicated E. coli to be possibly present in both Coles and Mud Island Creeks (see Figures 4-5). Fermentation of sugars in the Laurl Tryptose broth indicated coliform bacilli to be present in both creeks (see Figures 4-5). Colony growth on the Nutrient agar confirmed the presence of fecal contamination in both creeks. The National Park Service also found E. coli in Mud Island Creek in various amounts for each month checked (McInnis, 2013). The presence of E. coli and coliform bacilli may be the reason why Coles Creek was labeled as degraded and Mud Island Creek was labeled as potentially degraded by the National Park Service (Meiman, 2006).

In the previous seasonal study by Hopkins and Acholonu (2005) of Coles and Mud Island Creeks, the chemical and the physical profiles were checked, but not the biological profile. Ninety per cent of the parameters checked had decreased and 10% increased. This is seen when comparison was made between the results of 2002-03 (Hopkins and Acholonu, 2005) study and present study of both creeks. Physical parameters stayed within the range set by the MSWQC / EPA. Mud Island Creek's DO for the 2012-13 study was 7.42 ppm (see Table 1) and for 2002-03 it was 11.0 ppm (see Table 4). Coles Creek's DO for the 2012-13 study was 8.93 ppm and it was 6.9 ppm for 2002-03, which was above the threshold of 5 ppm. Both of these creeks' surface water temperatures did not exceed the 32 °C MSWQC / EPA standard for each of the study years. Mud Island Creek's surface water temperature was 25.6 °C and Coles Creek's surface water temperature was 20.2 °C for study year 2012-13. The study year 2002-03 temperatures was 17.13 °C for Mud and 16.9 °C for Coles. The pH for Coles (2012-13 was 7.08) and Mud Island Creeks (2012-13 was 7.1). During study year 2002-03 the pH was 6.68 (Mud) and 6.26 (Coles) staying within the range of 6.5 to 9.0 set by the State of MS.

For both study years of Mud Island Creek, alkalinity and hardness remained above the MSWQC / EPA standard, however they decreased (see Tables 1&4). For study year 2002-03 alkalinity was 174.3 ppm, then deceased by 45 ppm to 129.06 ppm for 2012-13 study year, which was not a significant decrease (p > 0.05). Hardness for study year 2002-03 was 126.3 ppm then decreased by 21 ppm to 104.53 ppm, which is also not a significant decrease (p > 0.05). Carbon dioxide started below the state's standard in 2002-03 study at 4.9 ppm then increased significantly (p < 0.05) to 28.13 ppm in 2012-13 which caused it to become above the standard (see Figure 10). All other parameters remained below the MSWQC / EPA standard.

Alkalinity and hardness decreased but, remained above the MSWQC / EPA standards for both study years of Coles Creek. Hardness was 89.1 ppm for 2002-03. It decreased by 10.7 ppm to 78.4 ppm for 2012-13 which did not show a significant change (p > 0.05). Alkalinity was 130.4 ppm (2002-03) which decreased by 57.34 ppm to 73.06 ppm (2012-13), not a significant decrease (p > 0.05). Carbon dioxide level was above the standard for 2012-13 study year. It increased to 17.66ppm (2012-13) from 5.1ppm (2002-03) a significant increase (p < 0.05) (see Figure 11). All other parameters remained below the MSWQC / EPA standard.

The National Park Service (NPS) 2012 monitoring and the current study on Mud Island Creek measured a few of the same parameters. They are pH, DO, turbidity and nitrate. The NPS results were as follow: pH 7.8, DO 9.25 ppm, turbidity 26.6 NTU and nitrate 3.6 ppm. The present study results were as follow: pH 7.1, DO 7.42 ppm, turbidity 59.85 NTU and nitrates 0.325 ppm. These results are below the MSWQC / EPA standard. Bodies of water with higher than state limits for contaminants may occur naturally, therefore, they are not subject to meeting the criteria set by the state (Quality criteria, 1986).

Two observations were made by this study about Mud Island and Coles Creeks. First, alkalinity, hardness and carbon dioxide reached levels above the MSWQC / EPA standards. Second, the high measurements may be a natural occurrence thus, are not subject to meeting the standards set by the state (Quality criteria, 1986). It is necessary to continue to monitor the water quality of these freshwater bodies by future investigators to answer the question, "Are the high levels of alkalinity, hardness and carbon dioxide natural or caused by pollutants from a point source or non-point source".



Figure 8: Mud Island and Coles Creeks test results compared with MSWQC / EPA standards.



Figure 9: Mud Island Creek 2002-03 test results compared with Mud Island Creek 2012-13 test reslts



Figure 10: Coles Creek 2002-03 test results compared with Coles Creek 2012-13 test reslts

Parameters	Fall	Winter	Spring	Average	MSWQC/ EPA
Chemical ppm					
Alkalinity *	85.3	126.6	180.0	129.0	3.08
Ammonia-N	1.0	0.88	0.5	0.9	10.0
Calcium	36.0	53.3	126.7	64.5	200.0
Carbon dioxide*	41.3	25.9	21.3	28.1	10.0
Chloride	21.3	21.7	28.0	23.7	230.0
Copper	0.0	0.0	0.0	0.0	
Hardness*	56.0	101.3	162.7	104.5	50.0
Iron	0.0	0.0	0.0	0.0	0.2
Magnesium	20.0	81.3	36.0	40.0	150.0
Phosphate	0.0	0.0	0.5	0.16	0.1
Silica	20.0	20.0	20.0	20.0	75.0
Nitrate-N	0.25	0.25	0.25	0.25	10.0
Dissolved oxygen	8.6	7.4	6.3	7.4	5.0
Salinity	0.13	0.166	0.1	0.1	

Table 2: Seasonal distribution of pollutants in Mud Island Creek, 2012-2013

Parameters	Fall	Winter	Spring	Average	MSWQC /EPA
Physical					
Turbidity NTU	98.7	63.4	9.9	59.8	50.0
Odor	None	None	None	None	
Surface H ₂ O temp°C	16.2	17.03	18.0	25.6	32.2
рН	6.9	7.0	7.5	7.1	6.5-9.0

Parameters	Fall	Winter	Spring	Average	MSWQC /EPA
Biological	coliform	coliform	coliform	coliform	Col/mL
Lauryl Trptose Broth	detected	detected	detected	detected	200-400
Nutrient Agar	detected	detected	detected	detected	200-400
MaConkey Agar	detected	detected	detected	detected	200-400

Parameters	Fall	Winter	Spring	Average	MSWQC/ EPA
Chemical ppm					
Alkalinity *	70.7	65.8	97.3	73.1	3.08
Ammonia-N	1.0	0.83	0.5	0.5	10.0
Calcium	41.3	54.2	45.3	46.9	200.0
Carbon dioxide*	17.3	18.4	15.7	17.66	10.0
Chloride	52.0	23.5	24.0	29.6	230.0
Copper	0.0	0.0	0.0	0.0	
Hardness*	68.0	80.9	81.3	78.4	50.0
Iron	0.0	0.0	0.0	0.0	0.2
Magnesium	26.7	29.8	36.0	30.4	150.0
Phosphate	0.0	0.0	0.5	0.16	0.1
Silica	20.0	20.0	20.0	20.0	75.0
Nitrate-N	0.1	0.2	0.2	0.5	10.0
Dissolved oxygen	11.0	8.77	7.3	8.9	5.0
Salinity	0.3	0.186	0.1	0.193	

 Table 3: Seasonal distribution of pollutants in Coles Creek, 2012-2013

Parameters	Fall	Winter	Spring	Average	MSWQC /EPA
Physical					
Turbidity NTU	42.8	84.9	20.0	63.8	50.0
0.1	NT.	N	N	N	
Odor	INOne	None	None	None	
Surface H ₂ O temp°C	18.0	11.3	18.3	15.86	32.2

Parameters	Fall	Winter	Spring	Average	MSWQC /EPA
Biological	coliform	coliform	coliform	coliform	Col/mL
Lauryl Trptose Broth	detected	detected	detected	detected	200-400
Nutrient Agar	detected	detected	detected	detected	200-400
MaConkey Agar	detected	detected	detected	detected	200-400

Parameters	Mud Island Creek	Coles Creek	MSWQC/ EPA
Chemical ppm			
Alkalinity*	174.3	130.5	20.0**
Ammonia-N	0.5	0.57	10.0
Calcium			
Carbon dioxide*	4.9	5.1	10.0
Chloride	29.0	49.8	250.0**
Hardness*	126.3	89.1	50.0
Iron	2.53	2.53	0.2
Magnesium	36.7	43.0	150.0
Phosphate	0.41	0.13	0.1
Silica	29.4	24.7	75.0
Nitrate-N	0.22	0.19	10.0
Dissolved oxygen	11.09	6.98	5.0
Salinity	0.12	0.10	

Table 4: Mud Island and Coles Creeks, compared to MSWCQ/EPA, 2002-2003

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Parameters	Mud Island Creek	Coles Creek	MSWQC/E PA
Physical			
Turbidity NTU	50.55	82.05	50.0
Odor	None	None	None
Surface H ₂ O °C	17.3	16.9	32.2
pH	6.68	6.29	6.5-9.0

<u>Note</u>: Single asterisk = parameters above the standard set by MSWQC/EPA. Double asterisk = Standards the MSWCQ EPA decreased after this study was done. Dash = not found.

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Ms. Gerri Wilson Mississippi Academy of Sciences Post Office Box 55907 Jackson, MS 39296-5907

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- The Academy provides poster backboards. Each backboard is 34" high by 5' wide. Mount the poster on the board assigned to you by your Division Chairperson. Please do not draw, write, or use adhesive material on the boards. You must provide your own thumb tacks.
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Author Guidelines

Editorial Policy. The Editorial Board publishes articles on all aspects of science that are of general interest to the scientific community. General articles include short reviews of general interest, reports of recent advances in a particular area of science, current events of interest to researchers and science educators, etc. Research papers of sufficiently broad scope to be of interest to most Academy members are also considered. Articles of particular interest in Mississippi are especially encouraged.

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Format

Abstract. In 250 or fewer words summarize any new methods or procedures critical to the results of the study and state the results and conclusions.

Introduction. Describe the knowledge and literature that gave rise to the question examined by, or the hypothesis posed for the research.

Materials and methods. This section should describe the research design, the methods and materials used in the research (subjects, their selection, equipment, laboratory or field procedures), and how the findings were analyzed.

Results. The text of the results should be a descriptive narrative of the main findings, of the reported study. This section should not list tabulated data in text form. Reference to tables and figures included in this section should be made parenthetically in the text.

Discussion. In this section compare and contrast the data collected in the study with that previously reported in the literature. Unless there are specific reasons to combine the two, as explained by the author in the letter of transmittal, Results and Discussion should be two separate sections.

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Literature cited. List references alphabetically. Cite references in the text by author and year of publication (e.g., Smith, 1975; Black and Benghuzzi, 2011; Smith et al., 2010; Smith, 2011a, 2011b). The following examples illustrate the style to be used in the literature list.

Black DA, Lindley S, Tucci M, Lawyer T, Benghuzzi H. A new model for the repair of the Achilles tendon in the rat. J Invest Surg. 2011; 24(5): 217-221.

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Bold, H.C., C.J. Alexopoulos, and T. Delevoryas. 1980. Morpholgy of plants and fungi, 4th ed. Harper and Row, New York. 819 pp

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