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Geographic Disparities in the Prevalence of Nonfatal Heart Disease and Stroke in Adults in the Mississippi Delta, Mississippi Non-Delta, and in the United States

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ABSTRACT

Objective: The primary purpose of this study was to assess geographic disparities in the prevalence of nonfatal heart disease and stroke and their risk factors between the Mississippi Delta, Mississippi non-Delta, and the United States.
Design and Setting: For this study, we aggregated and analyzed 2005 and 2007 data from the Behavioral Risk Factor Surveillance System (BRFSS).
Participants: Only non-institutionalized adults ages 18 and older were eligible to participate in the BRFSS.
Main Outcome Measures: We estimated weighted crude and adjusted prevalence rates and odds ratios of nonfatal heart disease and stroke and their risk factors in the Mississippi Delta, Mississippi non-Delta, and the United States.
Results: Compared to other Mississippians, adults in the Mississippi Delta were more likely to be diagnosed with coronary heart disease and obesity, less likely to be diagnosed with diabetes and high blood pressure, and just as likely to be diagnosed with a heart attack or stroke. Compared to the U.S. adult population, adults in the Mississippi Delta had greater odds of physical inactivity within a given month.
Conclusion: National- and state-level surveillance data are inadequate to fully assess the public health burden that heart disease and stroke place on rural and impoverished communities like the MS Delta. Within the state it is important to analyze regional or local surveillance data and identify regional or county-level disparities in cardiovascular disease prevalence and mortality, especially when the state's overall cardiovascular disease death rate is high. Public health efforts and resources can then be targeted at regions within the state, such as the Delta, or even at specific counties with the most disparate rates.

INTRODUCTION

The Mississippi Delta (MS Delta) lies between the Mississippi and Yazoo Rivers in the northwest of the state and covers about 11,000 square miles or almost 23% of the state's total area. It is primarily rural and includes the following counties: Washington, Tate, Humphreys, Carroll, Issaquena, Panola, Quitman, Bolivar, Coahoma, Leflore, Sunflower, Sharkey, Tunica, Tallahatchie, Holmes, Yazoo, Grenada, and Warren (Delta Health Alliance, 2009).

Approximately 60% of the MS Delta population is African American compared to 36% of the total state population and 12% of the total national population. (Delta Health Alliance, 2009). Compared to other areas of Mississippi, the Mississippi Delta has higher per capita rates of illiteracy, teen pregnancy, and high school dropouts (Mississippi Delta Fact Sheet, 2004). Several of the MS Delta counties rank among the nation's top 100 poorest counties (U.S. Department of Commerce, 2006).

Mississippi's health and healthcare issues cannot be separated from its socioeconomic issues. In 2001, the estimated cost of cardiovascular disease (CVD) in Mississippi was about $3.7 billion (Mississippi Chronic Disease Fact Sheet, 2009). In 2004, Mississippi's cardiovascular disease death rate was the highest in the country, and in 2005 was 25% higher than the U.S. rate (CDC, 2005). Mississippi's coronary heart disease (CHD) death rate ranks 43rd and its stroke death rate ranks 45th in the nation (Heart disease and stroke statistics, 2009). More Mississippians die each year from CVD than

from all types of cancer, traffic injuries, suicides, and AIDS combined (CDC, 2008). Without the MS Delta region, Mississippi would rank average or near average in almost all major health indicators (Lower Mississippi Delta Region Area Map, 2001).

The extent of disparities in the prevalence of nonfatal heart disease and stroke between the Mississippi Delta, Mississippi non-Delta (MS non-Delta), and the rest of the United States are less well known. The primary purpose of this study was to determine the geographic disparities in the prevalence of nonfatal heart disease and stroke and their risk factors (diabetes, obesity, physical inactivity, high blood pressure, high blood cholesterol levels, and smoking cigarettes) between the MS Delta, MS non-Delta, and the rest of the United States.

MATERIALS AND METHODS

Sampling and data collection

State health agencies in collaboration with the Centers for Disease Control and Prevention (CDC) conduct the Behavioral Risk Factor Surveillance System (BRFSS), a standardized telephone survey. An independent probability sample of non-institutionalized adult residents aged 18 years or older with telephones is selected in participating states. Data are collected monthly through telephone interviews using a multistage design based on random digit dialing methods. For this study we aggregated data collected by the BRFSS during 2005 and 2007 (CVD questions were not asked in 2006).

The 2005 and 2007 BRFSS MS Delta **response** rates were 70% in 2005 and 75% in 2007; rates for Mississippi were 72% in 2005 and 76% in 2007. The 2005 and 2007 BRFSS United States median **response** rates were 75% in 2005 and 72% in 2007. A 2005 and 2007 aggregated total of 12,146 responders residing in Mississippi completed the BRFSS interview. MS Delta residents completed 2,767 BRFSS interviews; MS non-Delta county residents completed 9,379 BRFSSS interviews. Fewer than 50 responders completed questionnaires in the following MS Delta counties: Issaquena (n=15), Sharkey (n=43), and Tunica (n=48) (Table 1). Excluding data collected in Mississippi, 345,462 responders in the United States, Puerto Rico, and the Virgin Islands completed the 2005 BRFSS interview, and 416,622 responders completed the 2007 BRFSS interview. These state-level BRFSS data were pooled to produce national estimates.

TABLE 1. Mississippi Delta Sample

County Name	No. Respondents	%
Bolivar	197	7.1
Carroll	74	2.7
Coahoma	166	6.0
Grenada	136	4.9
Holmes	166	6.0
Humphreys	63	2.3
Issaquena	15	0.5
Leflore	232	8.4
Panola	225	8.1
Quitman	74	2.7
Sharkey	43	1.6
Sunflower	186	6.7
Tallahatchie	91	3.3
Tate	183	6.6
Tunica	48	1.7
Warren	368	13.3
Washington	329	11.9
Yazoo	171	6.2

Geographic strata

We created the variable **"**region**"** to categorize respondents residing in the three geographic strata of interest: the MS Delta, consisting of the 18 counties listed previously in the Introduction; MS non-Delta, consisting of all other MS counties; and the rest of the United States and the District of Columbia. The phrase "in the United States," refers to each state in the United States of America and the District of Columbia with the exception of the state of Mississippi.

BRFSS Questionnaire

We determined the prevalence of nonfatal heart disease and stroke by estimating the prevalence of self-reported diagnosed myocardial infarctions (heart attack), stroke, and coronary heart disease. We collected these data by asking respondents, "Has a doctor, nurse or other health professional ever told

you that you had …" each specific condition separately. The answers to these outcomes were coded by interviewers as "yes," "no," "do not know," or "refused."

We determined the prevalence of heart disease and stroke risk factors by estimating the prevalence of self-reported smoking, obesity, physical inactivity, diagnosed diabetes, high blood pressure, and high blood cholesterol. We collected these data by asking respondents, "Has a doctor, nurse or other health professional ever told you that you had…" each specific condition separately. We determined current smoker status by asking two questions: "Have you smoked at least 100 cigarettes in your entire life" and "Do you now smoke cigarettes everyday, some days or not at all." A current smoker is defined as adults who smoked at least 100 cigarettes in their entire life, and either smokes everyday or some days. We assessed the status of an individual's physical inactivity/no exercise from the question, "During the past month, other than your regular job, did you participate in any physical activities or exercises such as running, calisthenics, golf, gardening, or walking for exercises?" The answers to the above outcomes were coded by interviewers as "yes," "no," "do not know," or "refused." Obesity status was based upon BMI calculated from reported height and weight. We calculated BMI as weight (kilograms) divided by height (meters squared) based on self-reported weight and height. Participants were classified as obese if their BMI was ≥30.

Respondents who reported being white and not of Hispanic, Latino, or Spanish descent were considered to be white; similarly, respondents who reported being black or African-American and not of Hispanic, Latino, or Spanish descent were considered to be black. Respondents who reported being employed for wages or self-employed was considered employed. Respondents' annual income was categorized as less than $15,000, $15,000 to $34,999, $35,000 to $49,999, and $50,000 or more. Respondents' highest educational attainment was categorized as less than high school graduate, high school graduate, college 1–3 years, and college 4+ years.

Weighting BRFSS Data

The BRFSS sample for each state and the District of Columbia was weighted to the respondent's probability of selection. Respondents' sampling weight for each state, with the exception of Mississippi, was further adjusted by age, sex or age, sex, and race-specific weighting adjustment classes. In contrast; sampling weights for respondents in Mississippi were adjusted by geographical region, age, sex, and race-specific weighting adjustment classes. The marginal totals for each weighting adjustment class were based on population totals as reported in the 2005 and 2007 Census projections for each state. A detailed description of BRFSS survey methods is available elsewhere (Nelson et al, 1998).

Statistical Analysis

For each geographic stratum we estimated weighted prevalence rates and corresponding 95% CIs using SAS callable SUDAAN (SAS System, 2008). Odds ratios (OR) were calculated comparing MS Delta vs. MS non-Delta and MS Delta vs. rest of the U.S.. Using logistic regression, prevalence and odds ratio estimates were adjusted initially for demographic variables (race, age, sex) and SES (highest educational attainment, annual income, employment status, and access to healthcare). In a second step, further adjustment was then made for smoking, lack of physical exercise, obesity, diabetes, high blood pressure, and high blood cholesterol levels. We interpreted OR estimates as an approximation for prevalence ratio estimates, due to the infrequent and rare occurrence of heart disease and stroke in the general population (Kleinbaum, et al, 1982). We excluded respondents categorized as a refusal, or response unknown for either of the demographic, SES, or risk factor variables from the logistic regression analyses.

RESULTS AND DISCUSSION

Description of Sample

In the MS Delta sample, 48.1% were black and 31.4% were male (Table 2). The mean age was 53.7 years (SD 17.3 years, min. 7 years, max. 96 years). Seventy-six percent had at least a high school diploma, 48.8% were employed, and 21.4% earned less than $15,000 annually. In the MS non-Delta sample, 23.1% were black, 30.9% were male, and the mean age was 53.3 years (SD 17.0 years,

min. 7 years, max. 99 years). Eighty percent had at least a high school diploma, and **16.2**% earned less than $15,000 annually (Table 2). In the U.S. sample, 8.2% were black, 37.9% were male, and the mean age was 52.6 years (SD 17.4 years, min. 7 years, max. 99 years). Ninety percent had at least a high school diploma, and 11.2% earned less than $15,000 annually (Table 2).

TABLE 2. Description of MS Delta , MS Non-Delta, U.S. Samples

Characteristic	MS Delta			MS non-Delta			U.S.		
	n	unweighted percentage	weighted percentage	n	unweighted percentage	weighted percentage	n	unweighted percentage	weighted percentage
Race									
White non-Hispanic	1,282	46.3	44.9	6,728	71.7	73.2	608,438	91.8	88.1
Black non-Hispanic	1,331	48.1	55.1	2,167	23.1	26.8	54,157	8.2	11.9
Gender									
Male	868	31.4	47.0	2,894	30.9	38.3	288,187	37.9	48.6
Female	1,899	68.6	53.0	6,431	68.6	61.7	473,240	62.2	51.4
Age (years)									
18-34	403	14.6	33.1	1,459	15.6	24.3	120,948	16.0	30.4
35-54	1,019	36.8	36.9	3,518	37.5	37.7	283,343	37.5	39.0
55 +	1,331	48.1	30.0	4,329	46.2	38.0	351,008	46.5	30.6
Education level									
< HS Graduate	663	24.0	22.2	1,896	20.2	19.7	35,841	10.2	11.8
HS Graduate	882	31.9	36.1	3,400	36.3	37.0	106,529	30.8	29.4
College 1-3 (years)	595	21.5	21.2	1,713	18.3	20.0	90,903	26.4	26.2
College 4+ (years)	625	22.6	20.6	2,190	23.4	23.3	111,328	32.7	32.6
Employment									
Unemployed	1,413	51.1	44.5	4,250	45.3	42.3	341,785	45.0	38.6
Employed	1,349	48.8	55.5	5,073	54.1	57.7	417,116	55.0	61.4
Income									
< $15,000	591	21.4	21.0	1,516	16.2	18.4	73,542	11.2	10.1
$15 - 34,999	907	32.8	39.3	2,512	26.8	34.8	202,589	30.8	28.1
$35 - 59,999	295	10.7	13.1	873	9.3	13.2	109,551	16.7	15.8
$60,000+	587	21.2	26.6	2,436	26.0	33.6	271,668	41.3	46.1

Cardiovascular Disease

Heart Attack

The crude weighted prevalence of diagnosed heart attack was 4.7% (95% CI: 3.7, 5.8) in the MS Delta, 4.9% (4.5, 5.5) in the MS non-Delta, and 4.1% (4.1, 4.2) in the United States (Table 3). Adults in the MS Delta were just as likely to be diagnosed with a heart attack as other adult Mississippians (odds ratio

[OR]=0.9 (0.7, 1.2)) and adults in the United States (OR=1.1 (0.9, 1.4)) (Table 3). These ORs changed only slightly after adjustment for demographic and SES factors (Table 4) and for demographic, SES, and risk factors (Table 5).

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TABLE 3. Prevalence and Odds Ratio Crude Estimates

	Location	n	%	95% CI	$OR_{Delta\ vs\ MS}$	95% CI	$OR_{Delta\ vs\ USA}$	OR_2_95% CI
Heart Attack	Delta	2,748	4.7	(3.7, 5.8)	0.9*	(0.73, 1.21)	1.1*	(0.90, 1.43)
	MS	9,339	4.9	(4.5, 5.5)				
	USA	757,579	4.1	(4.05, 4.20)				
Stroke	Delta	2,760	4.3	(3.4, 5.4)	1.2*	(0.88, 1.51)	1.6*	(1.28, 2.10)
	MS	9,351	3.7	(3.3, 4.2)				
	USA	759,283	2.7	(2.58, 2.71)				
Coronary Heart Disease	Delta	2,715	4.7	(3.8, 5.8)	1.0*	(0.79, 1.27)	1.1*	(0.88, 1.35)
	MS	9,272	4.7	(4.3, 5.2)				
	USA	754,507	4.3	(4.2, 4.4)				
Diabetes	Delta	2,764	11.3	(9.9, 12.9)	1.1*	(0.94, 1.31)	1.5*	(1.25, 1.68)
	MS	9,371	10.4	(9.7, 11.0)				
	USA	760,681	8.2	(8.1, 8.3)				
Smoking	Delta	2,757	26.3	(23.6, 29.1)	1.2	(1.0, 1.4)	1.4	(1.2, 1.7)
	MS	9,340	23.5	(22.3, 24.7)				
	USA	758,197	19.9	(19.7, 20.1)				
No Exercise	Delta	2,765	35.1	(32.4, 37.9)	1.2	(1.0, 1.4)	1.7	(1.5, 1.9)
	MS	9,364	31.4	(30.2, 32.6)				
	USA	760,597	24.7	(24.5, 25.0)				
Obese	Delta	2,651	36.1	(33.2, 39.1)	1.3	(1.1, 1.5)	1.7	(1.5, 1.9)
	MS	9,021	30.9	(29.7, 32.2)				
	USA	726,392	25.3	(25.1, 25.5)				
High Blood Pressure	Delta	2,764	36.4	(33.8, 39.1)	1.1	(1.0, 1.3)	1.6	(1.4, 1.8)
	MS	9,257	33.1	(31.9, 34.3)				
	USA	760,027	26.9	(26.7, 27.1)				
High Cholesterol	Delta	2,223	36.3	(33.4, 39.3)	0.9	(0.8, 1.0)	1.0	(0.9, 1.1)
	MS	7,646	38.6	(37.3, 39.9)				
	USA	632,552	36.6	(36.4, 36.9)				

* Interpreted as crude prevalence ratio estimates

TABLE 4. Adjusted Prevalence and Odds Ratio Estimates: Adjusted by Demographic and SES Factors

	Location	%	95% CI	$OR_{Delta\ vs\ MS}$	95% CI	$OR_{Delta\ vs\ USA}$	95% CI
Heart Attack	Delta	4.2%	(3.1, 5.2)	0.8*	(0.6, 1.1)	1.0*	(0.7, 1.3)
	MS	4.9%	(4.2, 5.6)				
	USA	4.3%	(4.2, 4.4)				
Stroke	Delta	3.2%	(2.3, 4.1)	1.0*	(0.7, 1.4)	1.3*	(0.9, 1.7)
	MS	3.2%	(2.7, 3.7)				
	USA	2.6%	(2.5, 2.7)				
Coronary Heart Disease	Delta	4.7%	(3.6, 5.7)	1.7*	(1.3, 2.5)	1.0*	(0.8 1.4)
	MS	2.8%	(4.1, 5.0)				
	USA	4.5%	(4.4, 4.6)				
Diabetes	Delta	8.3%	(7.1, 9.6)	0.7*	(0.6, 0.9)	1.0*	(0.9, 1.3)
	MS	11.0%	(10.2, 11.8)				
	USA	8.0%	(7.9, 8.1)				
Smoking	Delta	21.9%	(19.4, 24.3)	0.9	(0.8, 1.1)	0.9	(0.8, 1.1)
	MS	19.5%	(18.2, 20.7)				
	USA	20.8%	(20.6, 21.1)				
No Exercise	Delta	26.1%	(23.6, 28.6)	1.0	(0.9, 1.3)	1.3	(1.1, 1.4)
	MS	25.4%	(24.3, 26.6)				
	USA	21.9%	(21.7, 22.1)				
Obese	Delta	30.3%	(27.5, 33.1)	1.7	(1.4, 2.0)	1.3	(1.1, 1.4)
	MS	20.7%	(19.5, 22.0)				
	USA	26.2%	(26.0, 26.4)				
High Blood Pressure	Delta	32.8%	(30.2, 35.4)	0.9	(0.8, 1.1)	1.3	(1.1, 1.4)
	MS	34.1%	(32.9, 35.4)				
	USA	28.1%	(27.9, 28.3)				
High Cholesterol	Delta	38.1%	(35.0, 41.3)	0.9	(0.8, 1.1)	1.0	(0.9, 1.3)
	MS	40.3%	(38.7, 41.9)				
	USA	37.0%	(36.7, 37.2)				

* Interpreted as adjusted prevalence ratio estimates

** Adjusted for demographic (age, race, sex), and SES (employment status, annual income, educational attainment, access to healthcare),

TABLE 5. Adjusted Prevalence and Odds Ratio Estimates: Adjusted by Demographic and Risk Factors

	Location	%	95% CI	$OR_{Delta\ vs\ MS}$	95% CI	$OR_{Delta\ vs\ USA}$	95% CI
Heart Attack	Delta	4.6%	(3.5, 5.7)	1.1*	(0.8, 1.4)	1.1*	(0.8, 1.4)
	MS	5.0%	(4.4, 5.5)				
	USA	5.0%	(4.9, 5.1)				
Stroke	Delta	3.3%	(2.4, 4.1)	0.9*	(0.7, 1.3)	0.9*	(0.7, 1.3)
	MS	3.1%	(2.7, 3.5)				
	USA	3.0%	(2.9, 3.1)				
Coronary Heart Disease	Delta	5.6%	(4.4, 6.9)	1.7*	(1.3, 2.5)	1.1*	(0.8, 1.4)
	MS	3.4%	(2.9, 3.9)				
	USA	5.3%	(5.2, 5.4)				
Diabetes	Delta	9.0%	(7.7, 10.3)	0.5	(0.4, 0.7)	0.9*	(0.8, 1.1)
	MS	14.5%	(13.4, 15.5)				
	USA	9.3%	(9.2, 9.5)				
Smoking	Delta	20.7%	(17.8, 23.7)	1.4	(1.1, 1.7)	1.3	1.0, 1.4)
	MS	16.4%	(15.1, 17.6)				
	USA	18.3%	(18.1, 18.5)				
No Exercise	Delta	24.8%	(22.1, 27.4)	1.1	(0.9, 1.4)	1.3	(1.1, 1.4)
	MS	22.7%	(21.4, 23.9)				
	USA	21.2%	(21.0, 21.4)				
Obese	Delta	28.4%	(25.4, 31.4)	2.0	(1.7, 2.5)	1.0	(0.9, 1.3)
	MS	17.5%	(16.2, 18.7)				
	USA	27.6%	(27.3, 27.8)				
High Blood Pressure	Delta	35.0%	(32.2, 37.6)	0.7	(0.6, 0.8)	1.3	(1.0, 1.4)
	MS	42.8%	(41.3, 44.2)				
	USA	31.9%	(31.7, 32.2)				
High Cholesterol	Delta	37.2%	(34.1, 40.2)	0.9	(0.8, 1.1)	1.0	(0.8, 1.1)
	MS	38.8%	(37.2, 40.4)				
	USA	37.1%	(36.8, 37.3)				

* Interpreted as adjusted prevalence ratio estimates

** Adjusted for demographic (age, race, sex), SES (employment status, annual income, educational attainment, access to healthcare), and risk factors (diabetes, smoking, no physical exercise, obese, high blood pressure, and high blood cholesterol)

Stroke

The crude weighted prevalence of diagnosed stroke was 4.3% (3.4, 5.4) in the MS Delta, 3.7% (3.3, 4.2) in the MS non-Delta, and 2.7% (2.6, 2.7) in the United States (Table 3). Adults in the MS Delta were just as likely to be diagnosed with a stroke as other adult Mississippians (OR=1.2 (0.9, 1.5)); however, adults in the MS Delta were more likely to be diagnosed with a stroke when compared to adults in the United States (OR=1.6 (1.3, 2.1)) (Table 3). These ORs were attenuated and no longer statistically significant after adjustment for demographic and SES factors (Table 4) and for demographic, SES, and risk factors (Table 5).

Coronary Heart Disease (CHD)

The crude weighted prevalence of diagnosed CHD was 4.7% (3.8, 5.8) in the MS Delta, 4.7% (4.3, 5.2) in the MS non-Delta, and 4.3% (4.2, 4.4) in the United States (Table 3). Adults in the MS Delta were just as likely to be diagnosed with CHD as other adult Mississippians (OR=1.0 (0.8, 1.3)) and adults in the United States (OR=1.1 (0.9, 1.4)) (Table 3). After adjustment for demographic and SES factors, adults in the MS Delta were 1.7 (1.3, 2.5) times more likely to be diagnosed with coronary heart disease when compared to other adults Mississippians, and just as likely to be diagnosed with coronary heart disease when compared to adults in the United States (OR=1.0, (0.8, 1.4)) (Table 4). There was little or no change in these ORs after further adjustment for demographic, SES, and risk factors (Table 5).

Cardiovascular Disease Risk Factors

Diabetes

The crude weighted prevalence of diagnosed diabetes was 11.3% (9.9, 12.9) in the MS Delta, 10.4% (9.7, 11.0) in the MS non-Delta, and 8.2% (8.1, 8.3) in the United States (Table 3). Adults in the MS Delta were just as likely to be diagnosed with diabetes as other adult Mississippians (OR=1.1 (0.9, 1.3)); however, adults in the MS Delta were 1.5 (1.3, 1.7) times more likely to be diagnosed with diabetes when compared to adults in the United States (Table 3). After adjustment for demographic and SES factors, adults in the MS Delta were less likely to be diagnosed with diabetes as other adult Mississippians (OR=0.7 (0.6, 0.9)), and just as likely to be diagnosed as adults in the United States (OR=1.0 (0.9, 1.3)) (Table 4). These ORs were further attenuated after adjustment for demographic, SES, and risk factors (Table 5).

Current Smoking

An estimated 26.3% (23.6, 29.1) of adults in the MS Delta, 23.5% (22.3, 24.7) of adults in the MS non-Delta, and 19.9% (19.7, 20.1) of adults in the United States are current cigarette smokers (Table 3). Compared to adults in the MS non-Delta, adults in the MS Delta had a slightly increased odds of being current cigarette smokers (OR=1.2 (1.0, 1.4)). After adjustment for demographic, SES, and risk factors this OR increased to 1.4 (1.1, 1.7) (Table 5). Compared to adults in the United States, adults in the MS Delta had a 1.4 (1.2, 1.7)) times greater odds of being current cigarette smokers. After adjustment for demographic, SES, and risk factors this OR increased to 1.3 (1.0, 1.4) (Table 5).

No Exercise

An estimated 35.1% (32.4. 37.9) of adults in the MS Delta are physically inactive in a given month, compared to 31.4% (30.2, 32.6) of adults in the MS non-Delta and 24.7% (24.5, 25.0) of adults in the United States (Table 3). Compared to adults in the MS non-Delta, MS Delta adults had a slightly higher odds of being physically inactive in a given month (OR=1.2 (1.0, 1.4)); this was attenuated slightly after adjustment for demographic and SES factors (OR=1.0 (0.9, 1.3)) and after adjustment for demographic, SES, and risk factors (1.1 (0.9, 1.4)). Compared to adults in the United States, the odds of being physically inactive in a given month was 1.7 (1.5, 1.9) times greater for adults in the MS Delta. After adjustment the OR was 1.3 (1.1, 1.4).

Obesity

An estimated 36.1% (33.2, 39.1) of MS Delta adults are obese, compared to 30.9% (29.7, 32.2) of adults in the MS non-Delta and 25.3% (25.1, 25.5) of adults in the Unites States. Adults in the MS Delta had 1.3 times greater odds of being obese (OR=1.3 (1.1, 1.5) when compared to adults in the rest of Mississippi. This OR increased to 1.7 (1.4, 2.0) after adjustment for demographic and SES factors and 2.0 (1.7, 2.5) after adjustment for for demographic, SES, and risk factors. Compared to adults in the United States, adults in the MS Delta

had 1.7 times greater odds of being obese (OR=1.7 (1.5, 1.9)) (Table 3). This OR was attenuated after adjustment for demographic and SES factors (OR=1.3 (1.1, 1.4)) and was no longer statistically significant after adjustment for demographic, SES, and risk factors (OR=1.0 (0.9, 1.3)).

High Blood Pressure

An estimated 36.4% (33.8, 39.1) of MS Delta adults have been diagnosed with high blood pressure, compared to 33.1% (31.9, 34.3) of adults in the MS non-Delta and 26.9% (26.7, 27.1) of adults in the United States (Table 3). Compared to adults in the MS non-Delta, MS Delta adults had a slightly increased odds of being diagnosed with high blood pressure (OR=1.1 (1.0, 1.3)). This decreased after adjustment for demographic and SES factors (OR=0.9 (0.8, 1.1) (Table 4) and decreased further after adjustment for demographic, SES, and risk factors (OR=0.7 (0.6, 0.8). Compared to adults in the United States, adults in the MS Delta were more likely to have high blood pressure (OR=1.6 (1.4, 1.8). This OR was attenuated but still statistically significant after adjustment (Tables 4 and 5).

High Blood Cholesterol

An estimated 36.3% (33.4, 39.3) of MS Delta adults have been diagnosed with elevated blood cholesterol levels, compared to 38.6% (37.3, 39.9) of MS non-Delta adults and 36.6% (36.4, 36.9) of adults in the United States. Adults in the MS Delta did not have increased odds of being diagnosed with high blood cholesterol levels when compared to those in the rest of Mississippi (OR=0.9 (0.8, 1.0) or in the United States (OR=1.0 (0.9, 1.1) (Table 3). These ORs did not change after adjustment (Tables 4 and 5).

DISCUSSION

The primary purpose of this study was to determine geographic disparities in the prevalence of nonfatal heart disease and stroke and their risk factors between the MS Delta, MS non-Delta, and the rest of United States. National- and state-level surveillance data are inadequate to fully assess the public health burden that heart disease and stroke place on rural and impoverished communities like the MS Delta. National- and state-level rates can either overestimate or underestimate the burden of heart disease and stroke and their risk factor prevalence on local communities. The results of this study illustrate this to some extent.

Compared to other Mississippians, adults in the MS Delta had an increased likelihood of being diagnosed with coronary heart disease but were not more likely to be diagnosed with a heart attack or stroke. This latter finding was unexpected, as at least half of the 18 MS Delta counties (including Carroll, Issaquena, and Tunica counties) have the highest mortality rates of heart diseases and stroke in the state (CDC MAPS, 2009). It should be noted that the mortality rates of heart diseases includes heart attacks, CHD, and other diseases of the heart. This latter finding may also be due to a lack of access to vitally important emergency/trauma centers in the MS Delta as compared to the rest of Mississippi. In this study we used prevalence as the sole measure of disease burden, yet prevalence does not fully capture the public health impact of a disease or the barriers to care. For example, it is well known that residents in the MS Delta counties of Carroll, Issaquena, and Tunica do not have access to emergency/trauma care hospitals, and most hospitals in the state of Mississippi do not have neurologists or neurosurgeons on staff to provide urgent care for heart disease and stroke survivors (CDC MAPS, 2009). Lack of access to emergency medical care/trauma centers and distance from the hospital were significant barriers to the prompt emergency medical care necessary for acute stroke therapy (Menon et al., 1998).

Compared to other Mississippians, adults in the MS Delta were more likely to be obese but less likely to have diabetes or high blood pressure. However, it should be noted that obese adults who were also diagnosed as having diabetes or high blood pressure were more prevalent in the MS Delta than in the rest of Mississippi, Appendix A. Diabetes and high blood pressure are primarily preventable diseases, and are not the sole result of being obese. Strong evidence suggests that sufficient exercise and proper nutrition are more predictive of improvements in overall health than loss of body fat (Campos et al, 2005).

A diagnosis of high blood pressure or diabetes is dependent on adults in the MS Delta having reasonable access to healthcare professionals who would conduct those evaluations. Adults in the

MS Delta, were less likely to have access to healthcare than other Mississippians (PR=0.9 (o.86, 0.93)), see Appendix B. The consequence of this imbalance in healthcare access may result in a significant underestimation of total diabetes, or total high blood pressure prevalence among adults in the MS Delta.

LIMITATIONS

The sample size of data collected from the MS Delta counties was considerably smaller than that collected from the MS non-Delta counties or in the United States. As a result, the MS Delta sample produced larger standard errors and wider 95% confidence intervals. This may have contributed to conclusions of no statistical difference for the MS Delta sample even when point prevalence or odds ratio estimates were approximately equal to those of the MS non-Delta and/or U.S. populations. The diagnosis of heart disease and stroke was not based on a review of medical records, but on the accuracy of self reporting by BRFSS responders. Similarly, all demographic and risk factor data are self-reported, which may lead to bias in the prevalence estimates.

BRFSS data are cross-sectional; therefore, they do not determine cause-and-effect relationships. BRFSS data are not collected from institutionalized adults, as a result, point prevalence or odds ratio estimates of heart disease and stroke may be underestimated. Persons characterized as living in rural areas, poor, less-educated, or non-white were more likely to be without residential telephone landlines, a group currently not included in the BRFSS (Ford et al, 1998). The disparity in the prevalence of nonfatal heart disease between MS Delta and non-Delta counties, or between the MS Delta and U.S. adult populations might be greater than the findings reported in this study.

IMPLICATIONS

The state currently has several programs that address heart disease and stroke, for example, the Cardiovascular Health Program, the Chronic Illness Coalition, and the Task Force on Heart Disease and Stroke Prevention (CDC, 2005). The Task Force has developed explicit action plans to increase physical activity and the consumption of healthy diets, while decreasing the number of tobacco users (MSCVD, 2001). CDC, the American Heart Association, and other partners have also developed heart disease and stroke intervention strategies that promote smoking cessation, consuming five or more servings of fruits and vegetables per day, reducing stress, moderate weight loss, high fiber and low fat diets, and increasing leisure-time physical activities (CDC, 2008; AHA, 2009). To reduce the disparities in heart disease and stroke prevalence between the MS Delta and non-Delta, public health partners, school districts, religious institutions, and civic organizations that serve those communities should continue to advocate for prevention strategies that promote moderate weight loss, high fiber and low fat diets, and increased leisure-time physical activities. The findings in this study support the targeting of resources for developing and sustaining heart disease and stroke intervention programs at regions within the state, such as the Delta, or even at specific counties with the most disparate rates.

CONCLUSIONS

Within the state it is important to analyze regional or local surveillance data and identify regional or county-level disparities in cardiovascular disease prevalence and mortality, especially when the state's overall cardiovascular disease death rate is high. The disparities in coronary heart disease, obesity, diabetes, high blood pressure, and smoking prevalence between the MS Delta and the rest of the state identified in this study indicate where efforts and resources should be concentrated to reduce premature death from cardiovascular diseases.

DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily reflect the official position of the Centers for Disease Control and Prevention

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APPENDIX A. Crude Obesity Prevalence Estimates while controlling for Geographic Location and Age Group

Characteristics		Coronary Heart Disease			High Blood Pressure			Diabetes	
		Delta	MS		Delta	MS		Delta	MS
Age 18-34	**n**	150	360	**n**	150	360	**n**	150	359
	%	2.0	0.0	**%**	18.7	16.4	**%**	6.0	5.6
	95% CI	(0.7, 6.0)	(0.0, 0.0)	**95% CI**	(13.2, 25.7)	(12.9, 20.6)	**95% CI**	(3.2, 11.1)	(3.6, 8.5)
Age 35-54	**n**	418	1,122	**n**	426	1,123	**n**	425	1,124
	%	5.0	3.5	**%**	53.8	47.0	**%**	15.3	16.0
	95% CI	(3.3, 7.6)	(2.6, 4.7)	**95% CI**	(49.0, 58.4)	(44.1, 49.9)	**95% CI**	(12.2, 19.0)	(14.0, 18.3)
Age 55+	**n**	379	860	**n**	396	875	**n**	396	875
	%	13.2	10.6	**%**	79.0	63.5	**%**	41.2	25.8
	95% CI	(10.1, 17.0)	(8.7, 12.8)	**95% CI**	(74.6, 82.8)	(60.3, 66.7)	**95% CI**	(36.4, 46.1)	(23.0, 28.8)

APPENDIX B. Access to Healthcare

Delta			MS			$PR_{(Delta\ vs.\ MS)}$	
n	%	95% CI	n	%	95% CI	PR	95% CI
2,288	76.6	(73.7, 79.3)	8,152	85.4	(84.4, 86.4)	0.9	(0.86, 0.93)

Modified Atmosphere Storage Influences Quality Parameters and Shelf Life of 'Tifblue' Blueberries

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ABSTRACT

Rabbiteye blueberries(Vaccinium ashei L. c.v. 'Tifblue') were placed in plastic clamshell boxes, loaded into Shelf Life Extender (SLX^R)- Modified Atmosphere Storage (MAS) containers, gas flushed and saturated with either of 15.5% Ozone(O_3), 193 $mg.L^{-1}$ sulfur dioxide(SO_2), 18.8% carbon dioxide (CO_2), or normal air (control). Containers were then stored at 2^0C and sampled periodically over a period of 48 days. Regardless of gas treatment, percent decayed fruit did not differ up to 34 days. At 48 days of storage, weight loss was less for berries stored in SO_2 and O_3 than the control and CO_2. Moisture percentage and decayed fruit percentage not influenced by any of the treatments prior to 48 days. However, chroma and Hue values of berries were increased by SO_2 compared to the remaining treatments, indicating a shift from blue to blue red. Ozone increased storage life of blueberries with no detrimental effect.

INTRODUCTION

Blueberry acreage in Mississippi was approximately 667 ha in 2002 (Myles et al., 2004) and is predicted to increase to 1011 ha by the year 2008 (Spiers *et al*., 1998; Strik and Bañados, 2005; MDAC, 2008).Approximately 50% of Mississippi Rabbiteye and Southern Highbush blueberries are processed, and the remaining 50% are marketed as fresh fruit (48%) or sold on a pick-your-own basis (2%). Total blueberry production contributed $7 million to the state's economy in 2005 (Braswell, 2008) with fresh blueberries demanding the highest price and yielding larger profit. Since 50 % of fresh blueberries are immediately stored and shipped to market, extending shelf life is necessary because blueberries have a relatively short storage life, and will deteriorate rapidly after harvest (Bounous *et al.*, 1997; Jay, 1996; Delong *et al*., 2003; Magee, 1999; Beaudry *et al*., 1998; Rosenfeld *et al*., 1999; Sanford *et al*., 1991). Wills *et al.* (1998) stated that because of the soft texture of berries with high water content (83%), they are probably the most perishable of all fruits and vegetables, having limited storage life even under optimal conditions. When blueberries are removed from the plant, they should be cooled and marketed as soon as possible. Even though 'Tifblue' Rabbiteye blueberries had longer storage life (approx. 17 days) at 5 °C (Basiouny and Woods, 1992) than Highbush blueberries (14 days) at 0 ºC and 90-95% RH (Wills *et al*., 1998; Silva *et al*., 2005), both berries need to be kept under optimal storage conditions to slow down the rate of undesirable changes. Silva *et al*. (2005) explained that skin toughness of Rabbiteye blueberries could contribute to longer fresh shelf life than Highbush blueberries. Ballinger *et al*. (1978) reported that blueberries for the fresh market should not be exposed to temperatures exceeding 10 ºC and preferably should be held at or near 1ºC.

Mitra (1997) suggested that it is not possible to improve the quality of produce after harvest, but it is possible to slow the rate of undesirable changes, and maintain quality of produce for a longer time.

Growers or shippers need harvest and handling techniques that allow quality to be retained over an increasingly longer period. Extended shelf life of blueberries under optimal storage conditions would also be significant to their market storage and reaching more distant markets. Low temperature at -0.5 ~ 1.0 ºC and high humidity storage at 90 ~ 95% RH have been examined for extending shelf life of Rabbiteye blueberries during storage (Basiouny and Woods, 1992; *Venegas and* Núñez. 1998).

The use of modified atmosphere packaging (MAP) is a proven method to extend shelf life of fruits with differing results (Smittle, 1998; Song *et al*., 1992; Cameron *et al*., 1994; Blasing, 1993; Bounous *et al*., 1997). Earlier research involved using MAP gases such as CO_2 (Al-quarshi, 2002; Lee *et al*., 1995; Zagory, 1995); CO_2/SO_2 (Phillips, 1996; Amanatiduo *et al*., 1999); SO_2 (Palou *et al*., 2002; Soomro, 1998); or N_2 (Moleyar and Narasimham, 1994). However, these studies did not look at modified atmosphere storage (MAS) or other gases such as ozone. The use of Shelf Life Extender (SLX)-Modified Atmosphere Storage (MAS) containers has been limited in scope with no reports given in the literature. This study was conducted to determine the effects of ozone, SO_2 and CO_2 treated MAS on various quality parameters of fresh-packed stored 'Tifblue' rabbiteye blueberries.

MATERIALS AND METHODS

Five kilograms of rabbiteye blueberries (*Vaccinium ashei* L. cv. 'Tifblue') were hand harvested at full blue maturity from a commercial orchard (Reese Orchard, Starkville, Mississippi) on July 18, 2003. After damaged and dull fruit were removed, fruit were kept in an ice chests and transported to the Food Science and Technology Processing Laboratory at Mississippi State University for further work on the same day (<4h). Berries were not washed or treated with any fungicide prior to storage. Fruits which were harvested from different plants were randomly divided into three replications, and placed in 170g (6 oz) plastic (crystal polystyrene) perforated clamshell packages (Ivex Packaging Corp, Ohio).

Three Shelf Life Extender-Modified Atmosphere Storage containers per treatment (0.51m x 0.61m x 0.20m) (SLX® International Inc., San Luis Obispo, CA) were used as replications in a randomized complete block design on each treatment including control. Each treatment replicated three times consisted of twelve clamshells (100 fruit per clamshell). The containers were evacuated of air and flushed with the following treatments, 15.5 % O_3; 193 $mg.L^{-1}$ SO_2; 18.8 % CO_2 or normal air (control) by using a gas delivery system (SLX International Inc., CA) which automatically optimized the gas concentrations in each SLX® container. The concentrations used were based on previous work by atta() who identified such concentration as optimal. Ozone was produced using an ozone generator Model T-816 Ozonator (Welsbach Ozone System Corp., Philadelphia, PA). All containers were placed in a refrigerated walk-in room at 2 ºC and 95% relative humidity.

After 34 days, temperature fluctuation to simulate fruit handling during distribution and retail was introduced by placing MAS containers at room temperature up to 10 ºC. Temperature and relative humidity were controlled by the refrigeration system and monitored with a thermometer and a sensor (Rotronic Hygrometer, series 1H4, Rotronic Instrument Corp., Huntington, NY) during the entire storage period. Samples (three clamshells per container) were removed from each storage container every four days until the 20^{th} day, then every 7 days until the 48^{th} day. After each sampling interval the atmosphere inside the containers was re-established to its original gas concentration as previously earlier described. As specified below, data on quality parameters were recorded at each sampling interval.

Fruit fresh weight was determined by weighing one hundred fruit from three clamshells of each treatment. Weight loss was estimated by subtracting sample data weight from the initial weight and expressed as accumulated weight loss percentage per unit time (Al-qurashi, 2002).

To measure microbial berry decay, three replications of 600 fruit (6 clamshells) were placed in separate clamshell packages and percentage fruit decay was accumulated every sampling date. Berries that showed evidence of mold, spotting or leaky symptoms were considered spoiled fruit (Austin and Williamson, 1977).

Berry firmness was measured by using an

Instron Universal Testing Machine Model 1011 (Canton, Mass). Compression force (N) was determined by using a 20-mm-diameter cylindrical probe on the equatorial end of the berry at a crosshead and chart speed of 50 mm/min (Silva *et al*., 2005). A berry was placed on its side on a flat steel washer and simply centered against the load cell. The force was recorded when the berries started to release juice (Huang, 1996). Ten fruit were used for each replication.

Berry color was evaluated using a Hunter Color Difference meter (Model 6000 0/45* Spectrophometer, Fairfax, VA). Each blueberry was placed on a 10mm diameter port. Three readings were taken on the sample after rotating the berry (Silva *et al*., 2005). Thirty berries were used in each replication (10 berries x 3 replications). The reflectance values of 'L' (brightness), 'a' (redness+/ greenness-), 'b' (yellowness+/ blueness-) values, Huge angle values ($\tan^{-1}$ b/a), and chroma or saturation index ($SI=(a^2 + b^2)^{1/2}$ were calculated (Setser, 1984).

For statistical analysis, all experiments were set in a two-way (treatment x storage time) factorial arrangement. The differences between means were determined using the Student-Newman-Keuls test (SNK) at $p \leq 0.05$ (Kuehl, 2000).

RESULTS AND DISCUSSION

Weight loss increased with time regardless of treatment (Table 1). Percentage weight loss was greater in normal air (control) and CO_2 after 48 days, while SO_2 showed the least weight loss (Table 1). Weight loss could be due to an increase in respiration and transpiration, and as a result of leaky berries. Jackson *et al*. (1999), Huang (1996) and Soomro (1998) found similar results, in that blueberry weight loss increased with storage time. Soomro (1998) concluded that a different respiration rate may have caused the difference in weight loss of blueberries. Inhibition of enzymatic activity of blueberry under SO_2 MAP could result in its reduced respiratory rate. Petri et al., (2008) showed that sodium metabisulfite reduced respiratory rate by inactivating enzymatic activities in fruit and vegetables. Bounous et al. (1997) studied Modified Atmosphere Packing (MAP) of highbush blueberries and reported that weight loss was greater for berries stored in CO_2 at the end of six weeks.

Table 1. Effects of modified atmosphere storage (MAS) and storage time on weight loss, color, moisture and decay of 'Tifblue' blueberries at a 2 ºC and 95% relative humidity.

MAS	Storage time (days)			
	0	16	34	48
	Weight loss (%)			
Normal air	0 Ba[xy]	2 Ba	3 Ba	9 Aab
CO2	0 Ca	2 Ba	4 Ba	11 Aa
SO2	0 Ca	2 Ba	3 Aa	4 Ac
O3	0 Ca	2 Ba	3 Ba	6 Abc
	Decayed fruit (%)			
Normal air	0 Ba	2 Ba	6 Ba	60 Aa
CO2	0 Ca	2 Ba	4 Ba	50 Aa
SO2	0 Ca	3 Ba	5 Ba	27 Ab
O3	0 Ca	2 Ba	3 Ba	38 Ab

	Chroma			
Normal air	0.5 Ba[xy]	0.7 Ba	2.0 Aa	1.8 Aa
CO2	0.8 Aa	1.1 Aa	2.0 Aa	1.6 Abc
SO2	0.6 Ca	1.2 Ca	3.7 Ba	6.1 Aa
O3	0.6 Ba	1.4 Aa	2.8 Aa	2.0 Abc
	Hue angle (degree)			
Normal air	233 Aa	208 Ab	216 Ab	216 Aa
CO2	223 Aa	197 Ab	225 Ab	200 Ab
SO2	213 Ba	290 Aa	314 Aa	270 Aba
O3	246 Aa	201 Ab	193 Ab	203 Ab

[x] Means within rows with the same capital letter are not different at P≤0.05 using Student- Newman- Keuls test (SNK).
[y] Means within columns with the same capital letter are not different at P≤0.05 using Student- Newman- Keuls test (SNK).

Regardless of treatment, MAS reduced microbial berry decay rates up to 34 days at 2 ºC storage temperature. Other studies (Basiouny and Woods, 1992; Nunes et al., 2004) showed 17 to 21 days shelf life in cold storage (1-5 ºC and 60 – 90 % relative humidity). Ritenour *et al*. (2004) reported that temperature is the most important in storage of blueberries. SO_2 and O_3 resulted in less fruit decay compared to normal air and CO_2 at 48 days of storage (Table 1). In a previous study, ozone in combination with controlled atmosphere (CA) increased the percentage of marketable fruit, did not effect notable profiles, and did not induce anthocyamins and phenolic compounds of highbush blueberries (Song et al., 2003). Bialka and Demirci (2007) studied the efficacy of gaseous ozone on *Escherichia coli* or *salmonella* and determined that ozone is good candidate for decontamination of highbush blueberries with no effect on color or sensory analysis. Ozone as a chemical oxidant improved the microbiological quality of lowbush blueberries and could be considered as chlorine-alternatives (Crowe, et al., 2007). Results of our study confirm the anti-microbial properties of ozone. In a previous study 'Tifblue' blueberries had also lower percentage of fruit decay when fumigated with 200 mg. L^{-1} SO_2 and 29% CO_2 (Soomro, 1998). Huang (1996) found that 193 mg. L^{-1} SO_2 was effective in reducing decay of blueberry fruit. Al-qurashi (2002) found that berries exposed to 20% CO_2 had a low incidence of decay. Barth *et al,* (1995) reported that continuous ozone storage at 0.1 and 0.3 ppm significantly extended the market life of thornless blackberries with no observable injuries or decrease in quality in 12 days. In this study compression force was not affected by the gas treatments or time in storage (data not shown).

Chroma of blueberries stored in normal air and CO_2 was not affected for 34 and 48 days of storage. However, chroma of berries treated with SO_2 increased with storage time. Chroma of berries under O_3 also increased 16 days of storage and remained unchanged thereafter (Table 2). Nunes et al. (2004) previously reported that chroma of highbush blueberries decreased up to 1.6 after 14-day storage without a modified air. However, our results showed increased chroma on blueberries treated with SO_2. This could be due to oxidation of the waxy outer skin and change in anthocyanins at low pH which could produce a more vivid appearance on blueberry surfaces. Hue value was not affected by storage time, except at 16 and 34 days of storage when berries under SO_2 MAS had an increase ($p<0.05$) in Hue value (Table 2). Sanford *et al*., (1991) showed that when Hue angle value increased to 314, the blue color changed to blue red. Visual observations (data not shown) showed that color of SO_2 treated fruit stored at 2 ºC changed to red after eight days of storage. Changes in berry color by SO_2 may be due to the effect of increased surface acidity shifting the color of the anthocyamine from deep purple to red (Magee, 1999). Similar results were previously recorded by Sanford *et al*., (1991). Although SO_2 was effective in

decreasing decay and weight loss of blueberries, the change in color had a negative impact, resulting in an unacceptable fruit.

CONCLUSIONS

In this study, SO_2 and O_3 reduced weight loss and fruit decay of blueberries indicating that these gases increased quality and storage life of the berries. However, SO_2 increased chroma and Hue value indicating a change in color from blue to blue-red. This color change may render the berries less attractive to the consumer. Berry weight loss, moisture percentage and decayed fruit were not affected by CO_2. Ozone had no detrimental effect on the fruit and increased berry quality and storage life, thus, ozone is considered to be a viable gas treatment when storing blueberries.

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Cadmium Uptake by Collard and Indian Mustard Plants Grown in Cadmium Contaminated Soil

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Abstract

The growth responses of *Brassica oleracea* (Collard) and *Brassica juncia* (Indian Mustard) plants to soil cadmium (Cd) and bioaccumulation of Cd in plant tissues were investigated. Plants were grown in the laboratory under color corrected lights in soils containing 0 ppm, 250 ppm, 500 ppm, and 1000 ppm Cd. Plants were harvested on day 30 of the experiment. Dried plant samples were acid digested for tissue Cd analysis. Tissue Cd analysis was performed using an atomic absorption spectrophotometer. Data were analyzed for biomass production and tissue Cd accumulation. Results indicated that plant biomass was reduced in Cd treated plants. *B. oleracea* plants treated with 500 ppm and 1000 ppm Cd, did not survive the metal toxicity. And bioaccumulation of Cd in the plant tissue was dose related. Overall data indicated that *B. juncia* plants tolerated higher Cd levels in soil and accumulated higher levels of Cd in plant tissue as compared to *B. oleracea* and, therefore, it may have a better potential in the remediation of Cd contaminated soil.

INTRODUCTION

Heavy metal pollution of soils is a major environmental problem facing the modern world. Such metals tend to accumulate in the soil, may translocate into plant tissues, enter the food web, and then threaten the human health. Some heavy metals are linked to produce various diseases in humans (Adriano 1992, Zaman and Zereen 1998, ASTDR 2004). Cadmium is a tasteless and odorless natural element in the earth's crust. Cadmium is naturally present in low concentrations in soil (<10 ppb), however, its concentration can reach 100 ppm or above in areas adjacent to mines, smelters, battery plants, ammunition plants, etc. (ATSDR 2004). Soil Cd concentrations in industrialized areas have significantly increased, posing a serious health risk to humans. Cadmium is suspected to be an etiological factor for various human pathological conditions such as testicular and other cancers, hypertension, arteriosclerosis, growth inhibition, brain damage, kidney damage, low body iron storage and osteoporosis (Jarup 1998). According to the Mississippi Department of Environmental Quality, soils containing 100 ppm of Cd or above should be considered toxic and must be remediated before the land can be used for public use. Phytoremediation, an innovative technology, is considered to be a "green revolution" to remediate metal contaminated soils. This idea of using metal accumulating plants to remove soil pollutants have existed for over 300 years (Chaney et. al. 1997). The success of phytoremediation depends upon selecting plant species that can tolerate and accumulate high concentration of contaminants. Studies indicate that certain plant species are able to tolerate and bioaccumulate heavy metals from soil (Reeves and Brooks 1983, Vassil et al. 1998, Baker et al. 2000, Zaman 2003, Chaney et al. 2007, Mingorance et. al. 2007). In this study, we investigated the responses of *B. juncia* and *B. oleracea* plants to soil Cd and bioaccumulation of Cd in plant tissues.

MATERIAL AND METHODS

B. juncea and *B. oleracea* plants were grown in Memphis silt loam soil under laboratory conditions. Soil was collected from an undisturbed forest area of Alcorn State University campus located in southwest Mississippi. The soil contained about 70% silt, 20% clay, 9% sand, and 1% organic

matter with a pH of 6.9 (Panicker 1992). This soil covers approximately 3.2-million acres of land in Mississippi, Louisiana, Alabama, and Tennessee.

Plant seeds were grown in soils containing 0, 250, 500, and 1000 ppm Cd with 12 plants per group. Cadmium was mixed with the soil in the form of Cd $(NO_3)_2$ (Fisher Scientific, New Jersey, USA). Plant seeds were placed in 6.5-ounce porous bottom planters containing 150 grams of soil (dry weight) per planter. A one-centimeter depression was made in the center of the soil and one pre-germinated seed, with about 1 mm radicle length, was placed into each depression and then covered with soil. The planters were placed in reservoir trays. Each treatment group had its own separate reservoir tray. The plants were then placed for 16-hour light and 8-hour dark cycles under color corrected lights with a light energy of 1.4 quanta/ sec/cm^2. Watering was done every alternate day or as needed with distilled water and once a week with modified Hoagland solution (Hoagland and Arnon 1950). The plants were maintained under laboratory conditions at 21.9 ± 0.50° C and a relative humidity of 59.0 ± 4.0%. Plants were harvested on day 30 of the experiment. After harvesting, the plants were washed with distilled water and then completely dried at 75º C for 96 hours in a laboratory oven. Oven dried plant materials were weighed to determine the plant biomass.

For plant tissue Cd determination, the USEPA Method 3050A (USEPA 1986) was used. To perform this procedure, 0.25 g of oven dried plant samples were transferred to 125 ml Erlenmeyer flasks. To each flask, 15 ml of nitric acid (HNO_3) and 10 ml of deionized water were added. The samples were then heated on a hot plate for 45 minutes at medium heat. Then the samples were allowed to cool for 3 minutes and after adding 5 ml of HNO_3, the samples were then refluxed again for 30 minutes. The last step was repeated to ensure complete oxidation. The samples were then heated (without boiling) to evaporate to 5 ml. Following this, the samples were allowed to cool again, and 2 ml of deionized water was added along with 3 ml of 30% hydrogen peroxide (H_2O_2) to each sample. The samples were then heated to start the peroxide reaction. Hydrogen peroxide was continually added in 1 ml aliquots until the effervescence became minimal. The acid-peroxide digestates were heated for a final time to reduce the digestates down to 5 ml. After cooling, the samples were diluted to a total volume of 100 ml with deionized water. The digestates were then filtered using a Whatman Number 1 filter paper (Fisher Scientific, New Jersey, USA) to remove any particulates that may have been present in the samples. The filtrates were then ready for heavy metal analysis using an Atomic Absorption Spectrometer. The samples were sent to Environmental Chemistry Laboratory at Waterways Experiment Station, Vicksburg, MS, for Cd analysis. Data obtained in this study were analyzed by one-way Analysis of Variance (ANOVA) and the Tukey test.

RESULTS AND DISCUSSION

Both *B. juncea* and *B. oleracea* plants treated with Cd, showed dose related growth deficiencies as compared to control groups, indicating that Cd is phytotoxic. *B. oleracea* plants treated with 500 and 1000 ppm Cd did not survive the metal toxicity. *B. juncea* plants treated with such high levels of Cd survived the toxicity, but showed significant growth deficiencies (Fig 1 and 2).

Findings related to metal induced plant growth deficiencies also have been reported by other investigators (Athar and Ahmad 2002, Ismail, 2008). Our current findings are consistent with our previous studies where inhibition of plant biomass was observed in Cd and Pb treated radish plants (Zaman and Zereen 1998).

Tissue Cd accumulation in 250 ppm *B. oleracea* group was 165.20 mg/kg. This was the only Cd treated *B. oleracea* group that survived the metal toxicity (Fig 3). Cd accumulation in *B. juncea* was dose related and found to be 237.52, 631.67, and 3049.50 mg/kg in 500 and 1000 ppm Cd treated plants respectively (Fig 4).

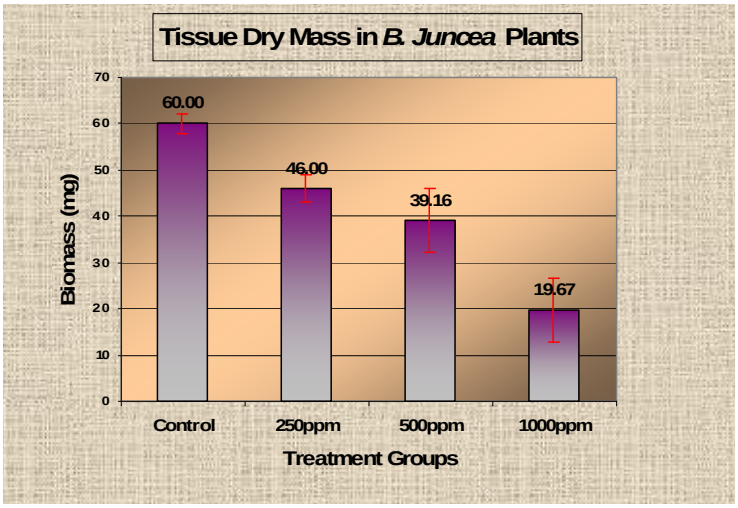

Figure 1. Tissue dry weight (mean ± SEM) of the Control and Cadmium treated *B. juncea* plants. All Cd treated groups are significantly different from the Control group at the 0.05 level: Tukey Test.

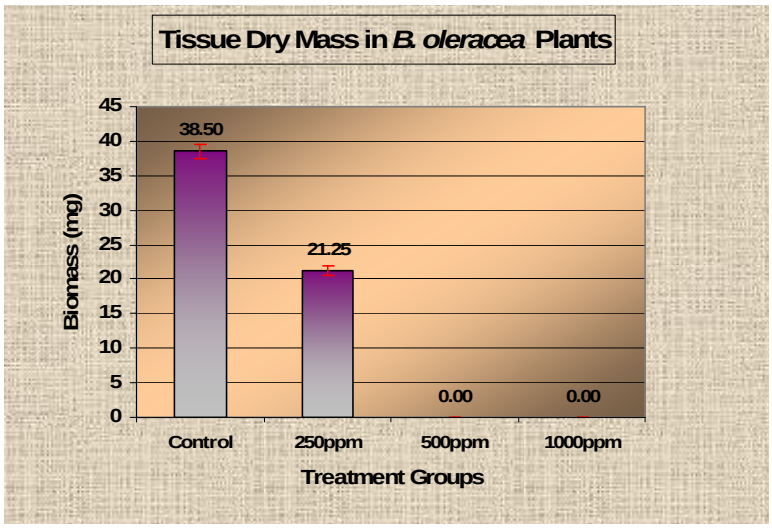

Figure 2. Tissue dry weight (mean ± SEM) of the Control and Cadmium treated *B. Oleracea* plants. 250 ppm Cd treated group is significantly different from the Control group at the 0.05 level: Tukey Test. Plants treated with 500 and 1000 ppm Cd, did not survive the toxicity.

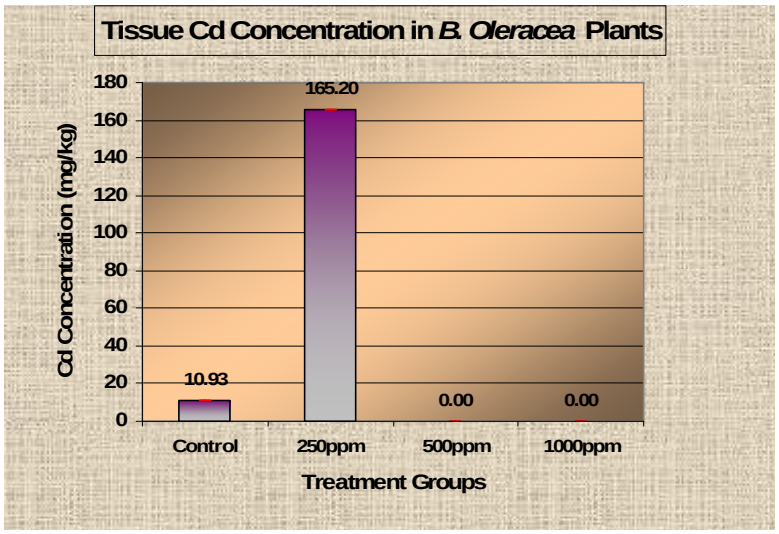

Figure 3. Tissue Cadmium concentration (mean ± SEM) of the Control and Cadmium treated collard plants. 250 ppm Cd treated group is significantly different from the Control group at the 0.05 level: Tukey Test. Plants treated with 500 and 1000 ppm Cd, did not survive the toxicity.

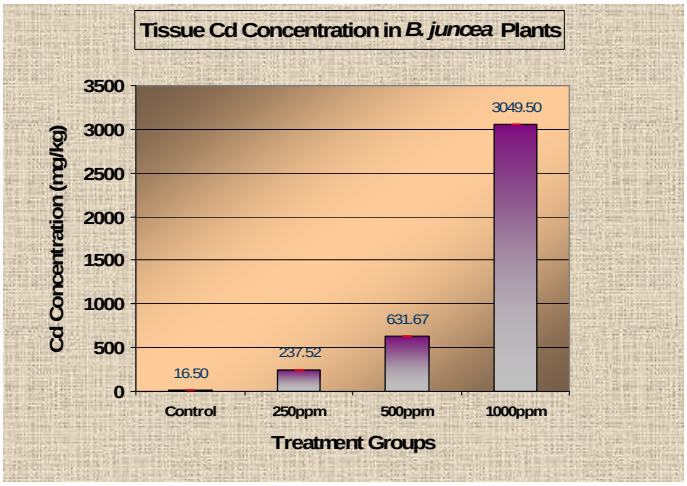

Figure 4. Tissue Cadmium concentration (mean ± SEM) of the Control and Cadmium treated Indian mustard plants. All Cd treated groups are significantly different from the Control group at the 0.05 level: Tukey Test.

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DISCUSSION

Uptake and accumulation of heavy metals by edible crops have been reported by many other investigators. However, since phytoremediation is an emerging science, research findings are not evolving in a congregated manner, and as a result, our knowledge in this field is not quite integrated yet. Smilde et. al. (1992) reported Zn (Zinc) and Cd absorption by lettuce, spinach, spring wheat, and maize grown in loam soil. Zinc and Cd were synergistic as Zn uptake by plants was enhanced by Cd in the soil. Athar and Ahmad (2002) observed the absorption of Cd, Cu (copper), Ni (nickel), Zn, Pb and Cr (chromium) uptake by wheat plants. Darmody et. al. (2004) reported elevated Cd and Cu uptake by lettuce and snap beans. Gabriella and Anton (2005) observed Cd, Cu, Pb, and Zn accumulation by maize and horseradish. Bose and Bhattacharyya (2008) studied Fe (iron), Ni, Mn (manganese), Cr, Cu, Zn, Pb, and Cd uptake by wheat plants grown in soil amended with industrial sludge.

Overall, the data indicate that *B. juncia* plants can tolerate higher concentrations of Cd as compared to the *B. oleracea* plants. Moreover, they are able to accumulate more Cd in the plant tissue, therefore, *B. juncia* has a better potential to remediate Cd contaminated soils as compared to *B. oleracea* plants. Studies will be continued in our laboratory to further investigate the phytoremediation potential of these two plant species with Cd and other heavy metals.

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Seasonal and Spatial Variations of Macrobenthic Invertebrates in Three Mississippi Gulf Coast Bayous

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ABSTRACT

Macrobenthic communities of three Mississippi Gulf Coast bayous Casotte, Cumbest and Heron, located within Grand Bay National Estuarine Research Reserve (NERR), were compared. From October 2003 to August 2004, macrobenthic invertebrate samples were collected from sampling sites in the bayous to compare densities and diversity among the bayous. Water quality parameters including water temperature, salinity, dissolved oxygen, pH, and turbidity were also monitored.

Annelids were the dominant phylum consisting of 68% of the total of 898 organisms. The highest value of total invertebrate density was found in Bayou Cumbest (168.73m^{-2}). The diversity of taxa as indicated by the Simpson Index varied between 1.00 and 2.1 bits. Bayou Cumbest had the highest invertebrate density, but contained smaller sized polychaetes, while Bayou Heron contained not only polychaetes, but larger organisms such as clams and Mollusks that have a higher biomass. Therefore, biomass can be significantly higher in Bayou Heron, and the macro benthic invertebrate abundance higher in Bayou Cumbest. The only water quality parameter that was significant in determining benthic invertebrate diversity (Simpson Index) was turbidity. Turbidity was statistically significant in determining Echinoderms and Crustaceans. Multiple regression analysis indicated that many physicochemical parameters could not be used in this study to explain the variations in the density and diversity of the macrobenthic communities. A more exhaustive approach must be taken. A sediment analysis must be performed to determine the type and abundance of heavy metals, pollutants contaminants, and perhaps other xenobiotic substances that may have entered the Grand Bay NERR ecosystem. Regarding the density and diversity of macro benthic invertebrates, relative biomass measurements will need to accompany the samples.

INTRODUCTION

Benthic invertebrates include a vast array of species consisting of crabs, worms and insects of various sizes and shapes. The word "benthic", originating from the Greek benthos, meaning bottom of the sea, and the word invertebrates that means without a backbone, describes these organisms' characteristics.

Benthic invertebrates live on or within sediments, where they influence sediment and bottom water chemistry, alter sediment organic content and structure, and serve as major prey species for fish and other benthic organisms (Cuomo and Zinn 1997; Covich et al. 1999). Benthic invertebrate species play key roles in the acceleration of detrital decomposition and other major ecological processes which include releasing nutrients into the aquatic environment as a result of feeding activities through excretion of waste (Covich et al. 1999). The bacteria and plants, in turn, quickly take up these nutrients, which results in accelerated microbial and plant growth (Ingham et al. 1985). Due to the increased biomass of microbes and plants, more food is readily available for herbivorous and omnivorous benthic invertebrates, which, in turn, serve as a food source to larger organisms that prey upon these benthic invertebrates. In most cases, the absence or abundance of these invertebrates is positively correlated with the

prevalence of larger organisms (Cuomo and Zinn 1997; Covich et al. 1999). An assessment of these benthic invertebrates can be used to estimate the abundance of larger prey as well as to predict future trends as a result of changes in benthic invertebrate population.

The taxonomic diversity of a benthic invertebrate community also reflects the water quality conditions, since generally a high diversity indicates a high water quality (Hynes 1984). For that reason, assessments of the benthic invertebrate communities are often used in biomonitoring, monitoring of the organisms that live in a particular environment in order to assess the continuing quality of the ecosystem (Phillips and Rainbow 1994; Bockelmann et al. 2004). Although plankton, fish, and invertebrates can all be used as bioindicators, benthic invertebrates are favored for this task for many reasons: they are omnipresent, relatively sedentary, and they reflect site-specific conditions because they respond to changes in water quality that occur at the time of sampling as well as changes that have occurred within a longer period before sampling (Roldán 2003). This is partially due to the fact that benthic invertebrates tend to feed on and in sediments where contaminants usually accumulate, which results in a quick response to changes in the environment caused by pollutants lodged in the sediments. Additionally, because macrobenthic invertebrates are situated between the sediment and water column, they integrate the characteristics of both sub systems (Blanchet et al. 2007). Because of these attributes, they can be used as references to prevent further contamination, which could spread to larger less sensitive organisms.

This study is designed to evaluate the benthic invertebrates of three Mississippi Gulf Coast bayous, Bayous Casotte, Cumbest, and Heron, that are under the pressures of recreational, industrial and residential activities. These bayous are located within Grand Bay National Estuarine Research Reserve (NERR). Grand Bay NERR is located in southeast Mississippi in Jackson County and contains approximately 7446.2 hectares of areas (NOAA 2006), some of which are surrounded by residential establishments. The objectives of this study were to: (1) compare densities and diversities of macrobenthic invertebrates among Bayou Casotte, Bayou Cumbest, and Bayou Heron located within Grand Bay NERR; and (2) determine the relationship between physicochemical water quality parameters and macrobenthic invertebrate abundance and density.

MATERIAL AND METHODS

Study sites

Grand Bay National Estuarine Research Reserve is located in the south-east section of Mississippi in Jackson County. The National Estuarine Research Reserve (NERR) system, established under the Coastal Zone Management Act (CZMA) of 1972, is a partnership between the National Oceanic Atmospheric Administration (NOAA) and the states that reside along coast lines (NOAA 2006). The Grand Bay reserve is the 24th NERR among the 27 reserves in the nation.

The geographic coordinates of the sampling sites are presented in Table 1. There are three distinct bayous sampled in Grand Bay NERR. Bayou Casotte is located in the vicinity of the Chevron Oil Refinery and is categorized as an industrial area. Bayou Casotte's sediments have a muddy composition and the faint but distinct smell of petroleum. Bayou Heron, a recreational area, is located in the vicinity of a major boat dock. In this bayou, it is not uncommon to witness boat fishing, jet skiing, and other recreational activities. As a result, the petroleum burning engines could add to the water pollution by deposition of hydrocarbons (Voudrias and Smith 1986) and increase turbidity (Murphy and Eaton 1983) in the area. Hydrocarbons in the aquatic environment are either absorbed in the sediments or incorporated into particulate matter (Voudrias and Smith 1986). These hydrocarbons can remain in the sediment for years and be incorporated into the benthic ecosystem (Voudrias and Smith 1986). Lastly, Bayou Cumbest flows through the center of a small residential community and is plagued by pollution in the form of raw sewage emanating from homes that occupy either side of the bayou which can lead to changes in pH, dissolved oxygen (DO), and other physicochemical parameters that influence abundance and diversity in the surrounding bayou.

Macrobenthic invertebrate sampling

Macrobenthic invertebrates are the invertebrates that can be seen with the naked eye and can be captured in mesh sizes between 200-500 microns (NCSU Water Quality Group). The Macrobenthic invertebrates in this study are categorized as follows: Annelids consist of about 15,000 species (Campbell 1996) including segmented worms, polychaetes, as well as earth worms and leeches (Hickman 2006); Echinoderms, meaning *spiny skin* in Greek, consist of about 7,000 different species (Nichols 1969); Crustaceans consist of about 52,000 species (Hickman 2006) including shrimp, lobsters, crayfish, and crabs (Day et al. 1989; Hickman 2006); Mollusks consist of about 112,000 species (Feldkamp 2002) that encompass clams and snails (Hickman 2006).

From October 2003 to August 2004, macrobenthic invertebrate samples were collected from the sampling sites in Bayous Casotte, Cumbest, and Heron to compare densities and diversity among the bayous. There were seven sampling periods (Oct 03, Dec 04, Mar 04, Apr 04, May 04, Jun 04, and Aug 04, sampling periods 1-7, respectively) in Bayou Cumbest and Bayou Heron. Due to the shallow water depth (< 0.3 m), it was not possible to sample in Bayou Casotte in periods 4, 5, and 6 (Apr – Jun 2004).

A total of two sediment grab samples were taken per sampling at each of the 11 sites. Each sediment grab was collected with a petite Ponar grab ($19.2 m^{-2}$), placed in a Ziploc™ bag (1grab sample/bag), and preserved in 10 % formalin (Puget Sound Water Quality Authority 1987). A grab was rejected if it was not completely closed due to obstructions in the jaw, partially full due to wash, or canted (Puget Sound Water Quality Authority 1987).

Samples were then taken to the Marine Biology laboratory at Jackson State University, Jackson, Mississippi. In the laboratory, the samples were washed free of fine sediments using a 500-micron (0.5 mm) sieve (Cuffney et al. 1993; EPA 2002), and then sorted to the major groups: Annelids, Echinoderms, Mollusks, Crustaceans, and others. The group "others" consisted of flat worms and other rarely encountered benthic invertebrates not fitting into the ladder groups. The sorted macrobenthic invertebrates were then preserved in 20% alcohol (Puget Sound Water Quality Authority 1987).

Monitoring of water quality parameters

Water quality parameters including water temperature, salinity, dissolved oxygen (DO), pH, and turbidity were measured using an YSI 556-02 water quality mulitparameter instrument with Conductivity, Temperature, and Dissolved Oxygen (CTD) attachment (5563-20). The instrument was calibrated at the beginning of each sampling day as manufacturer's manual instructions suggested. At each site, the probe was lowered in the water enough to be completely submersed and allowed to collect the physicochemical data at least two times to test for consistency and accuracy. The data were then transferred to Microsoft Excel by means of PC adapter (RS-232).

Data Analyses

Density (number of organisms per square meter) was calculated by multiplying the raw invertebrate counts per sample by the conversion factor specified by the Ponar sediment sampler ($19.2 m^{-2}$). The means of the two replicate sediment grabs taken from each site were used to estimate total organism density among bayous and total organism abundance per phylum among bayous. The means were then used to determine proportions (p_i) of the species *(i),* in the total sample of individuals (Ricklefs and Miller 2000). The proportions were used to calculate the Inverse Simpson's index ($D = 1/\Sigma p_i^2$) (Simpson 1949) that is often used to quantify the biodiversity of a habitat.

Data sets, including invertebrate groups and physicochemical parameters, were plotted in SPSS 16.0.1 and visually examined for normality. Throughout the analyses, a p value of equal or less than 0.05 was considered statistically significant.

Two-way analysis of variance (ANOVA) was performed to determine the effects of sites, sampling periods, and site x sampling period interactions on diversity and abundance of the macrobenthic invertebrates. The fixed variables were site and period; and the dependent variables were Simpson Index, and each of the major macrobenthic invertebrate groups (Annelids, Echinoderms, Mollusks, Crustaceans, and others).

Multiple regression analysis was performed in SPSS 16.0.1 to determine the relationship

between physicochemical water quality parameters (explanatory variable) and the abundance of the macrobenthic invertebrates could be used to explain any variations observed in the diversity and abundance of the macrobenthic invertebrates. The explanatory physicochemical variables that were used include: dissolved oxygen (DO %), pH, salinity (ppt), turbidity (NTU), and water temperature (^{o}C). The observed variables consisted of the Inverse Simpson Index $D = 1/\Sigma p_i^2$ (Simpson 1949); and each of the major macrobenthic invertebrate groups: Annelids, Echinoderms, Mollusks, Crustaceans, and others.

RESULTS

Abundance and density of macrobenthic invertebrates

The totals of 898 organisms were sampled during the study period which included the following main phyla: Annelids, Mollusks, Crustaceans, Echinoderms, and other. Annelids were the dominant phylum comprising of 68% of the total abundance (Fig. 1).

The highest values of total invertebrate density on average were found in Bayou Cumbest (168.73 m^{-2}), followed by Bayou Heron (85.78 m^{-2}), and Bayou Casotte (76.05 m^{-2}). Due to the missing samples from Bayou Casotte, it is not possible to determine a fair comparison between the three bayous, however, throughout the sampling period; Bayou Cumbest contained higher benthic invertebrate counts than Bayou Heron throughout the study period (Fig. 2)

Diversity of macrobenthic invertebrates

The mean Simpson's index values for each sampling period are presented in Fig. 3. The overall diversity of taxa estimated by the Simpson Index varied between 1.00 and 2.1 bits for the entire study area (Fig. 3). Again, it is not possible to make a fair comparison between the three bayous due to the missing samples in Bayou Casotte, however, throughout the study, the mean Simpson Index for Bayou Heron was (1.79) followed by Bayou Cumbest (1.77) and Bayou Casotte had the lowest mean Simpson index for the entire study (1.28).

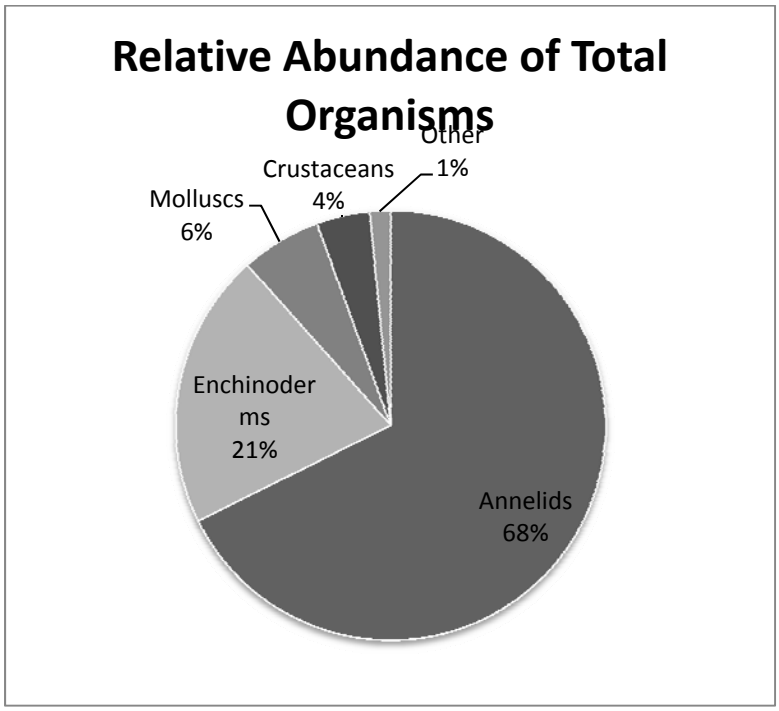

Figure 1. Relative abundance of total organisms across the three bayous during the study period

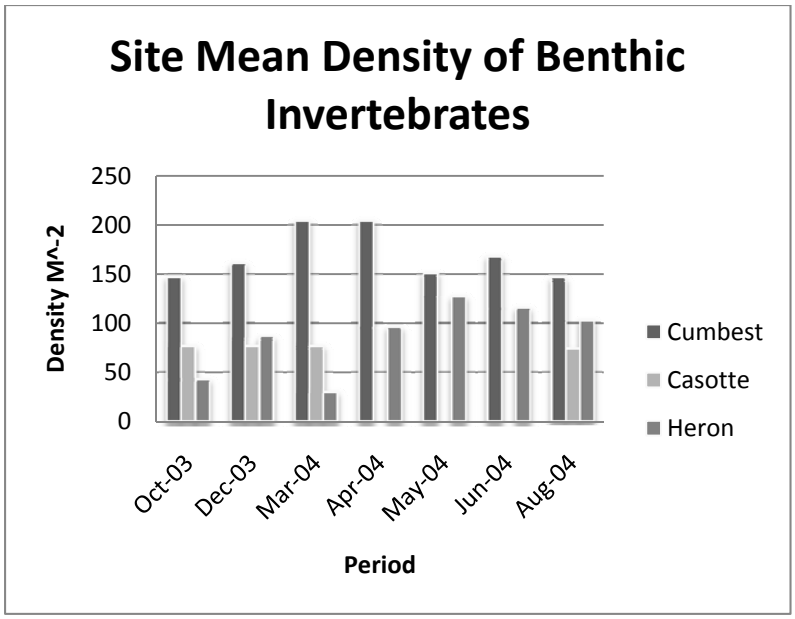

Figure 2. The site mean density of benthic invertebrate throughout study period. Period 1 is October, 2 is December, 3 is March, 4 is April, 5 is May, 6 is June, and 7 is August. In Bayou Casotte there is no data for periods 4, 5, and 6, due to water depth < 0.61 meters.

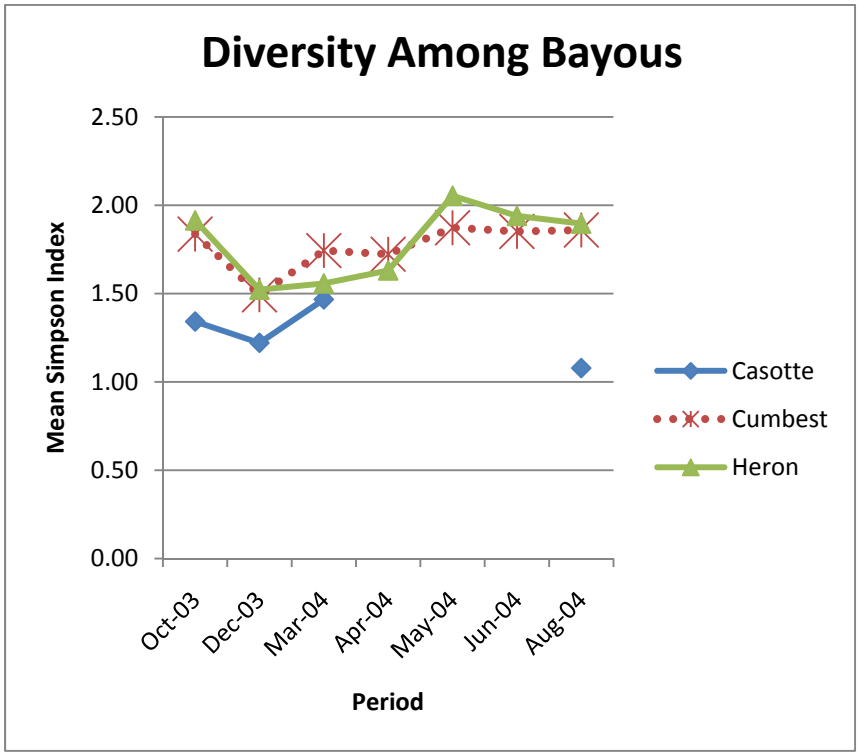

Figure 3. Mean Simpson Index among bayous by period. A higher index number represents greater diversity. Period 1 is October 03; 2 is December 03; 3 is March 04; 4 is April 04; 5 is May 04; 6 is June 04; and 7 is August 04. In Bayou Casotte there is no data for periods 4, 5, and 6, due to shallow water depths < 0.6 meters.

Macrobenthic invertebrates among sites and period

The diversity (Simpson Index values) was different significantly among the sites, but not among the study periods (Table 2). The two way effect of site and period was not the important factor to describe the Simpson Index variation. Annelids contained the highest number of organisms per period for all sites (Figs. 4a through 4e). The abundance of Annelids differs significantly among the sites (Table 2). The effects of site and site x period were significant on the abundance of Echinoderms (Table 2; Fig. 4b), and they displayed higher abundance in periods 3 ($60m^{-2}$) and 4 ($50m^{-2}$) for Bayou Cumbest while Bayou Heron displayed a higher abundance in periods 5 ($60m^{-2}$) and 6 ($50m^{-2}$). In Bayou Casotte, Echinoderm abundance remained low (< $10m^{-2}$). Mollusks abundance significantly differed among sites, but not among periods (Table 2). The site x period interaction was not significantly influencing Mollusk abundance. Abundance of Mollusks remained consistent throughout the periods and relatively low for all sites (< $15m^{-2}$) (Fig. 4c). Crustacean abundance was also site dependent ($p \leq .05$) and remained consistently low (< $10\ m^{-2}$) for all sites and for all study periods (Figs. 4d). There was no site, period, or site x period interactions observed for "Others" which also had relatively low abundance for the entire study for all sites and periods (< $5\ m^{-2}$) (Fig. 4e).

Table 2. Two-way ANOVA results for the Simpson Index, Annelids, Echinoderms, Mollusks, Crustaceans and Other. The diversity and abundance were compared for interactions among sampling sites, sampling periods, and the two way interaction of Sites and Periods. P values less than 0.05 are considered statistically significant.

Source	SS	df	Mean Square	F	P
Dependent Variable: Simpson					
Site	3.564	2	1.782	5.079	.008
Period	2.026	6	.338	.962	.454
Site * Period	1.345	9	.149	.426	.919
Error	41.401	118	.351		
Total	438.338	136			
Dependent Variable: Annelids					
Site	89870.219	2	44935.109	44.511	.000
Period	4181.915	6	696.986	.690	.658
Site * Period	4818.899	9	535.433	.530	.850
Error	119125.020	118	1009.534		
Total	1218780.880	136			
Dependent Variable: Echinoderms					
Site	13092.255	2	6546.128	5.684	.004
Period	4258.411	6	709.735	.616	.717
Site * Period	23308.470	9	2589.830	2.249	.023
Error	135908.780	118	1151.769		
Total	275531.680	136			

Dependent Variable: Mollusks					
Site	5111.047	2	2555.524	17.258	.000
Period	453.140	6	75.523	.510	.800
Site * Period	572.170	9	63.574	.429	.917
Error	17473.310	118	148.079		
Total	31795.440	136			
Dependent Variable: Crustaceans					
Site	1318.466	2	659.233	6.881	.001
Period	302.373	6	50.395	.526	.788
Site * Period	413.024	9	45.892	.479	.886
Error	11304.960	118	95.805		
Total	16957.440	136			
Dependent Variable :other					
Site	11.844	2	5.922	.165	.848
Period	114.213	6	19.035	.532	.783
Site * Period	250.672	9	27.852	.778	.637
Error	4224.000	118	35.797		
Total	5160.960	136			

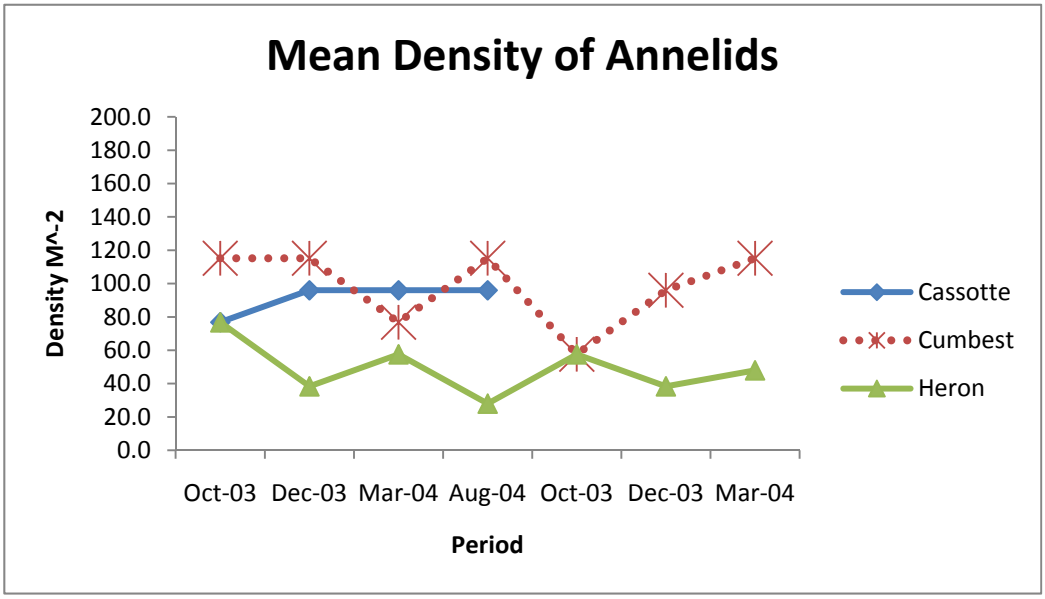

Figure 4a.

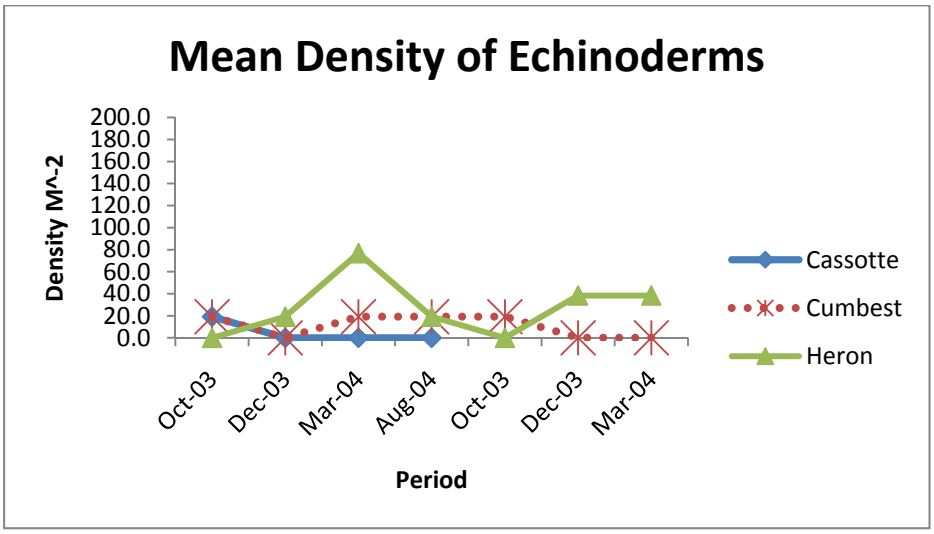

Figure 4b.

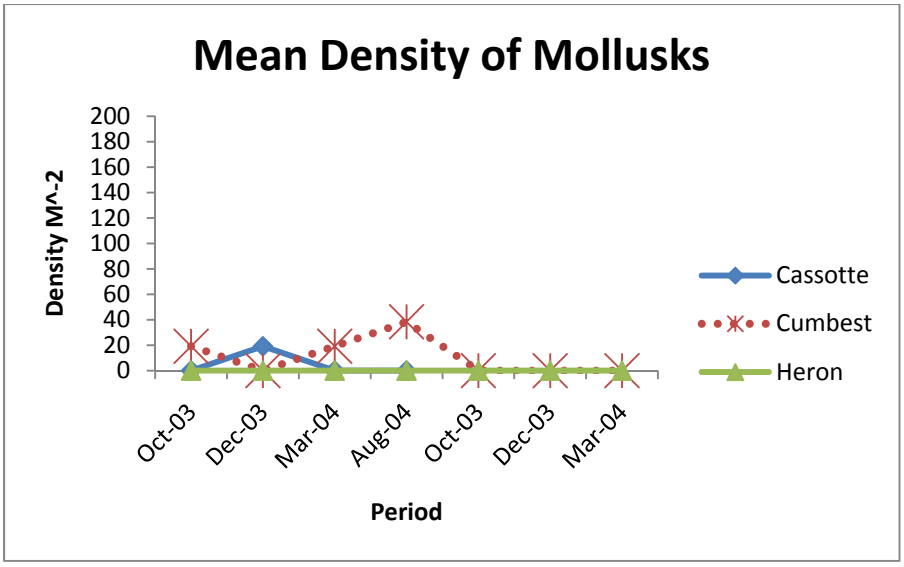

Figure 4c.

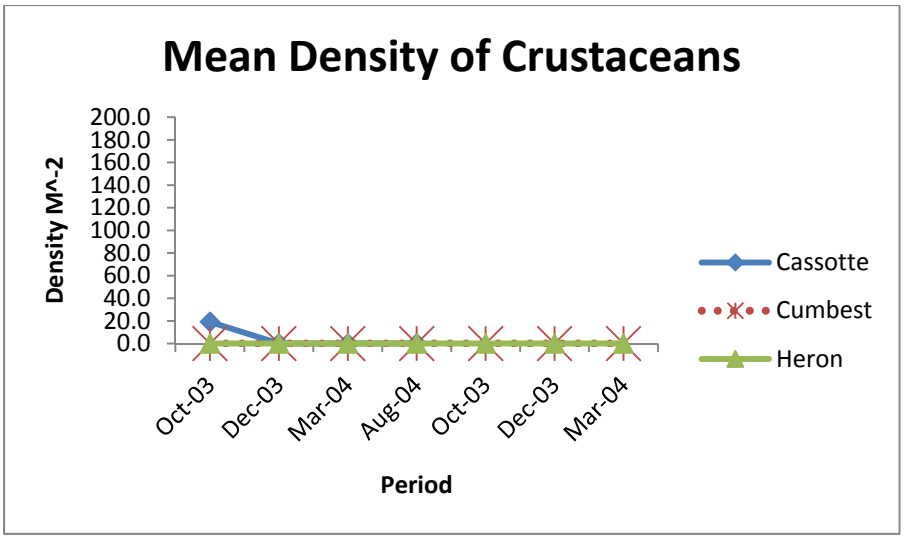

Figure 4d.

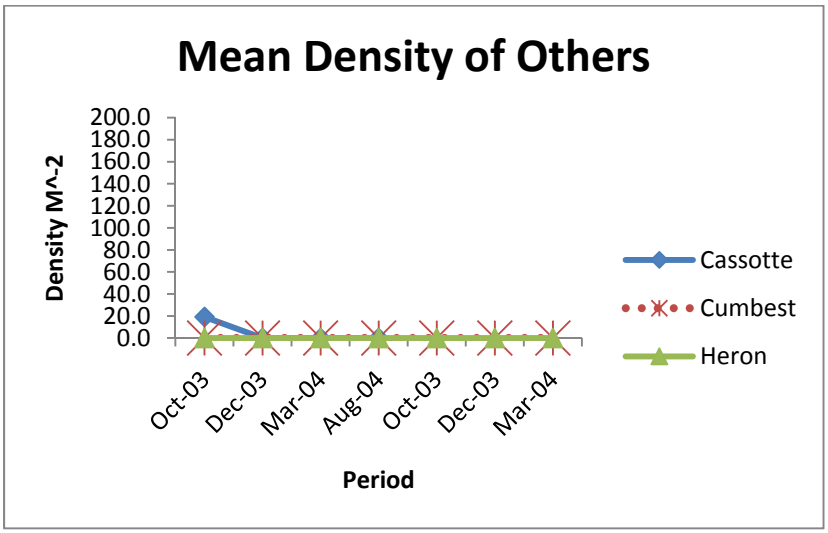

Figure 4e.

Figures 4a-e: Plots of Benthic macro invertebrate groups Annelids (a), Echinoderms (b), Mollusks (c), Crustaceans (d), and others (e), per sampling period in m^2. Period 1 is October 03; 2 is December 03; 3 is March 04; 4 is April 04; 5 is May 04; 6 is June 04; and 7 is August 04. In Bayou Casotte there is no data for periods 4, 5, and 6, due to water depth < 0.61 meters.

Water quality

Temperature, salinity, DO%, pH, and turbidity varied throughout the study periods, sites, and the effects of site x period were all statistically significant when compared using two-way ANOVA ($p \leq .05$) (Figs. 5a through 5e). Salinity was higher in

Bayou Casotte for the periods sampled ($\bar{X}$ = 24.6 ppt), or compared to Bayou Cumbest ($\bar{X}$ = 17.84) and Bayou Heron ($\bar{X}$ = 16.23). The pH was more alkaline in Bayou Casotte ($\bar{X}$ = 8.2) for periods sampled than that of Bayous Heron ($\bar{X}$ = 7.27) and Cumbest ($\bar{X}$ = 7.49). Turbidity was lowest in bayou Casotte ($\bar{X}$ = 4.2 NTU) probably due to its inaccessibility by boat. The water depth at Bayou Casotte was often lower than .6 meters. This resulted in less interference from constant human activities as well as artificial wakes created by speed boats and other recreational water crafts

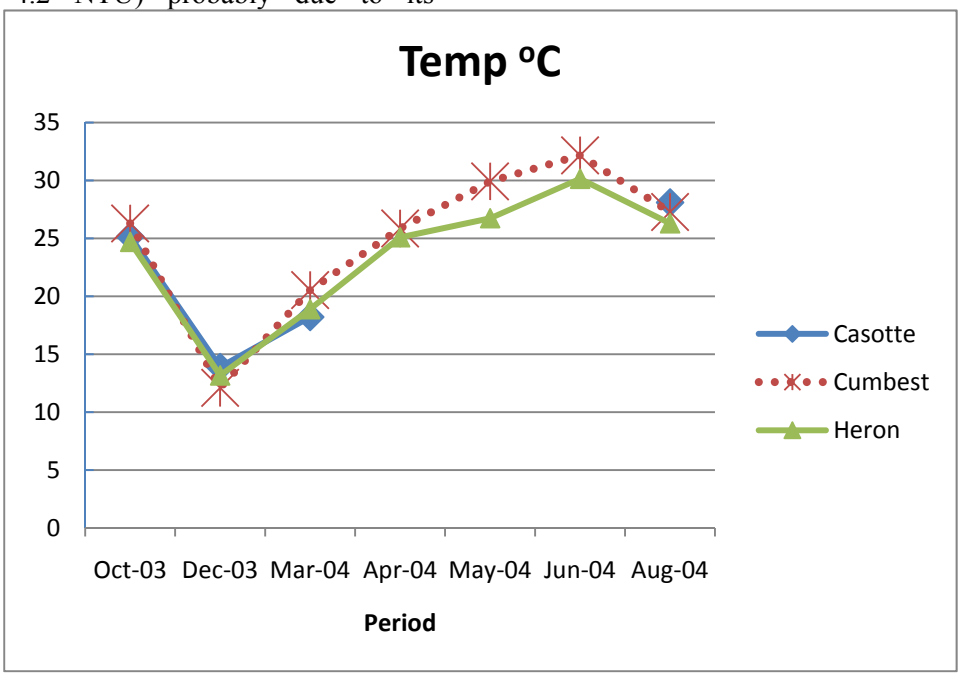

Figure 5a.

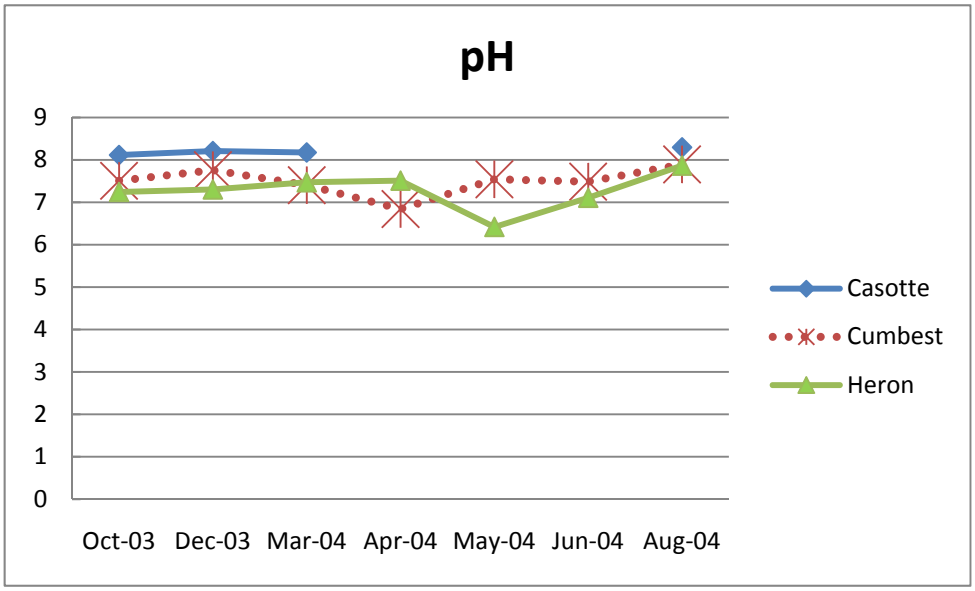

Figure 5b.

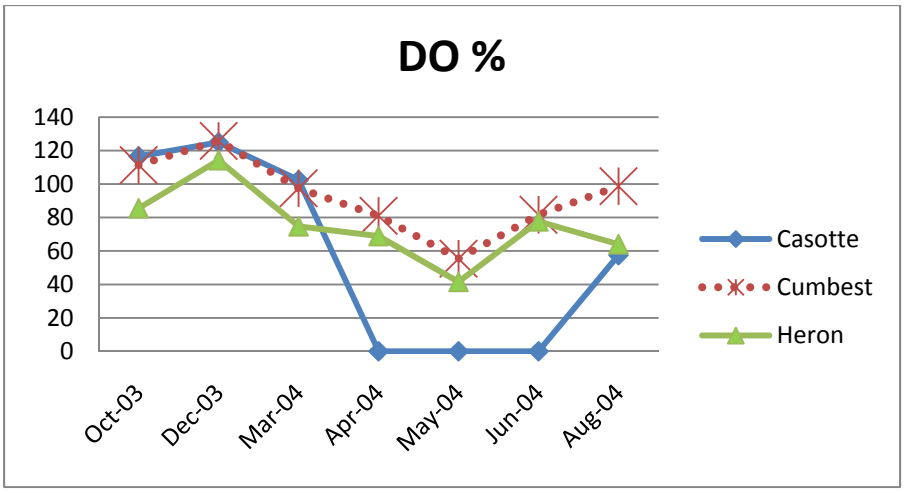

Figure 5c.

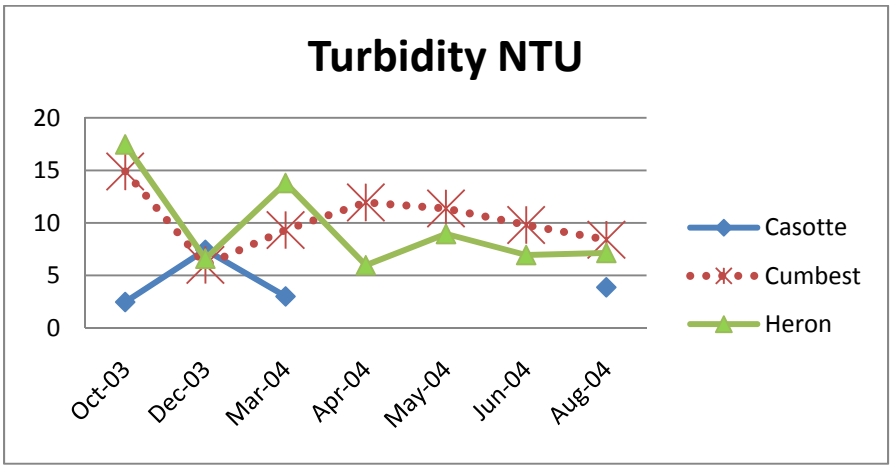

Figure 5d.

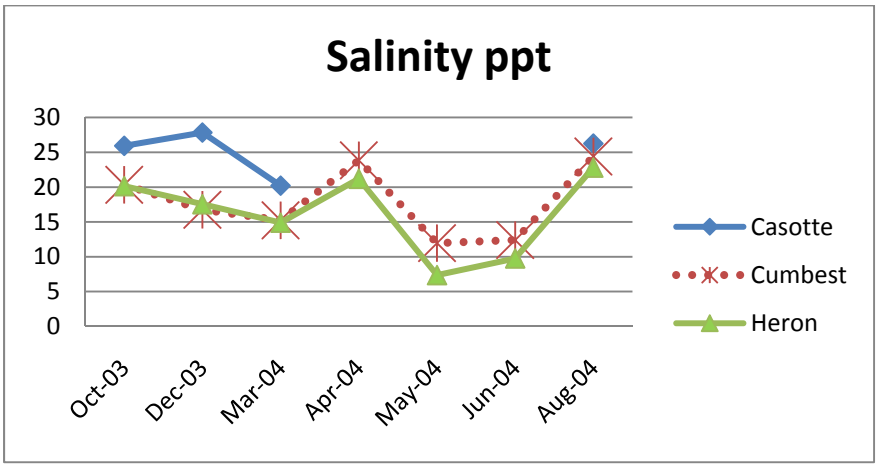

Figure 5e.

Figures 5a-e: Graphs of physiochemical water quality parameters including temperature (a), pH (b), DO% (c), turbidity (d), and salinity (e), throughout study. Period 1 is October, 2 is December, 3 is March, 4 is April, 5 is May, 6 is June, and 7 is August. In Bayou Casotte there is no data for periods 4, 5, and 6, due to water depth < 0.61 meters.

Effects of physicochemical parameters on macrobenthic invertebrates

Multiple regression analysis revealed physicochemical parameters that could be used to explain the variations in the density and diversity of the macrobenthic communities. Temperature was statistically significant in predicting Mollusks ($p = 0.000$) (Table 3). DO% was statistically significant in predicting Annelids ($p = 0.022$), and Mollusks ($p = 0.001$) (Table 3). Turbidity was statistically significant in determining Simpson Index (overall diversity) and abundances of Echinoderms and Crustaceans (Table 3). There was no statistically relevant physicochemical parameter that could explain variations observed in "Others". However, the R^2 values in multiple regression analysis indicated a relatively weak correlation with the highest observed value being less than 0.147.

Table 3. Results of multiple regression analysis. Physicochemical parameters including dissolved oxygen (DO%), pH, salinity (ppt), turbidity (NTU), and water temperature (^oC) are the independent variables and the Simpson index, Annelids, Echinoderms, Mollusks, Crustaceans, and Other are the dependent variables. P values less than 0.05 are considered statistically significant.

Model	Unstandardized Coefficients		Standardized Coefficients	t	P
	B	Std. Error	Beta		
Dependent Variable: Simpson R Squared = .099					
(Constant)	.899	.673		1.336	.184
Temp	.022	.014	.215	1.586	.115
Salinity	-.003	.009	-.034	-.327	.744
DO Conc.	.011	.030	.052	.373	.710
pH	-.001	.093	-.002	-.016	.988
Turbidity	.030	.010	.246	2.898	.004
Dependent Variable: Annelids R Squared = .106					
(Constant)	-26.159	45.035		-.581	.562
Temp	1.630	.928	.240	1.757	.081
Salinity	.388	.634	.064	.612	.542
DO Conc.	4.666	2.019	.325	2.310	.022
pH	3.069	6.191	.054	.496	.621
Turbidity	1.293	.682	.162	1.894	.060

Dependent Variable: Echinoderms R Squared = .114					
(Constant)	16.313	40.787		.400	.690
Temp	-.413	.840	-.067	-.492	.624
Salinity	-.141	.574	-.025	-.246	.806
DO Conc.	-2.922	1.829	-.224	-1.598	.113
pH	3.254	5.607	.063	.580	.563
Turbidity	1.999	.618	.276	3.235	.002
Dependent Variable: Mollusks R Squared =.147					
(Constant)	-28.526	14.446		-1.975	.050
Temp	1.133	.298	.506	3.808	.000
Salinity	.100	.203	.050	.491	.624
DO Conc.	2.239	.648	.473	3.457	.001
pH	-1.479	1.986	-.079	-.745	.458
Turbidity	.390	.219	.149	1.781	.077
Dependent Variable: Crustaceans R Squared =.095					
(Constant)	-18.570	11.168		-1.663	.099
Temp	.451	.230	.269	1.963	.052
Salinity	.170	.157	.113	1.083	.281
DO Conc.	.703	.501	.198	1.403	.163
pH	.228	1.535	.016	.148	.882
Turbidity	.378	.169	.192	2.235	.027
Dependent Variable: other R Squared =.019					
(Constant)	-6.391	6.823		-.937	.351
Temp	.105	.141	.107	.749	.455
Salinity	-.026	.096	-.029	-.271	.787
DO Conc.	.202	.306	.097	.660	.511
pH	.547	.938	.066	.583	.561
Turbidity	.099	.103	.085	.953	.342

DISCUSSION

This study was designed to compare macrobenthic communities of three Mississippi Gulf Coast bayous Casotte, Cumbest, and Heron, located within Grand Bay NERR where residential, industrial, and recreational activities occur. Bayou Casotte, located in the vicinity of the Chevron Oil Refinery, is influenced by industrial effluents. Bayou Cumbest flows through the center of a small residential community and is plagued by pollution in the form of raw sewage emanating from homes that occupy either side of the bayou which can lead to changes in pH, dissolved oxygen (DO), and other physicochemical parameters that influence abundance and diversity of aquatic communities in and surrounding the bayou. Bayou Heron is located in the area of a major boat ramp, and suffers from recreational activities such as sports fishing, speed boating and water skiing.

The seasonal and spatial variations of macrobenthic invertebrates in Bayou Casotte, Bayou Heron, and Bayou Cumbest were examined to determine the relationship between physicochemical parameters and macrobenthic invertebrate abundance and density and to compare densities and diversities of macrobenthic invertebrates. Elliot (2002) stated that several environmental factors, such as salinity, dissolved oxygen, turbidity, and temperature must be considered when assessing the

current conditions of the environment. Anthropogenic activities, such as bottom trawling, water sports and other recreational activities should also be taken into consideration. Elliot (2002) also suggested point source pollution from the discharge of cooling water from coastal factories, and sewage effluents from coastal residential homes can have negative impacts on the overall health of an ecosystem. Ismail and Awad (1986) found that benthic invertebrates found in areas near a sewage outfall station contained lower total number of individuals, number of species and species richness than that of the control site. In a study conducted by Burt et al. (1991), it was found that most impacted study sites occurred downstream of industrial and municipal sources and in depositional areas.

Anthropogenic activities and pollutions such as these listed above are evidenced in the three bayous of Grand Bay National Estuarine Research Reserve (GBNERR). Bayou Casotte, located in the vicinity of a major oil refinery, contained the lowest diversity and abundance. In a similar study, Muniz and Pires (2000) found that oily effluents deposited into water bodies have a significant impact on bottom fauna. In Bayou Heron, there are occurrences of recreational watercraft use, fishing, crabbing, and other activities that require the use of a boat. Bayou Cumbest had a foul odor, indicative of hydrogen sulfide (H_2S), which indicates anoxic/reduced conditions (McLusky 1989; Kennish 1992; Carpenter et al. 1998) that may be stressful to invertebrates such as Mollusks (Strum et al. 2006). This is further confirmed in Table 3 in this study, where Mollusks demonstrated a significant response to DO%. Surprisingly, Bayou Cumbest still contained the highest total invertebrate counts (168.7 m^{-2}) of the three bayous. However, invertebrate's community diversity was not statistically different between Bayou Heron (Simpson Index of 1.79 bits) and Bayou Cumbest (Simpson Index of 1.77 bits) (Fig. 3).

Biomass measurements were not taken in this study, however by visual observation, Bayou Cumbest contained smaller sized, more numerous polychaetes, while Bayou Heron contained not only polychaetes, but larger organisms such as clams and Mollusks that have a higher biomass. Therefore, biomass can be significantly higher in Bayou Heron, and the macro benthic invertebrate abundance higher in Bayou Cumbest. This indicates that biomass data must be collected in conjunction with macrobenthic invertebrate counts in order to more accurately assess and compare macro benthic invertebrates among sites.

The only physicochemical parameter that was significant in determining benthic invertebrate diversity was turbidity (Table 3). Turbidity is believed to be effective in controlling the benthic invertebrate community in two ways. Inorganic turbidity suppresses benthic secondary productivity by reducing the supply of food from the water column through a reduction of photosynthesis, and by smothering the fauna. This would lead to a reduction in diversity (Van de Meutter et. al. 2005). As diversity declines, the opportunistic Annelids (Diaz 1984; Levington and Kelaher 2004) thrive, while less tolerable Crustaceans and Mollusks either suffer from mortality or slowly migrate to more favorable habitats (Strum et al. 2006). Inversely, high diversity can prevent opportunistic species invasion and indicate better water quality (Davis 1980). This study shows some evidence of this phenomenon. Out of the 898 organisms which included Annelids, Crustaceans, Echinoderms, Mollusks, and "others", Annelids comprised of more than 68% of the total abundance (Fig. 1) in this study. Annelids are opportunistic organisms (Diaz 1984; Levington and Kelaher 2004), existing in extreme conditions such as frequently fluctuating physicochemical parameters and/or pollutant contaminated areas, and thriving when other organisms suffer from unfavorable physicochemical conditions (Dauer and Ranasinghe, 1992). Opportunistic organisms such as Annelids, more specifically polychaetes (Dauvin and Ruellet 2007), exhibit rapid growth, and thrive in dense assemblages (Grassle and Grassle 1974). In all three bayous studied, Annelids were the most represented phylum (Fig. 1).

Bayou Heron, a high traffic area consisting of number of recreational activities greatly influence and explain the high turbidity measured at the sites. Turbidity, in this study, was a statistically significant ($P<0.05$) negative impact in determining the abundance of Echinoderms and Crustaceans (Table 3). Even though there was usually very small occurrence of recreational activities and great effort was made on our part to minimize wake during the

time of sampling, one must take into consideration the fact that most boats that enter the bayou are loaded and unloaded in this area. Since the degree of turbidity caused by the boat propeller is inversely proportional to water depth, the area surrounding the shallow loading and unloading areas are more turbid. The engine propellers and boat wakes stir up bottom sediments which can remain suspended in the water column for a time proportional to the sediments size (Bilotta and Braizer 2008). Larger sediments tend to settle to the bottom of the bayou faster than the finer sediments. The settling time of these particles can range from several minutes to several weeks (Winterwerp and Van Kesteren 2004).

In this study, Dissolved Oxygen (DO) was a statistically significant parameter ($P<0.05$) in influencing Annelids and Mollusks. With a lower DO (range < 41% concentration), observation revealed a decrease in the abundance of Mollusks with an increase in annelid density. This suggests that the Annelids opportunistic nature (Grassle and Grassle 1974) played a key role in their ability to thrive when conditions were less favorable for the Mollusks. Temperature also had a statistically significant ($P<0.05$) impact on Mollusks. Many species of Mollusks have low tolerance of even slight physicochemical variations (Seto and Sato 2003).

CONCLUSIONS

This study compared benthic communities of three Mississippi Gulf Coast bayous Casotte, Cumbest and Heron, located within Grand Bay NERR where residential, industrial, and recreational activities occur. Annelids were the dominant phylum consisting of 68% of the total abundance. On Average, the highest value of total invertebrate density was found in Bayou Cumbest ($168.73 m^{-2}$). For the entire study, the diversity of taxa as indicated by the Simpson Index varied between 1.00 and 2.1 bits. There was no significant difference in diversity between Bayou Heron (1.79 bits) and Bayou Cumbest (1.77 bits). Multiple regression analysis indicated that many water quality parameters could not be used in this study to explain the variations in the density and diversity of the macrobenthic communities. In order to better understand why macro benthic invertebrate counts differ among Bayous, Casotte, Heron, and Cumbest, physicochemical parameters alone cannot be used as they did little to distinguish one site from the other. A more exhaustive approach must be taken. A sediment analysis must be performed to determine the type and abundance of heavy metals, pollutants contaminants, and perhaps other xenobiotic substances that may have entered the Grand Bay NERR ecosystem. Regarding the density and diversity of macro benthic invertebrates, relative biomass measurements will need to accompany the samples.

ACKNOWLEDGMENTS

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Mesoscale Modeling Investigation using PENN STATE/NCAR MM5 Model for Weather Simulation and Prediction

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ABSTRACT

The objective of the present study is to establish a mesoscale modeling investigation of severe weather events and to adopt the numerical weather prediction model for possible use in regions where solar equipment will be used. Accurate and reliable forecasting is crucial in regions that have limited resources; such as third world countries, where renewable solar energy can be utilized. Cloud cover, temperature, radiation, and precipitation are major factors that help the operation of such devices; therefore weather conditions must be predicted fairly well in advance so that appropriate measures can be taken to protect assets. The PSU/NCAR Mesoscale Model (MM5) is used to simulate and predict weather circulations and patterns. The Washington D.C. region was chosen as a case study where solar cookers were used for experimental studies by Howard University for last 5 to 7 years. The model was run for a 24-hour time period on September 16, 1999, when Hurricane Floyd made its way through the east coast. NCEP/NCAR global analysis data was used to construct the initial and boundary conditions. The model predicted parameters including sea level pressure, rainfall, temperature, radiation tendency, wind magnitude and direction. The results were then compared with station observations taken by the National Data Buoy Center (NDBC) and aircraft observations taken by the National Hurricane Center (NHC) and noted reasonably good agreement. The study aided in determining weather conditions well in advance to assess risks, so that appropriate measures can be taken in to account to forecast severe weather conditions.

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INTRODUCTION

Third-world countries depend heavily on natural resources for their ultimate survival; hence, gaining these forms of resources by all means would prove a great deal to their subsistence. Technology in this present atomic age has touched every nook and corner around the world - bringing enhancement in people's day-to-day lives. One such advancement is the use of solar energy in applications wherever possible; availability of adequate sunlight. Jackson State University, Howard University, and North Carolina State University have teamed up to make this potential effort achievable in two African countries namely Madagascar and Senegal.

The numerical model such as the PENN STATE/NCAR MM5 MODEL can be used to simulate and predict the weather over a region fairly well in advance. This could vastly impact regions' social and economic activities by substituting energy resources efficiently.

Previous studies by Loren and Reddy 2001; Reddy et.al., 2002 and Lu et.al., 2006, have shown that by analyzing storm's structure and dynamics its track and change in intensity of the storm can be predicted. A mesoscale modeling investigation of tropical cyclone/hurricane forecast over the Gulf of Mexico was established under the NASA/HBCU Renewable Energy and Technology Project to adopt the numerical weather prediction model for possible use in regions where solar equipment can be used.

Sea level pressure, rainfall, radiation precipitation and temperature are the major parameters simulated. The results of the model output were then compared with the National Data Buoy Center (NDBC) and aircraft observations taken by the National Hurricane Center (NHC), and noted reasonably good agreement.

MATERIALS AND METHODS

1. Model Overview

MM5 model has been developed for almost 30 years and the latest version released is version 3.7.2. This is a fairly sophisticated modeling system with full and explicit microphysics, a non-hydrostatic formulation, soil and vegetation parameterization and multiple nesting capabilities. The model consists of five modules: TERRAIN, REGRID, RAWINS/LITTLE_R, INTERPF and MM5. Of the entire set of programs, the MM5 module is the actual numerical weather prediction part of the modeling system. The output of the model was viewed using a graphical package GrADS (Gridded Analysis and Display System). Actual information on the MM5 model can found at www.mmm.ucar.edu/mm5/mm5-home.html (Wang et.al, 2001). Brief summaries of all the modules are presented in the following sections.

The most essential element needed to run any model is the data used as input into a system for setting up the initial, lateral and boundary conditions. Consistency, accuracy, and timeliness are what makes the data complete. Data sets that are required for running the MM5 modeling system include: (i) Land use and vegetation, (ii) Gridded atmospheric data and (iii) Observation data. At this time it is worth to mention that the use of synoptic data has produced better results than not performing objective analysis (Grell et al., 1994).

A. TERRAIN

The TERRAIN module creates input terrain elevation and vegetation (land use) data for each grid directly from source input datasets and horizontally interpolates (or analyzes) from a latitude/longitude mesh onto a chosen mesoscale gird. The main tasks for TERRAIN are:

- Select subsets of terrain and land use/cover data that covers a given model domain
- Create fields of topography and land use/cover either by interpolation or by objective analysis at domain grid points
- Reset topography and land use/cover data on nest grid boundaries and adjust them for overlapping nests
- Computes constants fields, such as latitude and longitude for each grid point for a given grid, map scale factor and Coriolis parameter

The input TERRAIN requires information about model domain dimensions (number of grid points and grid spacing) for each required grid. In addition, terrain and land use/cover data are required. There are several types of input datasets available in the MM5 model package for TERRAIN that includes: terrain elevation, land-use/vegetation, land-water mask, soil types, vegetation fraction and deep soil temperature. They differ in coverage and horizontal resolution. All data are available at six resolutions at 1 degree, 30, 10, 5, and 2 minutes, and 30 seconds. Each dataset recognizes 13 types of land use/cover ranging from urban to tropical forest.

B. REGRID

REGRID is the second module and it consists of two parts: pregrid and regridder. REGIRD handles pressure levels and surface analyses, and two-dimensional interpolation. The main task of pregrid is to extract data from appropriate NCAR archived gridded meteorological analyses (NCEP or ECMWF) that correspond to user determined time intervals that the user will be running. Resulting extracted files are used as inputs in regridder, the second part of the REGRID module. Regridder recombines data created by TERRAIN and REGRID/pregrid modules resulting in one data file for each nested grid domain. TERRAIN data includes information about domain size and topography; REGRID/pregrid provides atmospheric data that have at least these variables: sea-level pressure, horizontal wind components, temperature, relative humidity, height of pressure levels, sea-

surface temperature and snow-cover data and times covering MM5 model simulation. Other data levels maybe used as well, interpolated and passed on to the modeling system. REGRID/regridder will merge both data adjusting for topography and sea surface temperature (if applicable). Output from REGRID/regridder will be used as the first guess to an objective analysis (RAWINS/LITTLE_R), or as analyses, which are to be directly interpolated to the MM5 model levels for initial and boundary conditions for MM5 (INTERPF). In short, pregrid handles data input task and regridder handles horizontal interpolation to the MM5 grid.

C. RAWINS/LITTLE_R

Either RAWINS or LITTE_R module is run depending on user preference. LITTLE_R is alternative to RAWINS that was used in this case. This module provides input of upper atmosphere and surface observations. It reads rawinsonde and surface observation data from NCAR archive and extracts stations within modeling domains and simulation time. Its main function is to enhance the first-guess meteorological data or the low-resolution analyses output with observations. The first-guess could be either from REGRID or MM5 module. The goal is to improve coarse resolution of gridded data, which is typically 2.5 x 2.5 degree by including either rawinsonde or surface data. This may be important for model run over limited areas where gridded data cannot resolve horizontal and vertical variability at model domain's resolution. Neither vertical interpolation nor temporal interpolation is done by LITTLE_R and strictly gridded data alone is used for observational analysis. This module is optional and the data is not required for MM5 model run. The output data produced is on pressure-level.

D. INTERPF

INTERPF is the last module that processes input data before running MM5. Typically the modeling system gets and analyzes data on pressure surfaces, but these have to be interpolated to the model's vertical coordinate before being input into MM5. The output data generated by the preprocessors: REGRID/regridder or RAWINS/LITTLE_R for input is pressure-level meteorological fields, which are vertically interpolated into sigma levels. The sigma levels are user defined model sigma levels. INTERPF provides initial, lateral, and lower boundary conditions for each grid (main and nested).

E. MM5

MM5 calculates all required variables at each grid point during the time of simulation. The adopted grid is Arakawa B-grid staggered mesh that consists of dot (corner) and cross (center) grid points where vector and scalar data are defined respectively. In this module a user can specify various physical options and parameterizations. Different schemes could be used for different nesting, but it is better to keep the same for all. The major physics options are as follows:

- Microphysics – Explicit moisture schemes
- Cumulus parameterization – Represents sub grid scale vertical fluxes and rainfall due to convective clouds
- PBL schemes (Planetary Boundary Level)
- Radiation Schemes
- Surface Schemes – provides sensible and latent heat flux effects of land and water surfaces. Ground temperature based on heat budget using Radiative fluxes and atmospheric layer properties

Besides the selection of physical parameterization options a user may also select the duration of model runs for different nested grids. However, the main domain running time must be within the time period specified in the previous modules. Options in boundary conditions, nesting, and Four-Dimensional Data Assimilation (FDDA) can be selected.

HURRICANE FLOYD - History

This is a brief summary of Hurricane Floyd 1999 taken from the NHC/TPC archive of past hurricanes seasons:
Floyd was initially a disorganized storm that emerged as a tropical wave from western Africa on 2 September. It was a large and intense Cape Verde hurricane that pounded the central and northern Bahaman islands, seriously threatened Florida, struck the coast of North Carolina and moved up the

United States east coast into New England. It neared the threshold of category-five intensity on the Saffir/Simpson Hurricane Scale as it approached the Bahamas, and produced a flood disaster of immense proportions in the eastern United States, particularly in North Carolina. Floyd is estimated to have been a 90-knot hurricane at landfall in North Carolina. Totals of 12 to 14 inches of rain were observed in Maryland, Delaware, and New Jersey. Maximum sustained winds reached 135 knots.

As Floyd neared the North Carolina coast late on the 15th, its maximum winds decreased below category three-status. Figure 3 shows the NOAA-14 AVHRR HRPT multispectral false color image 2018Z of the storm structure [Pasch et.al, 1999].

At landfall near Cape Fear, as shown in Figure 4 (GOES-8 Colorized IR image), North Carolina around 0630 UTC 16 September estimated maximum winds reached near 90 knots. Floyd was losing its eyewall structure as it made landfall. Floyd then weakened to a tropical storm and moved swiftly up along the east coast. The storm's best track is shown in Figure 2.

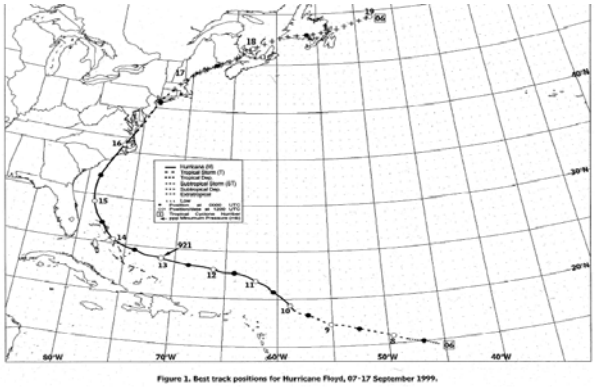

Figure 1. Best track positions for Hurricane Floyd, 07-17 September 1999.

Figure 1: Hurricane Floyd 1999 – Best Track (NHC)

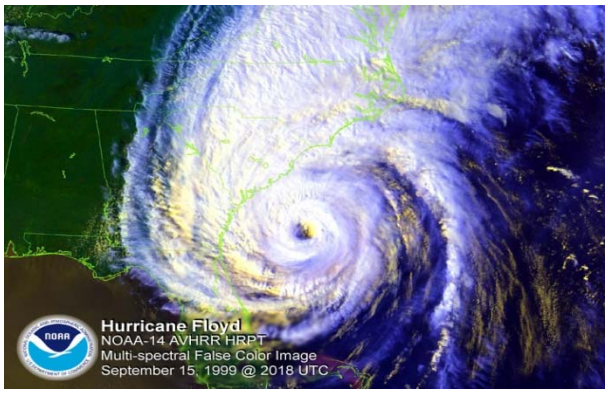

*Figure 2: NOAA-14 AVHRR HRPT **multispectral false color image 091599 at** 2018Z*

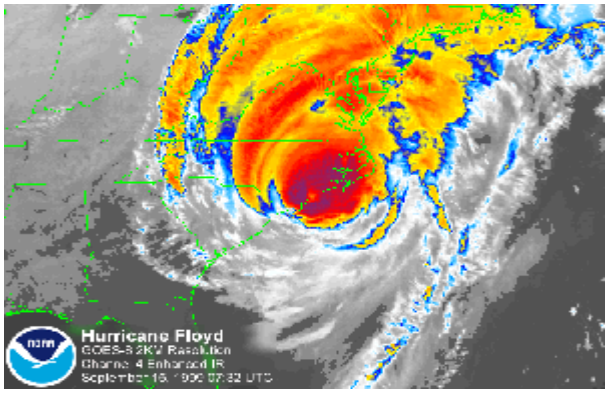

Figure 3: GOES-8 Colorized IR image 091699 at 0645Z

MODEL CONFIGURATION

The model was run for 24-hour time periods on September 15th and 16th, 1999; when Hurricane Floyd was making its way through the east coast. The settings are shown in Table 1 and the MM5 domain – 6 km over Washington D.C. is

shown in figure 4.

Dynamics	Non-hydrostatic
High Resolution	6km (Figure 1)
Vertical Layers	23
Forecast Time	24 hr
Initialization	NCEP Global Analysis
Explicit Schemes	Simple Ice
Cumulus Scheme	Grell
PBL Scheme	Blackadar
Radiation Scheme	Cloud
Soil Scheme	5-layer soil

Table 1 – Domain Configuration

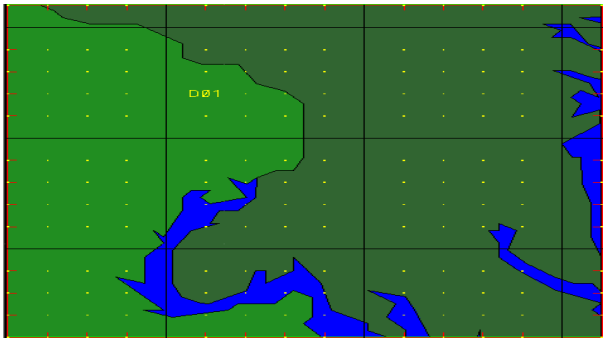

Figure 4: MM5 Domain - 6km over the Washington D.C. region

RESULTS AND DISCUSSIONS

The MM5 model output results for sea level pressure, accumulated precipitation, temperature, radiation tendency, wind magnitude and wind direction are shown in Figures 5 - 22 at times 12Z on September 15, 1999, 00Z and 12Z on September 16, 1999. Tables 2 and 3 provide comparisons of model simulations with observations. The study has shown the following:

The simulated parameters were in good agreement with the observations (Tables 1 and 2). The heavy precipitation was produced by the land falling hurricane Floyd which turned into tropical storm on 16th September, 1999 (Figures 7-10).

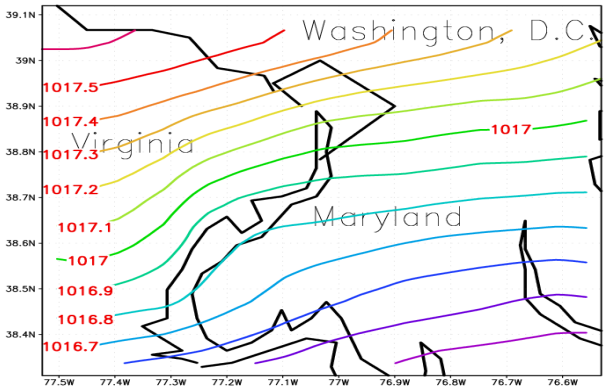

Figure 5: Sea Level Pressure (mb) 12Z on 091599

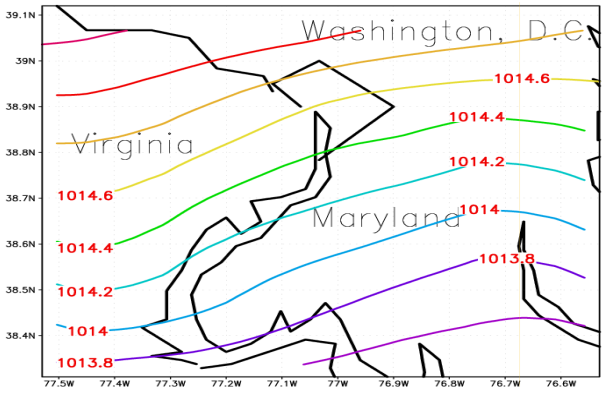

Figure 6: Sea Level Pressure (mb) 000Z on 091699

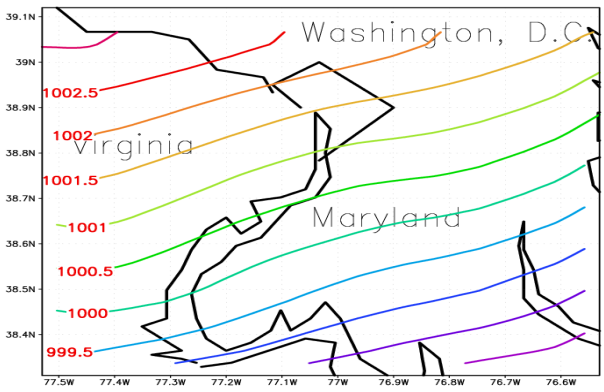

Figure 7: Sea Level Pressure (mb) 12Z on 091699

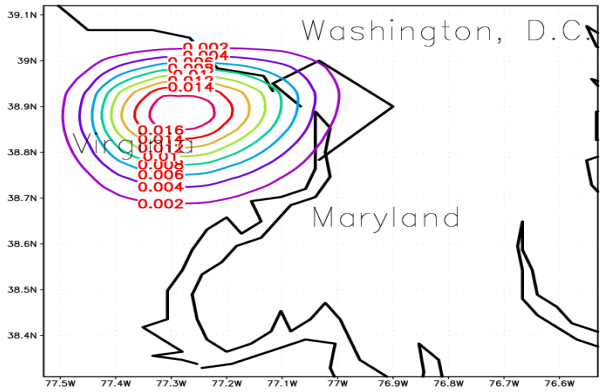

Figure 8: Total Non-Convective Precipitation (cm) 12Z on 091599

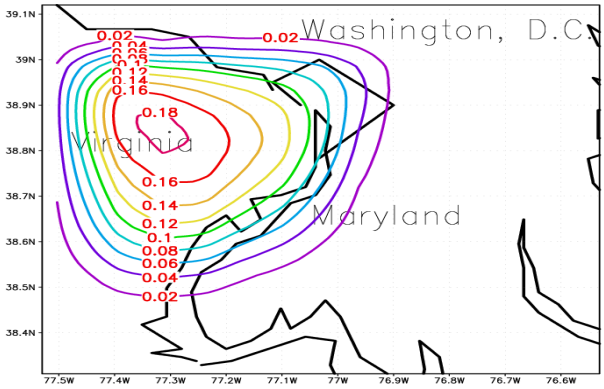

Figure 9: Total Non-Convective Precipitation (cm) 000Z on 091699

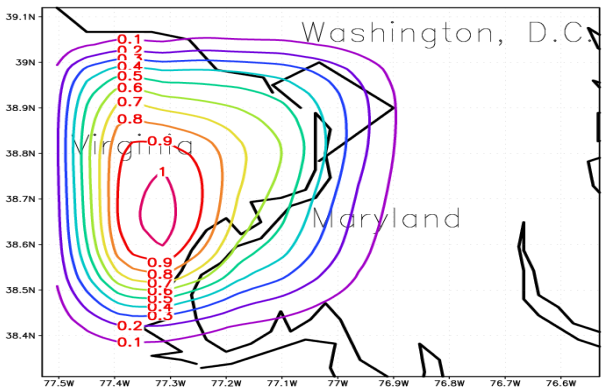

Figure 10: Total Non-Convective Precipitation (cm) 12Z on 091699

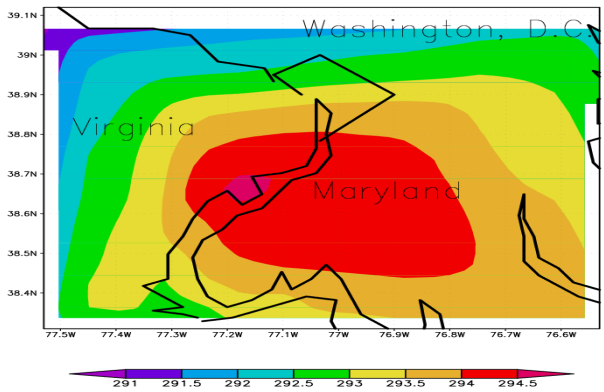

Figure 11: Temperature (K) 12Z on 091599

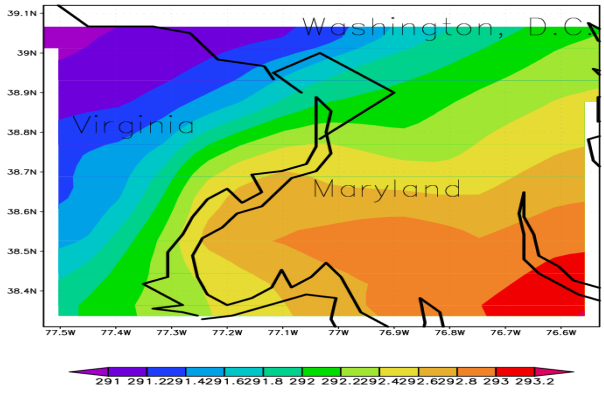

Figure 12: Temperature (K) 000Z on 091699

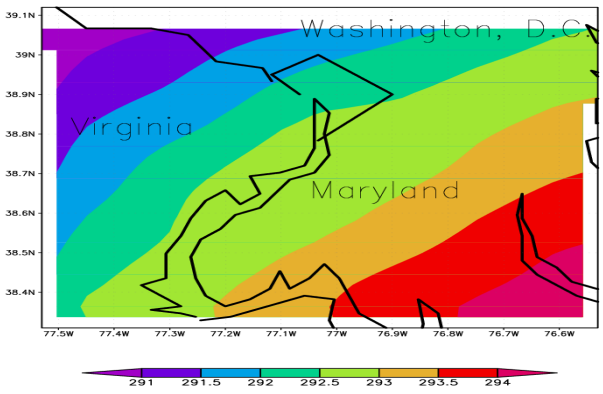

Figure 13: Temperature (K) 12Z on 091699

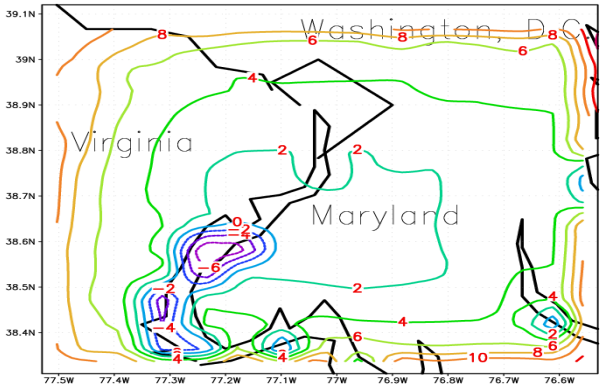

Figure 14: Radiation Tendency (K/day) 12Z on 091599

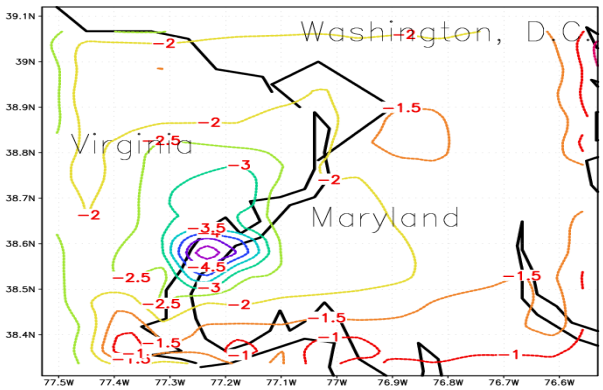

Figure 15: Radiation Tendency (K/day) 000Z on 091699

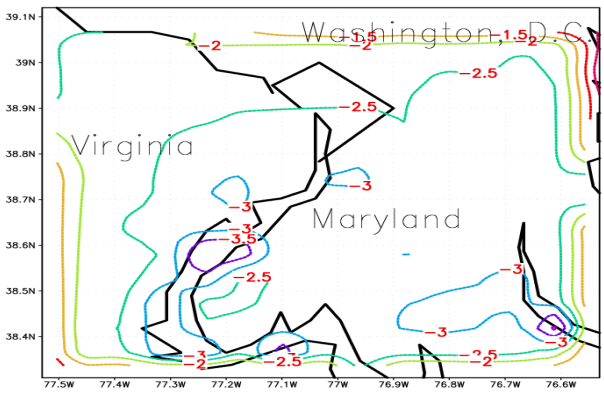

Figure 16: Radiation Tendency (K/day) 12Z on 091699

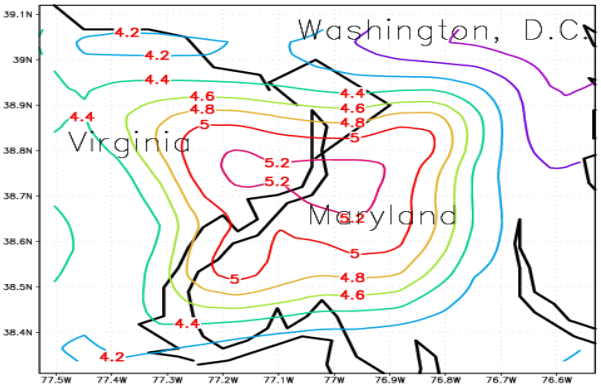

Figure 17: Wind Magnitude 12Z on 091599

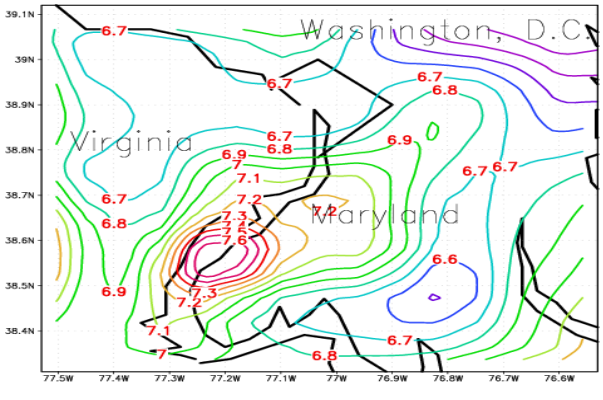

Figure 18: Wind Magnitude 000Z on 091699

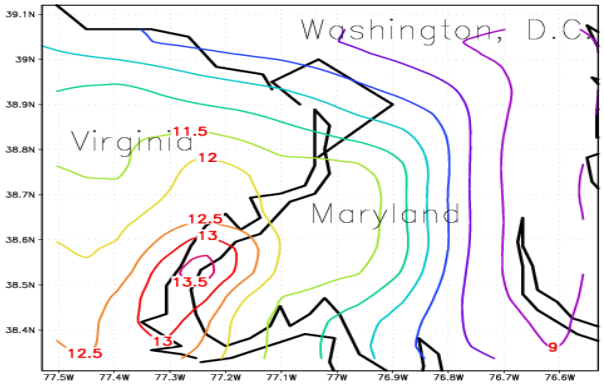

Figure 19: Wind Magnitude 12Z on 091699

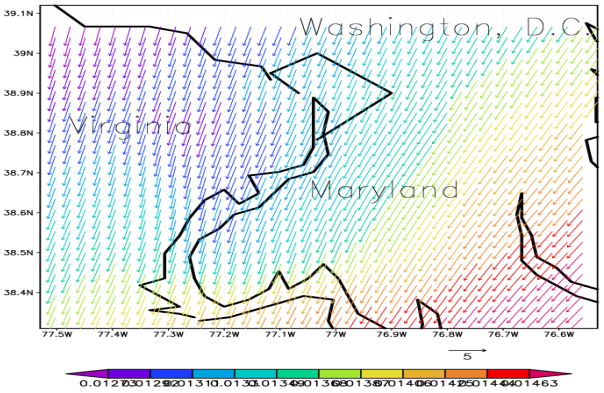

Figure 20: Wind Direction 12Z on 091599

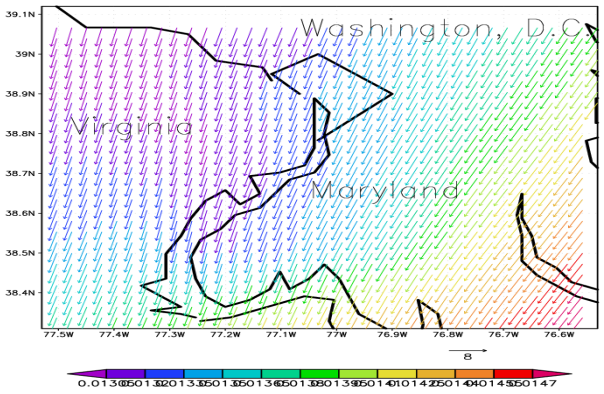

Figure 21: Wind Direction 000Z on 091699

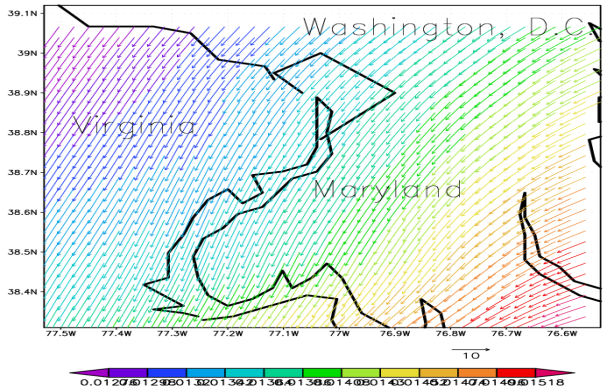

Figure 22: Wind Direction 12Z on 091699

Date	Hour (UTC)	Sea Level Pressure (mb)	
		Observed	Simulated
9/15/99	12	1017.8	1017.85
9/16/99	00	1014.4	1014.4
9/16/99	12	1001.5	1000.5

Table 2: Simulated sea level pressure vs. NDBC - Station TPLM2 Observations

Date	Hour (UTC)	Wind Speed (m/s)	
		Observed	Simulated
9/15/99	12	6.2	5.2
9/16/99	00	7.7	7.6
9/16/99	12	17.5	13.5

Table 3: Simulated wind speed vs. NDBC - Station TPLM2 Observations

Due to heavy precipitation associated with Hurricane Floyd, a significant drop in the radiation tendency (2k per day) and temperature (2 degrees Kelvin) was noticed (Figures 11-16).

MM5 model is suitable for studies of severe weather and associated precipitation and temperatures.

Model weather forecast could be utilized for creating public awareness and risk assessments, for making use of solar energy for cooking food, and also other appropriate measures may be taken to

protect solar assets.

The use of remotely sensed synoptic data is very crucial for close-to-reality weather prediction and simulation for model lateral and lower boundary conditions.

Krishnamurti et.al, 1998; Liu et.al, 1997; and Wu et.al, 1999, showed an understanding of the tropical cyclone structure, intensity change and track using numerical models. Their simulations showed the heavy precipitation associated with land falling tropical cyclones. The results of the present study are corroborating with the above results.

The MM5 output will be used to construct model initial and lateral boundary conditions for the Weather Research and Forecasting model (WRF) with grid spacing at resolutions one-three by weather regime and location. The data assimilation techniques such as 3DVAR to analyze most standard meteorological data to improve fine scale modeling will be introduced.

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The Effect of Morphine on Mitomycin C-Induced DNA Damage and Repair

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ABSTRACT

Peripheral blood lymphocytes obtained from heroin addicts show increased basal and mutagen-induced genetic damage, as well as diminished capacity for DNA repair, compared to those from control populations. Other studies have confirmed the ability of opiates to interfere with DNA repair processes. We report here on efforts to establish further the means by which morphine reduces DNA repair efficiency. Splenocytes from C57BL/6 mice were incubated with mitomycin C (3.0×10^{-6} M) or vehicle with or without subsequent addition of morphine sulfate (10^{-4} M). Morphine had no effect on the spontaneous frequency of micronucleated cells but increased that produced by mitomycin C treatment approximately 2-fold. Morphine sulfate (10^{-7} to 10^{-4} M) was without effect on spontaneous levels of ^{3}H-thymidine incorporation but was found to reduce mitomycin C-induced thymidine incorporation in a time- and concentration-dependent manner. Fluorometric analysis of DNA unwinding showed morphine did not interfere with induction of strand breaks in mitomycin C-treated cells. The persistence of mitomycin C-DNA adducts was followed by ^{32}P-postlabeling of DNA from mitomycin C-treated cells, and morphine was not found to alter the rate of adduct removal significantly. These results suggest it is the synthetic phase of nucleotide excision repair that is sensitive to inhibition by morphine.

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INTRODUCTION

Opioid analgesics have been shown to produce genetic damage in a variety of test systems (reviewed by Li and Lin (1998), although negative results have also been reported (reviewed in Madden et al., 1979). In addition, there is evidence that opioids can potentiate the genotoxicity of other agents. For example, morphine was shown to enhance the frequency of UV-induced sister chromatid exchanges (Shafer et al., 1983) and ethyl methanesulfonate-induced DNA damage and mutagenesis (Shafer et al., 1994). One explanation for the increased DNA damage in the presence of morphine is that the opioid interferes with repair processes (reviewed in Madden et al., 2002): DNA repair capacity, as measured by unscheduled DNA synthesis following exposure to UV light, is reduced in lymphocytes from heroin addicts (Madden et al., 1979) or those exposed to morphine in vitro (Madden and Falek, 1991). We report here on the effect of morphine on DNA damage and repair in murine splenocytes treated with mitomycin C.

MATERIALS AND METHODS

Chemicals. Bovine calf serum was obtained from HyClone Laboratories (Logan, UT). [Methyl-^{3}H]-thymidine was purchased from Amersham Life Science, Inc. (Arlington Heights, IL). [γ-^{32}P]ATP was a product of Dupont NEN Research Products (Boston, MA). Trichloroacetic acid, GF/C filters, Whatman filter papers, and liquid scintillation cocktail (ScintiVerse) were purchased from Fisher Scientific (Norcross, GA), and thin layer chromatography plates (PEI-cellulose) were obtained from Macherey-Nagel (Duren, GDR). Unless specified otherwise, all other chemicals used in these studies were purchased from Sigma Chemical Company (St. Louis, MO).

Splenocytes. The housing, treatment, and sacrifice of animals were in accordance with the University of Mississippi Medical Center Animal Care and Use Committee guidelines. Approximately 3-4 week old female C57BL/6 mice (Harlan Sprague Dawley, IN., Indianapolis, IN) were housed in

plastic cages with wood chip bedding and maintained in a temperature- and humidity-controlled facility on a 12 h lights-on schedule with unlimited access to rodent diet and tap water. Splenocyte suspensions were prepared as described previously (Sawant and Couch, 1995). Briefly, cell suspensions were prepared by manually dissociating spleen tissue through a wire screen, the cells washed twice and suspended at the desired density in RPMI 1640 medium containing 25 mM HEPES buffer, 2 mM glutamine, 10% heat-inactivated calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin.

Micronuclei production. The method of Erexson and Kligerman (1987) was used to assess micronuclei production in murine splenocytes. Cultures (5 x 10^5 nucleated cells/mL) were incubated at 37° in 5% CO_2 for 21 h in the presence of 5 μg/mL concanavalin A and mitomycin C or vehicle. Throughout these studies, 3 x 10^{-6} M mitomycin C and 10 μL/mL saline served as positive and negative controls, respectively. Cells were then harvested by centrifugation and re-suspended in medium containing 3 μg/mL cytochalasin B together with morphine sulfate (1.5 x 10^{-7} M) or vehicle. After 50 h total incubation, slides were prepared, stained with acridine orange, and randomized and coded prior to scoring. Four slides (2000 binucleated cells were scored per slide) were scored per group per experiment. Statistical comparisons were performed by analysis of variance on arcsine transformed data followed by Dunnett's test.

DNA strand breaks. The effect of morphine sulfate on DNA strand brakes appearing during the repair of mitomycin-DNA adducts was studied by estimating the amount of double strand DNA, as described by Birnboim and Jevack (1981). Splenocyte suspensions (10^6 cell/mL) were treated with mitomycin C or vehicle for 1 h at 37°C. A portion of the cells was used to determine the amount of double stranded DNA present initially, while other cultures were incubated an additional 3.5 h in medium containing morphine sulfate (10^{-4} M) or saline prior to assay. Three measurements were made per sample: fluorescence not due to ethidium bromide binding was estimated in cell extracts which are sonicated and placed in alkali to allow complete unwinding; a second sample not exposed to denaturing conditions was used to estimate total fluorescence; the third sample was exposed to denaturing conditions but not sonicated. Denaturation was stopped by lowering the pH, ethidium bromide added, and fluorescence measured (520 nm excitation, 590 nm emission). The percent double-stranded DNA was calculated from the ration of partially to totally denatured samples, corrected for background fluorescence. Comparisons between multiple means were done with two-way analysis of variance. Individual comparisons of effects of morphine sulfate and mitomycin C alone or in combination with saline treatment were done using Student's *t*-test.

DNA adducts. The persistence of mitomycin C-DNA adducts in the presence or absence of morphine sulfate was measured using ^{32}P-postlableing for the detection of adducted nucleotides, as described by Reddy and Randerath (1986). Cell suspensions were prepared as in the assay for unscheduled DNA synthesis and treated with mitomycin C or saline for 2 h at 37°C. Aliquots of the suspension were analyzed for adduct formation at this time, while other were treated with morphine sulfate or saline for an additional 2 h prior to analysis. Briefly, extracted DNA was hydrolyzed with micrococcal nuclease, spleen phosphodiesterase, and nuclease P1. The digest was dried, reconstituted in distilled water, and labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP. ^{32}P-Labeled adducts were separated by multi-directional polyethylenimine-cellulose thin layer chromatography. Chromatograms were exposed to a phosphor screen at room temperature for 4 h, scanned, and the image digitalized using a PhosphorImager (Model 425F, Molecular Dynamic, Sunnyvale, CA) and an image acquisition and analysis program (ImageQuant 3.3, Molecular Dynamics). For quantitation of signal intensities, the relationship between radioactivity (counts per minute) and signal intensity was obtained for aliquots of serially diluted [γ-^{32}P]ATP solutions. The adduct level, expressed as relative adduct labeling, was converted to fmol adduct per μg DNA as described by Gupta and Dighe (1984).

^{3}H-Thymidine incorporation. The method of Madden et al. (1979) was used with some modification to assess DNA synthesis in response to chemically-induced damage. Splenocyte

suspensions (10^6 cell/mL) were treated for 2 h with mitomycin C or saline at 37°C, then the cells were centrifuged (400*g* for 7 min) and re-suspended in medium containing either saline or morphine sulfate (10^{-7} to 10^{-4} M). ^{3}H-Thymidine (1μCi/mL) was added at various times after re-suspending the cells, and acid precipitable material collected on GF/C filters 3 h later for measurement of thymidine incorporation by liquid scintillation counting (Packard 2500 TR liquid scintillation analyzer, Packard Instrument Company, Meriden, CN). In other studies, the incorporation of thymidine after treatment with mitomycin C or vehicle in the presence or absence of 10^{-4} M morphine sulfate was followed up to 10 h following treatment. Results are expressed as percent incorporation of cell exposed only to saline. Statistical comparisons were by repeated measures analysis of variance and Student's *t*-test (time-course studies) or analysis of variance and Dunnett's test (concentration-response).

RESULTS

Micronuclei production. Morphine sulfate (10^{-4} M) alone had no effect on the observed frequency of micronucleated binucleate cells, whereas mitomycin C (1.5 x 10^{-7} M) increased the frequency approximately 9-fold (Table 1). Combined treated with morphine and mitomycin C produced a significantly higher frequency (~2-fold) of micronucleated binuclear cells than mitomycin C alone.

Table 1. The effect of morphine on micronuclei induction by mitomycin C.

Treatment	MBN/1000 BNC
SALINE (10μL/mL)	3.7 ± 0.3
MORPHINE SULFATE (10^{-4} M)	3.3 ± 0.3
MITOMYCIN C (3.0 x 10^{-6} M)	34.0 ± 1.2[a]
MORPHINE SULFATE + MITOMYCIN C	71.7 ± 2.3[a,b]

MBN: micronucleated binuclear cells; BNC: binuclear cells. Results shown represent the mean ± standard deviation from three independent experiments. [a]significantly different (P<0.01) from saline control; [b]significantly different (P<0.01) from morphine sulfate or mitomycin C alone.

^{3}H-Thymidine incorporation.

Splenocytes treated with 3 μM mitomycin C for 2 h incorporated significantly more (37%) ^{3}H-thymidine than cells treated with saline (Figure 1). Incubation of mitomycin-treated cells with 10^{-4} M morphine sulfate for 2, 6, and 10 h reduced thymidine incorporation to 64, 74, and 82%, respectively, of that in cells treated with mitomycin C alone, levels that are not different than cells treated with saline. Morphine did not influence thymidine incorporation in saline-treated cells. Incubation with 10^{-6} to 10^{-4} M morphine sulfate for 2

h reduced ^{3}H-thymidine incorporation in mitomycin C-treated cells in a concentration-dependent manner to 88-70% of the incorporation following treatment with mitomycin C alone (Figure 2).

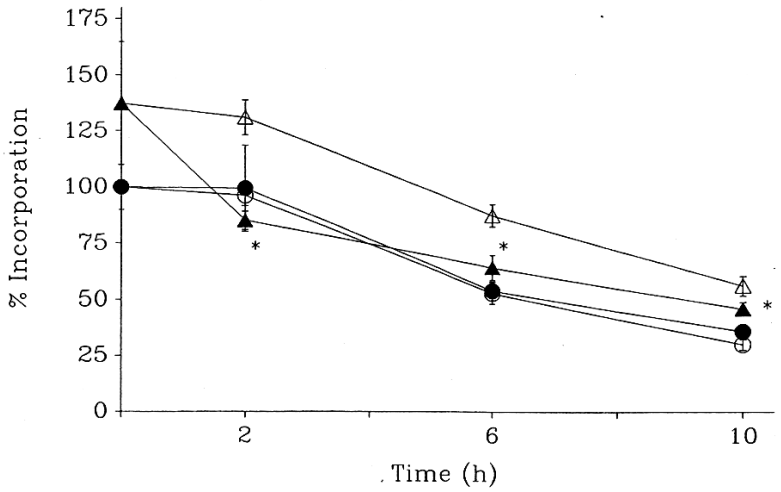

Figure 1. Incorporation of ^{3}H-thymidine as a function of time after mitomycin C (triangles) or saline treatment (circles) in splenocytes with (closed symbols) or without (open symbols) morphine sulfate. Results are expressed as percent of incorporation of saline controls (4.4 x 10^{-11} mol ^{3}H-thymidine/10^{6} cells). Each point represents the mean ± standard deviation of ten determinations. *Significantly different from corresponding controls (P<0.01).

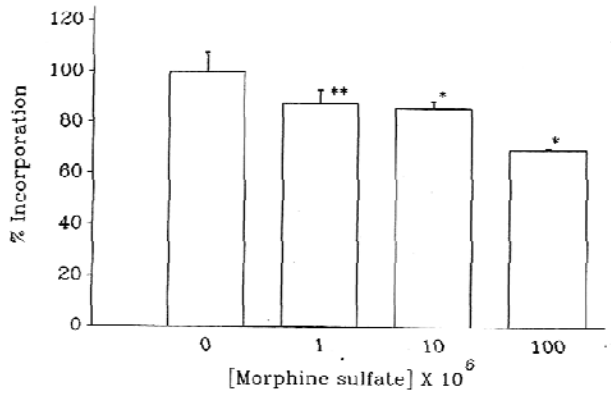

Figure 2. Incorporation of ^{3}H-thymidine in splenocytes treated with mitomycin C for 2 h followed by saline or morphine sulfate for an addition 2 h. Each point represents the mean ± standard deviation of 6 determinations. Single and double asterisks represent significant differences from mitomycin C treatment alone at P<0.01 and P<0.05, respectively, different from mitomycin C alone (P<0.01).

DNA strand breaks. Incubation of splenocytes with 3 μM mitomycin C for 1 and 4.5 h resulted in 23 and 32%, respectively, less double-stranded DNA than in saline-treated cells (Table 2). Morphine sulfate (10^{-4} M) did not affect the extent of double-stranded DNA in saline-treated cells, whereas addition of morphine sulfate to mitomycin C-treated cultures resulted in a significant decrease (24% of mitomycin C alone) in the fraction of double-stranded DNA obtained.

DNA adducts. Adducted nucleotides could be detected on chromatograms prepared from splenocytes treated with 3 μM mitomycin C for 2 h. The mean adduct level in these chromatograms corresponded to 1.0 fmole adducted nucleotides/μg DNA. No adducted nucleotides could be detected on chromatograms prepared from cells treated either saline or mitomycin C followed by incubated with either saline of morphine sulfate for an additional 2 h.

Table 2. The effect of morphine on DNA breaks following treatment with mitomycin C

Treatment	Percent double-stranded DNA
SALINE[A]	74.9 ± 1.5
MITOMYCIN C[A]	58.3 ± 2.0[a]
SALINE-SALINE[B]	76.1 ± 2.6
SALINE-MORPHINE SULFATE[B]	76.9 ± 3.0
MITOMYCIN C-SALINE[B]	51.9 ± 1.7[a]
MITOMYCIN C-MORPHINE SULFATE[B]	39.8 ± 1.3[a,b]

Prior to assay, splenocytes were incubated with (A) saline or 3 μM mitomycin C for 1 h or (B) saline or mitomycin C for 1 h followed by incubation with saline or 10^{-4} M morphine sulfate for an additional 3.5 h. Results shown are the mean ± standard deviation of 4 experiments. [a]Significantly different ($P<0.01$) from corresponding saline treatment; [b]significantly different ($P<0.01$) from mitomycin C alone.

DISCUSSION

Although morphine does not induce formation of micronuclei following in vitro exposure of splenocytes to the drug, incubation of cells with morphine following mitomycin C treatment did produce marked increases in the frequency of micronucleated cells (Table 1). Although somewhat high (10^{-4} M), the concentration of morphine used in these experiments is approximately equal to the maximal serum concentration obtained in mice (0.5 x 10^{-4} M) following a single subcutaneous administration of 20 mg/kg (Berkowitz et al., 1974).

Mitomycin C forms covalently bound DNA monoadducts and cross-links (Tomasz et al., 1986;

Basu et al., 1993) that are removed primarily by the nucleotide excision repair pathway (Bootsma and Hoeijmakers, 1994; Zheng et al., 2003). Measurement of thymidine incorporation was first used to determine if the synergistic effects of mitomycin C and morphine on DNA damage are consistent with interference with repair processes by the opioid. Morphine was found to inhibit mitomycin C-induced thymidine incorporation in a time- and concentration-dependent manner (Figures 1 and 2).

These results were obtained without the use of hydroxyurea or other means to reduce replicative synthesis. Although the fraction of proliferating, unstimulated splenocytes is low (Pabst and Fritz, 1988) and unscheduled DNA synthesis has previously been quantitated by liquid scintillation counting in mutagen-treated lymphocytes in the absence of hydroxyurea (Holmberg et al., 1988), it cannot be concluded the effects of morphine observed were solely due to effects on repair synthesis. The ability of morphine treatment to lower thymidine incorporation was, however, only evident in mitomycin C- and not saline-treated cells. In experiments conducted with 4 mM hydroxyurea in the medium, the amount of thymidine incorporation observed was markedly reduced compared to that in its absence, although the relative increase following mitomycin C treatment was similar (data not shown).

Since unscheduled DNA synthesis reflects the entire repair process (incision, excision, synthesis), exclusive of ligation, the effect of morphine on the induction of DNA strand breaks was then measured to determine if incision was affected. The appearance of DNA strand breaks following treatment with agents that do directly produce them is thought to result from repair processes (Fairbairn et al., 1995). Fluorometric analysis of DNA unwinding was used to measure the effect of morphine on DNA strand breaks, or incision. Although the assay for measurement of strand breaks employed yields a result that is the net effect of induction of breaks and their reannealing, the increase in DNA breaks due to incision at mitomycin C damaged sites (Table 2) indicates that morphine does not block incision but interferes with a subsequent step in excision repair.

Once incisions are made on either side of a DNA lesion, the damaged segment is removed. The effect of morphine on that step was studied by evaluation the rate of DNA adduct removal by ^{32}P-postlabeling. Spleens of rats treated with mitomycin C have been found to have 1 major adduct and 6 relatively minor ones (Reddy and Randerrath, 1987). The level of adduct formation found in this study was quantitatively similar to that of the major adduct produced in vivo, although other adducts were not detected. As neither incision nor excision appeared to be affected by morphine, it appears that the opioid interferes with nucleotide excision repair at a later step. An effect on ^{3}H-thymidine incorporation consistent with interference with repair patch synthesis was observed, but effects on ligation may also be possible.

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Camponotus planatus (Hymenoptera: Formicidae), an Exotic Carpenter Ant Found in Mississippi

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The compact carpenter ant, *Camponotus planatus* Roger (Hymenoptera: Formicidae), occurs in the Caribbean Region and ranges from Columbia to Texas, and it has been introduced into Florida, Hawaii, and the Galapagos Islands (Creighton 1950, McGlynn 1999, Wetterer and Wetterer 2003). In Florida, it is only known to occur in the southern half of the state (Deyrup 1991, Deyrup et al. 1988, Deyrup et al. 2000, Klotz et al. 1995) where it is considered an occasional structural pest (Warner and Scheffrahan 2005).

On 7 October 2009, workers of *C. planatus* were collected in Bay St. Louis, Hancock County, Mississippi at a nursery that specializes in palms (Arecaceae). Many of the palm trees at the nursery were imported from Florida, which has the highest number of exotic ants in the United States (Deyrup et al. 2000), several of which are known to be associated with palms. Nurseries such as this one have an increased likelihood of receiving introduced species of ants from Florida and may serve as a gateway for exotic ants entering Mississippi. In fact, a visit to this same nursery in April of 2008 by MacGown and J. G. Hill resulted in the discovery of another exotic species new for Mississippi, *Tapinoma melanocephalum* (Fabricius), the ghost ant (MacGown and Hill 2009). The carpenter ants were likely introduced to the nursery in palms shipped from Florida, as this species is known to nest in leaf axils of palms (Warner and Scheffrahan 2005). *Camponotus planatus* workers were observed moving in trails along the irrigation system on the ground and crawling up and down sabal palms (*Sabal palmetto* (Walt.) Lodd), which were planted directly in the soil (rather than in pots). Workers were fast moving and difficult to collect. Colonies were not discovered, but foraging workers were observed moving toward the uppermost parts of palms, where nests presumably were located. MacGown and Hill did not observe this species during their visit in 2008. Return visits to the area will be made to determine whether or not this species becomes established.

Camponotus planatus (Figure 1) can be easily distinguished from other carpenter ants in this region by the following characteristics: relative small size with workers ranging from 3 to 6 mm in overall length; the gaster black and the rest of the body reddish-brown; and presence of abundant, long, white setae on much of the body except for the scapes. Nests are often difficult to locate, but may be found in hollow twigs, old termite galleries in dead wood, grass culms, voids in tree trunks, and leaf axils of palms (Deyrup et al. 1988, Warner and Scheffrahan 2005).

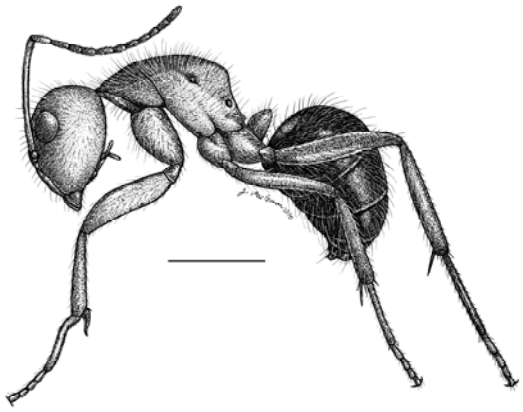

Figure 1. Profile view of a *Camponotus planatus* worker. Scale bar equals 1.0 mm.

In addition to this carpenter ant, four other exotic species were collected at the site, including *T. melanocephalum, Brachymyrmex patagonicus* Mayr (the dark rover ant), *Paratrechina longicornis* (the crazy ant), and *Solenopsis invicta* Buren (the red imported fire ant) all of which were found during the first visit to the nursery in 2008 by MacGown and Hill (MacGown and Hill 2009). Of note was the fact that *T. melanocephalum* was still present at the site, having survived targeted control efforts, several days of sub-freezing temperatures, and mild flooding, which occurred as a result of Hurricane Gustav in late August 2008. In fact, this species appeared to be much more abundant than the previous year and multiple colonies with dealate queens were discovered nesting under the bark of numerous palms. Foraging workers of *P. longicornis* were found at the site and were also found at a gas station approximately one mile east of the nursery. Both *B. patagonicus* and *S. invicta* are well established in southern Mississippi; thus, their high levels were expected.

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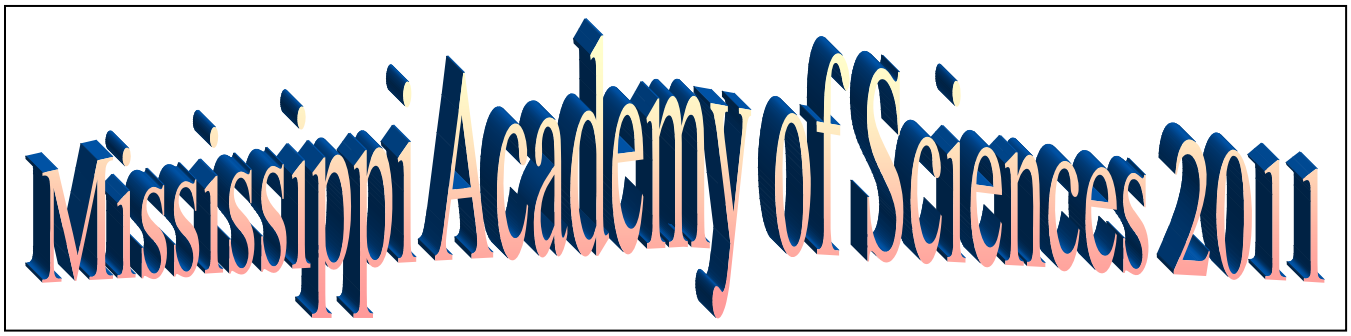

HATTIESBURG, MS

February, 2011

More Information will be available in the October issue

CALL FOR ABSTRACTS

MISSISSIPPI ACADEMY OF SCIENCES ABSTRACT FORM/MEMBERSHIP FORM

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Abstract title:_____
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If you are a student please fill-out the next line
Name of Mentor and e-mail of Mentor _____
(Presenter must be current (i.e., 2011 membership dues must be paid), student member, regular member or life member of the MAS)
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Check the division in which you are presenting

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Type of presentation
__ Poster presentation __ Workshop __ Lecture presentation __ Invited Symposium
If the presenting author for this paper will also present in another division, please list the other division_____

Audiovisual Equipment needs:
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Please complete the following:
__ Enclose copy of abstract (even if abstract has been submitted electronically)
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__ $25 per abstract
__ $25 regular membership fee OR $10 student membership fee (2007 membership must be paid for abstract to be accepted)
__ You must supply a check #_____ or P.O. #_____ (credit cards are not accepted)

In addition, you MAY preregister at this time to take advantage of the saving
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- Your paper may be presented orally or as a poster. Oral presentations are generally 15 minutes. The speaker should limit the presentation to 10-12 minutes to allow time for discussion; longer presentations should be limited accordingly. Instructions for poster presentations are linked here.
- Enclose a personal check, money order, institutional check, or purchase order for $25 publication charge for each abstract to be published, payable to the Mississippi Academy of Sciences. The publication charge will be refunded if the abstract is not accepted.
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- Attendance and participation at all sessions requires payment of registration.
- Note that three separate fees are associated with submitting and presenting a paper at the annual meeting of the Mississippi Academy of Sciences.
    1. An abstract fee is assessed to defray the cost of publishing abstracts and
    2. a membership fee is assessed to defray the costs of running the Academy.
    3. Preregistration payment ($25 regular; $10 student) may accompany the abstract, or you may elect to pay this fee before February 1, or pay full registration fees at the meeting.
- Abstracts may **only** be submitted on line via a link through the MAS website.The appropriate abstract fees can be paid via Paypal or sent via mail to Barbara Holmes at the Academy address .
- **Late abstracts will be accepted with a $10 late fee during November increased to $25 after that. Late abstracts will be accepted only if there is room in the appropriate division. They will be published in the April issue of the MAS JOURNAL.**
- Submit your appropriate fees NO LATER THAN NOVEMBER 1, 2010.

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

GUIDELINES FOR POSTER PRESENTATIONS

- 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.
- Lettering for your poster title should be at least 1" high and follow the format for your abstract. Lettering for your poster text should be at least 3/8" high.
- Posters should be on display during the entire day during which their divisional poster session is scheduled. They must be removed at the end of that day.
- Authors must be present with their poster to discuss their work at the time indicated in the program.