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Research Articles

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GREEN SALAMANDER (*Aneides aeneus*) POPULATIONS IN MISSISSIPPI: A PRELIMINARY UPDATE

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ABSTRACT

During 2008-2009 we surveyed sandstone outcrops for the state endangered Green Salamander (*Aneides aeneus*) in the four areas of Tishomingo County, Mississippi where the species had been reported previously to: 1) determine if populations persist; 2) make general comparisons between past and current abundance patterns; and 3) suggest conservation and management strategies specific to these populations. Surveys in 1990 suggested that *A. aeneus* had been extirpated from one of the historic sites (Cabin Cliffs); however, we found salamanders inhabiting each of the four locations. Additionally, population numbers of *A. aeneus* were similar at Springhill Cliffs between 1990 surveys and our surveys. Comprehensive surveys of the sandstone outcrops near the historic sites are needed as additional populations/ metapopulations likely exist. Development of a long-term monitoring program will be useful to assess the population trends of this secretive species.

INTRODUCTION

Worldwide many amphibian species have experienced population declines (Blaustein and Wake, 1990; Lannoo, 2005) and the current extinction rate appears to dwarf that of background levels (McCallum, 2007). While a global problem, amphibian population declines and losses are at times a result of complex local causes and are often regional (Lannoo, 2005). For example, Corser (2001) reported a significant (98%) decline in Green Salamander (*Aneides aeneus*) relative abundance within seven Blue Ridge Escarpment populations from 1970 throughout the 1990's, yet Tennessee and Kentucky populations apparently remained stable during the same time (Snyder, 1991). Differentiating between natural population fluctuations and those caused by human impacts (direct or indirect) can at times prove challenging (Pechmann and Wilbur, 1994). However, long-term monitoring of populations will help to make this distinction, and provide a better understanding of population trends.

The Green Salamander (*A. aeneus*) inhabits moist but not wet crevices of quartzite, limestone, dolomite, sandstone, granite, and schist formations (Netting and Richmond, 1932; Walker and Goodpaster, 1941; Schwartz, 1954; Bruce, 1968; Woods, 1968; Mount, 1975). The salamander's geographic range extends from extreme southwestern Pennsylvania to northern Alabama and extreme northeastern Mississippi (Pauley and Watson, 2005). While the species may be found in caves, under logs, or even in trees (Gordon and Smith, 1949; Gordon, 1952; Waldron and Humphries, 2005; Pauley and Watson, 2005), it is most frequently observed deep within the crevices of emergent rock outcroppings that are well shaded and located in high humidity forests. Compared to other genera in the family [i.e., Plethodontidae], members of the genus *Aneides* are considered specialized by virtue of their loss of the aquatic larval stage and advanced climbing abilities (Wake, 1966). Given its unique habitat requirements, natural history, and patchy distribution (Petranka, 1998), the Green Salamander is listed as a "species at risk" by the U.S. Fish and Wildlife Service (Waldron and Humphries, 2005), and is considered critically imperiled, imperiled, or vulnerable in each of the 13 states in which it occurs (Natureserve, 2011). In Mississippi, the Green Salamander is state endangered, and can only be found within, and immediately adjacent to, Tishomingo State Park, Tishomingo County (Ferguson, 1961; Woods, 1968; MMNS, 2001).

Unfortunately, a formal monitoring program does not currently exist for the Tishomingo population of *A. aeneus* (T. Mann, Mississippi Natural Heritage Program, pers. comm.). However, Cliburn and his students periodically visited this site during the late 1960's through the early 1990's and made numerous observations upon the population (Woods and Cliburn, 1967; Woods, 1968; Cliburn, 1977; Baltar, 1983; Cliburn and Porter, 1986; Cliburn and Blanton-Pihkala, 1986; Cliburn and Porter, 1987a; Cliburn and Porter 1987b; Nalepa, 1993; Cliburn, unpubl. data). These researchers identified three areas within the park where *A. aeneus* are found: 1) Cabin Cliff; 2) Spring Hill Cliffs; and 3) South Cliffs. Woods (1968) extensively surveyed the Cabin Cliff site and found that salamanders were extremely abundant. On the contrary, multiple visits to Cabin Cliff from 1987-1990 revealed that *A. aeneus* were no longer found at the site, and salamanders were more abundant at Spring Hill Cliffs (J.W. Cliburn, pers.comm.; Nalepa, 1993). While routinely observed at South

Cliffs, *A. aeneus* abundance at this site is low (J.W. Cliburn, pers. comm.; Nalepa, 1993). In addition to the sites within the park boundaries, a fourth site located on private property was identified, but the population is small and salamanders are infrequently observed (J.W. Cliburn, pers. comm.).

This population of green salamanders lies on the edge of their distribution at a time when many amphibians are declining (Lannoo, 2005). Additionally, Cliburn, (unpubl. data} found that the population of green salamanders was extirpated at one site (Cabin Cliffs) where they were abundant in the 1967 -1968 surveys. We hypothesized that the whole Mississippi population may be declining. The purpose of this study was to revisit the four main sites in Mississippi where *A. aeneus* have been reported to determine if populations still persist; make general comparisons between past and current abundance patterns; and suggest conservation and management strategies specific to the Mississippi populations.

MATERIAL AND METHODS

Study Area

Situated on the periphery of the Cumberland Plateau, Tishomingo State Park, Tishomingo County, MS encompasses an area of approximately 603.3 ha. The main topographic features of the park are the vertical rock walls cut in Highland Church sandstone by Bear Creek, the major stream of the area (Woods, 1968). The park abounds with seeps, springs, and boulder-strewn branches, which along with the sandstone outcrops, provide habitat for flora and fauna unique to Mississippi (Ferguson, 1961; Woods, 1968; MMNS, 2001). Detailed descriptions of the climate, geology, soils, flora, and fauna of the park and county have been reported elsewhere (Ferguson 1961; Woods, 1968; Miller, 1983; Merrill et al. 1988).

Surveys

We conducted surveys on six occasions from July 2008 through August 2009. Cabin Cliff and Spring Hill Cliffs as delineated by Woods (1968) were completely surveyed on each of the six visits, while South Cliffs and the private property site were surveyed three times and once, respectively. We tried to conduct surveys during optimal conditions (i.e., overcast and/ or trace precipitation), although logistical problems and time constraints often resulted in us conducting surveys in what would be considered suboptimal conditions (i.e., sunny and dry). We used flashlights during both day and evening surveys to scan crevices and faces of the sandstone outcrops. The methods of Woods (1968; page 21) were followed to insure consistent survey methodology, and the duration of surveys at each site survey were similar for the three survey periods (1968, 1990, and 2008-9). Species of amphibians and reptiles other than *A. aeneus* were identified and environmental conditions for survey years are in Table 1. The unpublished field notes of J.W. Cliburn (collected in 1990), and information found in Woods (1968) were used to make general comparisons with our survey data.

Table 1. Average environmental variables for the years in which surveys were conducted.

	1967	1990	2008	Historical Average
Mean Temp (° C)	15.4	17.2	15.1	15.4
Mean Precipitation (cm)	136.8	131.4	142.5	143.9

RESULTS AND DISCUSSION

We observed *A. aeneus* inhabiting each of the four known historic locations. Surprisingly we found 13 individuals at Cabin Cliff, a site where the species was thought to be extirpated. An individual *Eurycea longicauda* (Long-tailed Salamander) and 11 *Plethodon mississippi* (Mississippi Slimy Salamander) were also observed in the sandstone crevices at Cabin Cliff. We found 34 *A. aeneus*, 17 *E. longicauda*, 32 *P. mississippi*, and one *P. ventralis* (Southern Zigzag Salamander) at Spring Hill Cliffs. Four *A. aeneus*, two *E. longicauda*, and one *P. ventralis* were found at South Cliffs, and only one green salamander was observed at the private property site.

The number of individual *A. aeneus* varied by month and by observer at the two sites for which we have comparable data (Table 2). However, the total number of individuals observed at Spring Hill Cliffs did not vary considerably between what we, and Cliburn (unpubl. data) found in the 1990's (Table 2). Additionally, the fact that we found green salamanders at Cabin Cliff during our surveys, albeit at comparatively lower numbers than that observed by Woods (1968), is a promising sign that the species may successfully re-colonize the area. The precise reason why *A. aeneus* were not detected at Cabin Cliff during Cliburn's 1987-1990 surveys will remain a mystery, although several possible explanations have been proposed. An ice storm in the mid 1980's removed many of the canopy and sub-canopy trees surrounding the cliff (J.W. Cliburn pers. comm.; B. Brekeen, Park Manager, pers. comm.). This likely resulted in increased insolation (and possibly increased airflow), thus increasing temperatures and decreasing moisture, and ultimately drying of the foraging and nesting crevices. Furthermore, a common practice of park staff during much of the 1980's was to dump wood ash from cabin fireplaces over the cliff edge, which may have altered the pH or other microhabitat characteristics of the nesting and foraging crevices rendering them unsuitable/ unusable for *A. aeneus* (J.W. Cliburn pers. comm.). This practice was discontinued when brought to the attention of the park manager (B. Brekeen, Park Manager, pers. comm.).

Table 2. Number of *Aneides aeneus* found at two study sites in Tishomingo County, Mississippi

	Month							
Location	Jul	Aug	Nov	Feb	May	Aug*	Total	Reference
Cabin Cliffs	10	9	31	2	9	9	70	Woods (1968)
	0	0	0	0	0	0	0	Cliburn (Unpubl. data; 1990)
	0	1	3	1	4	4	13	This study (2008-2009)
Spring Hill Cliffs	0	7	18	0	0	7	32	Cliburn (Unpubl. data; 1990)
	3	2	14	5	6	4	34	This study (2008-2009)

*August data was collected only once by Woods and Cliburn for the year(s) indicated. The number of individuals the authors observed in their original August survey is repeated in the second August column.

The Green Salamander population of Tishomingo County is apparently isolated and represents the westernmost extent of the species' range (Woods, 1968; Gordon, 1967). The importance of peripheral populations has been highly debated. In some instances, these populations are considered less viable than those at the core of the species' range, constituting population sinks, and are of little conservation value (Peterson, 2001; Cassel and Tammaru, 2003). Alternatively, peripheral populations can be important reservoirs of unique alleles for a species (Lesica and Allendorf, 1995), and may serve as sites of future speciation events (Mayr, 1963); in these latter instances, peripheral populations have especially high conservation value (Lesica and Allendorf, 1995). *A. aeneus* found in Tishomingo County are apparently morphologically divergent from the more central populations within the species' range (Woods, 1968), but if this translates to a significant divergence in genetic/ allelic composition is unknown and warrants further investigation.

A more detailed and better understanding of the Green Salamanders current distribution within Tishomingo State Park is needed. For example, *A. aeneus* generally move short distances (< 3.7 m), and only in extreme cases make long distance movements (e.g., 76-91 m; Petranka, 1998 and references therein). Therefore, it seems more probable that recolonization of Cabin Cliff was a result of salamanders moving from a site yet unknown to researchers, as opposed to moving from the known site nearest Cabin Cliff (~250 m straight line distance), which is 2-3 times the maximum distance Green Salamanders have been reported to travel. A complete survey of all the sandstone outcrops within the park should be conducted to identify additional populations/ metapopulations. Development of a long-term monitoring program will be useful to assess the population trends of this state endangered species.

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EFFECTS OF RHIZOBACTERIA ON THE GROWTH, AND UPTAKE OF LEAD BY TALL FESCUE (*Festuca arundinacea*)

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ABSTRACT

Tall Fescue (*Festuca arundinacea* Schreb.) was grown in a naturally lit greenhouse to evaluate the effects of two rhizobacteria and ethylene diamine tetraacetic acid (EDTA) on the growth and lead (Pb) accumulation of this plant. Tall fescue seeds were inoculated with two strains of commercial bacteria, namely, Bacteria 1 (B1) *Pseudomonas monteilii* and Bacteria 2 (B2)*Wautersia metallidurans*. Inoculated seeds were planted in plastic tubes containing sterile or non-sterile soil mixture consisting of topsoil and peat (2:1, v: v) that had been spiked with various amounts (0, 500, 1000, 2000 mg Pb/Kg dry soil) of lead nitrate. The plants were allowed to grow for six weeks. Our results showed that there were higher Pb concentrations in shoots of plants treated with EDTA + B1 and EDTA + B2 that were grown in non-sterile soils as compared to treatments in sterile soils. Lead uptake by the roots also increased with increasing soil Pb concentrations especially with EDTA + B1+ B2 treatments. These results indicated that EDTA + certain Rhizobacteria may synergize to mobilize Pb in the soil for root uptake and translocation to the shoots of tall fescue.

INTRODUCTION

muscle and joint pain, and memory or concentration problems [2, 3].

According to the American Academy of Pediatrics (AAP), soil is contaminated by lead from various sources. Lead particles are deposited in the soil from flaking lead paint and incinerators, as well as from waste disposal [4]. Natural levels of lead in soil are usually below 50 ppm [5, 6].

Phytoremediation is an alternative way of reducing heavy metal contamination from the soil by using different plant species known as hyperaccumulators. Hyperaccumulators refer to plants that share the ability to grow on metalliferous soils and accumulate extraordinarily high amounts of heavy metals in the aerial organs without suffering phytotoxic effects. A strongly enhanced rate of heavy metal uptake, faster root-to-shoot translocation and a greater ability to detoxify and sequester heavy metals in leaves are the primary differences between hyperaccumulators and non-hyperaccumulators.

The combination of the two approaches, phytoremediation and bioaugmentation, results in a process known as rhizoremediation. During rhizoremediation, exudates derived from the plant can help to stimulate the survival and action of bacteria subsequently resulting in a more efficient degradation of pollutants. The bacteria are spread through the soil via the root system of plants penetrating otherwise impermeable soil layers[7].

It has been suggested that rhizosphere microbes may play an important role in phytoremediation. Microbes are ubiquitous in soils to which hyperaccumulators are native, even in those soils containing high concentrations of metals [8]. We envisioned that certain bacteria might have a beneficial effect on plant's growth especially root growth, an important component of phytoremediation. We also hypothesized that the presence of bacteria in the rhizosphere might increase the solubility of Pb in the soil, which may also increase Pb uptake by tall fescue.

Tall fescue (*Festuca arundinacea* Schreb.) is a robust long-lived, comparatively deep-rooted bunchgrass. It is adapted to cool and humid climates and will grow fairly well on soils low in fertility [9]. Fescue is distributed throughout the majority of the United States and it is easy to establish due to its rapid germination and good seedling vigor [9]. This plant is especially well adapted to acid, wet soils of shale origin and produces more forage than other cool-season grasses on soils with a pH of less than 5.5. Tall fescue has also been reported to be an efficient Pb-accumulating plant when coupled with other phytoextraction strategies such as lower pH, and the use of a chelate [10].

The two bacteria used in this study were *Pseudomonas monteilii* (ATCC 700476) and *Wautersia metallidurans* (ATCC 43123). *Pseudomonas monteilii* (ATCC 700476) is a gram-negative, rod-shaped bacterium that is nonhemolytic on blood agar and was isolated from clinical sources. Various rhizosphere bacteria are potential (micro) biological pesticides which are able to protect plants against disease and improve plant yield [11]. Gram-negative rods such as *Pseudomonas* spp. dominate the rhizosphere. The presence and survival of these beneficial rhizobacterial strains are, in contrast to the limited studies of rhizoremediation, presented in detail for processes such as biocontrol of soil borne plant diseases [12, 13], biofertilization, and phytostimulation [14]. Success of these important processes is based on the rhizosphere competence of the microbes [15], which is reflected by the ability of the microbes to survive in the rhizosphere, compete for the exudate's nutrients, sustain sufficient numbers, and efficiently colonize the growing root system [15]. *Wautersia metallidurans* (ATCC 43123) is thought to be resistant to zinc, cobalt, nickel, mercury, cadmium, and produces a zinc-binding protein. In broth, the cells may appear as short to long rods. These soil-borne bacteria with plant-growth promoting effects are generally termed plant growth-promoting rhizobacteria (PGPR) [11, 16].

MATERIAL AND METHODS

Chemical Reagents and Other Materials

Lead nitrate [($Pb(NO_3)_2$], and other chemicals were purchased from Sigma Chemical (St. Louis, MO), and Fisher Scientific (Houston, TX). Delta top soil, humus peat, and seeds of tall fescue were purchased from Hutto's Garden Supply (Jackson, MS). Deepot D40 656 mL planting tubes (referred to hereafter as planting tubes or tubes) and support trays were purchased from Stuewe and Sons, Inc. (Corvallis, OR). Delta top soil and peat were weighed on a Brainweight 1500 scale, Model B1500 (Ohaus Scale corp.).

Dry tissue biomass was determined using a Metler AE260 analytical balance. Reagents were weighed with a Wards Electronic top loading balance (Model 15 W6201). Plant tissue samples were dried in a Precision Thelco Model 18 convection oven at 75º C for approximately 48 hours. Lead concentration of plant tissues were analyzed with a Perkin Elmer Optima 3300 DV Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) and were expressed as µg Pb/g dry weight (ppm). Modified Hoagland's nutrient solution containing buffers and trace elements were prepared in the laboratory.

Soil and Growth Medium Preparation

Appropriate amounts of lead nitrate [$Pb(NO_3)_2$] were mixed with delta top soil and humus peat. Delta top soil is a silt loam soil, a member of the fine, kaolinitic, thermic typic kandiudult soils [17]. Representative samples of the prepared soil mixture were sent to Mississippi State University Soil Testing Laboratory, Mississippi State, MS for determination of its physical and chemical characteristics.

Approximately 550 g of the dry, sieved delta topsoil, peat mixture (2:1 v/v) were placed in a plastic zip lock bag and amended with either 0, 500, 1000, or 2000 mg Pb/kg dry soil mixture using lead nitrate. Deionized distilled water (DDW) was added to the bags to adjust the soil moisture content to approximately 30% field capacity. The bags of soil were left to equilibrate (age) on a laboratory bench in the greenhouse for six months. The bags were occasionally turned and mixed during the incubation period to ensure thorough mixing.

Sterile soils were prepared by placing 550g of each soil mixture with the appropriate metal concentration into separate brown paper bags and autoclaved at 121º C, 15 psi, for one hour on three consecutive days. The autoclaved soil in each bag was dispensed into each sterile 660 mL black plastic planting tubes, and pre-treated, bacterial-inoculated seeds of tall fescue were planted. All treatments were carried out in three replicates.

Preparation and Inoculation of Seeds

Tall fescue seeds were surface-sterilized by shaking in 70% ethanol for one minute then rinsed five times with sterile water. A germination test was conducted for axenic seeds and non-sterile seeds to determine their viability.

Two strains of commercial bacteria were used: *Pseudomonas monteilii* (B1 [Bacteria 1]) and *Wautersia metallidurans* (B2 [Bacteria 2]) based on a viability study previously conducted in our laboratory (data not presented). Following the procedure of Whiting and his colleagues [8], a mixed inoculum of these two bacterial strains was prepared for seed inoculation. One loopful of each bacterial strain was added to 5 mL of sterile 0.85% saline (NaCl) solution in a sterile plastic 15 mL BD Falcon tube and vortexed to suspend the bacteria. Specifically, one mL of this suspension was added to 4 mL of sterile 0.5% methylcellulose solution prepared with 0.85% sterile saline solution to provide a bacterial suspension for inoculation of seeds.

To estimate the number of bacteria in the culture, serial dilutions of each of the bacterial solutions were spread on 1.5% trypticase soy agar (TSA) plates and incubated at 25º C for 7 days. The live bacterial suspension contained a total of 2.52×10^8 colony-forming units (CFU) per milliliter. One set (n=144) of sterile fescue seeds were soaked in the bacterial solution containing B1 (*Pseudomonas monteilii*). A second set (n=144) of sterile fescue seeds were soaked in a bacterial solution containing B2 (*Wautersia metallidurans*). A third set (n=144) of sterile fescue seeds were soaked in bacterial solution containing both B1 and B2. After 20 minutes of soaking, all seeds were removed from the bacterial solutions and dried on sterile filter papers in a laminar-flow cabinet. All subsequent seed transfers from the filter papers to the growth media were performed using flamed, autoclaved forceps in a laminar-flow cabinet.

Planting, Plant Establishment and Maintenance, and

Harvesting

After the seeds of tall fescue were inoculated with *Pseudomonas monteilii* and *Wautersia metallidurans* , they were planted in sterile plastic D40 planting tubes containing sterile or non-sterile metal-contaminated soil. Each sterile planting tube was filled with 550 g of the appropriate sterile Pb-spiked soil mixture (0, 500, 1000, 2000 mg Pb/kg dry soil) previously prepared. Eight of the pre-treated seeds were sown in each of the planting tubes and watered with 10 mL autoclaved water.

Emerged seedlings were thinned out to a desired population density of four plants per tube at 5 days after emergence. In this experiment, each treatment replicate consisted of one tube containing four plants. Any symptoms of metal toxicity (e.g., discoloration, pigmentation, yellowing, stunting) exhibited by plants were noted during the experimental period. Additionally, the position of each D40 tray was rotated one position clockwise each week to prevent any growth discrepancies that may occur due to shadowing by another plant. Plants were maintained at the naturally lit Jackson State University greenhouse throughout the experimental period.

Each planting tube was watered with 10 mL of autoclaved water as needed. After 15 days, the plants were watered with modified Hoagland’s nutrient solution on alternating days. The chelating agent ethylenediaminetetraacetic acid (EDTA) was applied to the soil one week before harvest. The plants were allowed to grow for six weeks. After harvesting, the root and shoot lengths were measured, roots and shoots were separated and roots were washed with DDW to remove any adhering soil particle and debris. The roots and shoots were placed separately in brown paper bags and oven dried at 75º C for 48 hours. The dry weight of roots and shoots were determined using a Metler AE260 analytical balance.

Lead Extraction and Analyses

Lead was extracted from all root and shoot samples using a nitric acid-hydrogen peroxide procedures with slight modifications [18]. Lead contents of digestates were quantified using ICP-OES (Perkin Elmer Optima 3300 DV). In general, experimental units were arranged in a randomized complete block design (RCBD) with three replications. Data were analyzed using SAS V9. Statistical analysis of variance was performed on all data sets. Least significant difference (Fisher's LSD) was used for multiple comparisons between different treatments and control groups. Results of the statistical data were summarized in figures and tables. In this study, a probability of 0.05 or less was considered to be statistically significant.

RESULTS

The delta top soil used in this study was classified as a silt loam soil, belonging to the family of fine, Kaolinitic, thermic typic Kandiudult soils [17]. The soil had a sandy loam texture with a cation exchange capacity (CEC) of 17.6 and a pH of 6.3. The extractable magnesium was considered to be very high at 726 lbs/acre.

Tall fescue plants grown in sterile Pb-contaminated soils with treatments of EDTA and bacteria (previously described) indicated that shoot Pb concentrations were highest in treatments of 2000 mg Pb/kg dry sterile soil (Figure 1). Shoot Pb concentrations of tall fescue grown in non-sterile soil followed the same trend as shoots of tall fescue grown in sterile soil (Figure 2).

Figures 3 and 4 show the root Pb concentrations of tall fescue plants grown in sterile and non-sterile soils, respectively. Root Pb concentrations in sterile soils were highest in treatments using bacteria alone without the addition of EDTA at 2000 mg Pb/kg dry soil treatment, though not significantly so. Root Pb concentrations of plants inoculated with B2 and B1 + B2, and were significantly higher than treatments with EDTA + B1 and EDTA + B2 +B2. (Figure 3).

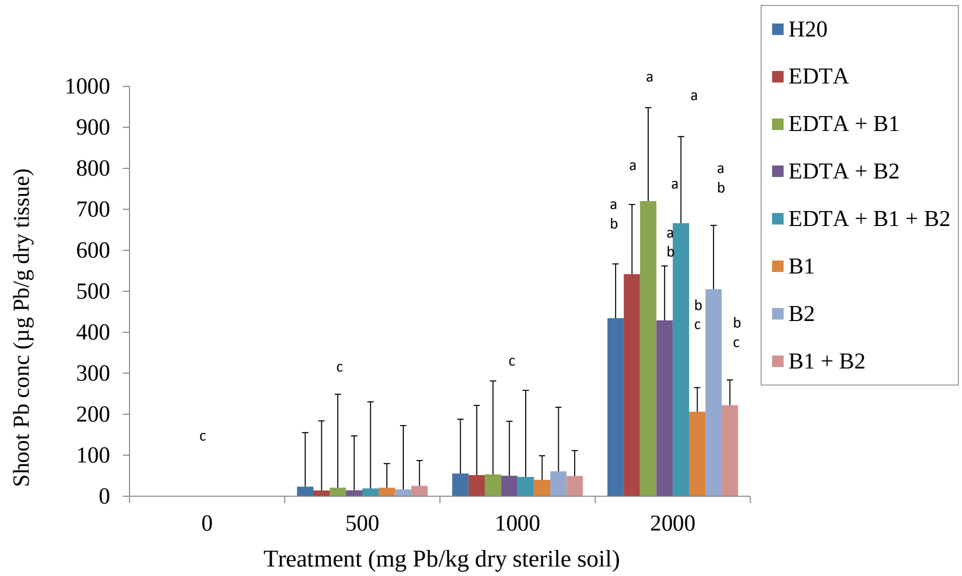

Figure 1. Effects of lead and bacteria on shoot Pb concentrations (µg Pb/g dry wt) of tall fescue grown in sterile soils. Values and error bars represent means and standard errors of 3 replicates. Treatments with common letters do not differ significantly from other treatments according to Fisher's LSD ($p < 0.05$).

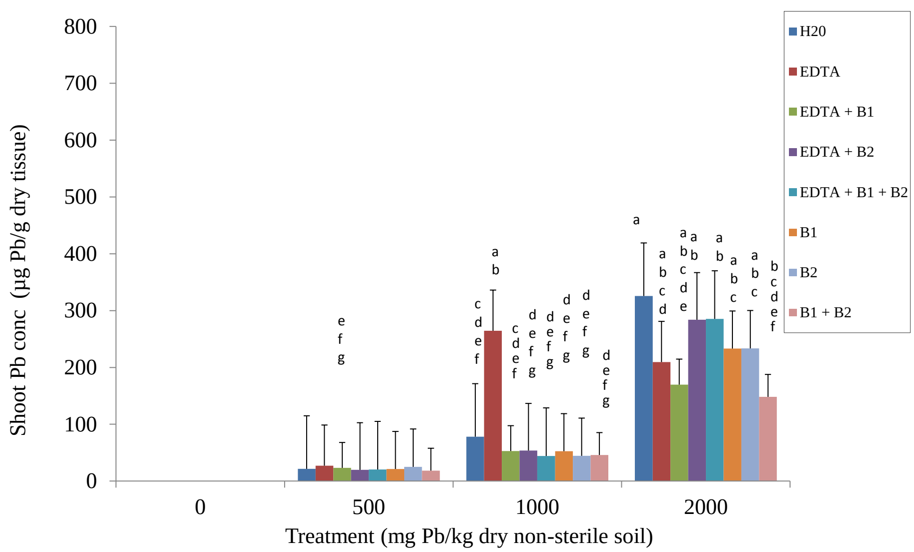

Figure 2. Effects of lead and bacteria on shoot Pb concentrations (µb Pb/g dry wt) of tall fescue grown in non-sterile soils. Values and error bars represent means and standard errors of 3 replicates. Treatments with common letters do not differ significantly from other treatments according to Fisher's LSD *($p < 0.05$)*.

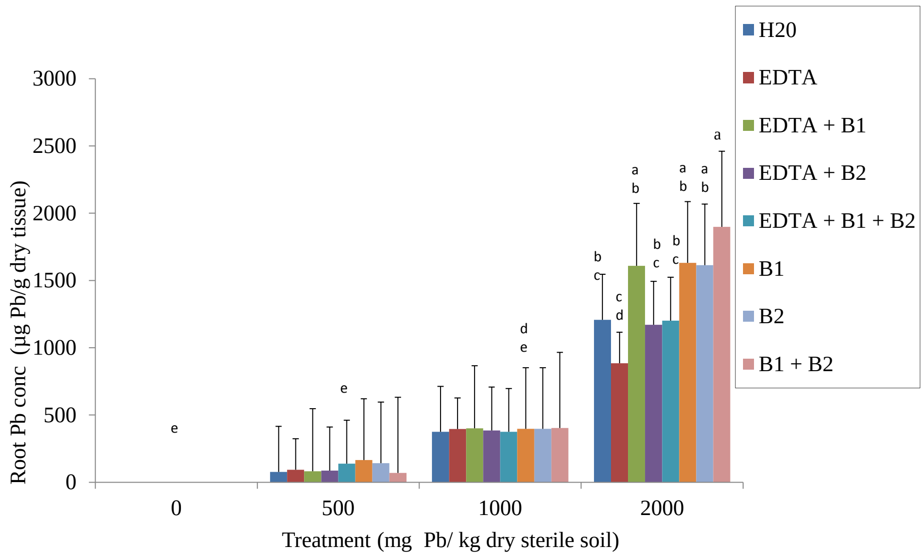

Figure 3. Effects of lead and bacteria on root Pb concentrations (µg Pb/g dry wt) of tall fescue grown in sterile soils. Values and error bars represent means and standard errors of 3 replicates. Treatments with common letters do not differ significantly from other treatments according to Fisher's LSD ($p < 0.05$).

Root Pb concentrations of tall fescue that were grown in non-sterile soils were generally higher in treatments with bacteria + EDTA as compared to bacterial treatments without EDTA (Figure 4). The highest concentrations were observed in plants grown in non-sterile soil with treatments of EDTA and B1 + B2 treatments.

Shoot and root biomass of tall fescue plants grown in sterile soils were lowest at 2000 mg Pb/kg dry soil. Conversely, shoot biomass was highest in 1000 mg Pb/kg dry soil across all treatments (Figure 5). On the other hand, shoot biomass of tall fescue grown in non-sterile soil were generally greater in plants exposed to 500 and 1000 mg Pb/kg non-sterile soil (Figure 6) compared to the control and 2000 mg Pb/kg treatments

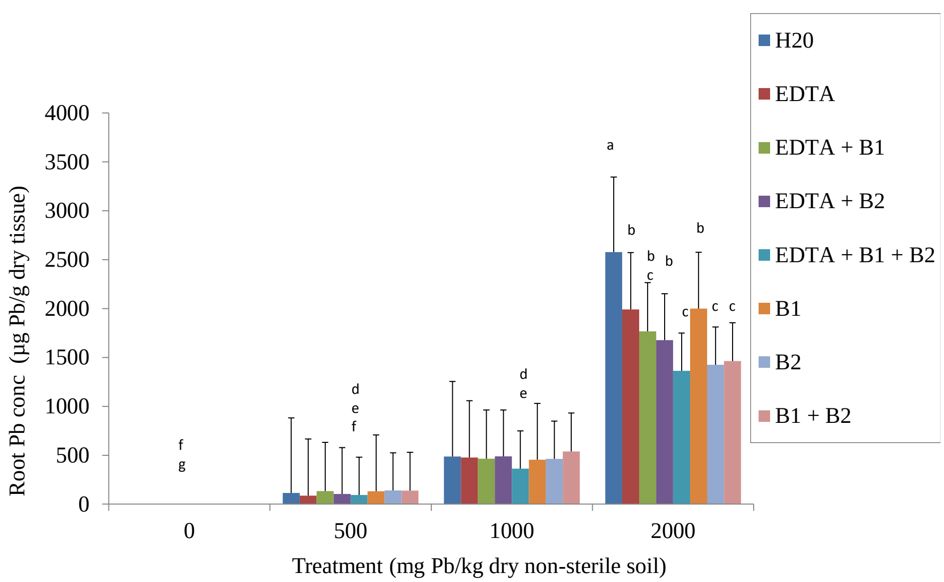

Figure 4. Effects of lead and bacteria on root Pb concentrations (µg Pb/g dry wt) of tall fescue grown in non-sterile soils. Values and error bars represent means and standard errors of 3 replicates. Treatments with common letters do not differ significantly from other treatments cording to Fisher's LSD ($p < 0.05$).

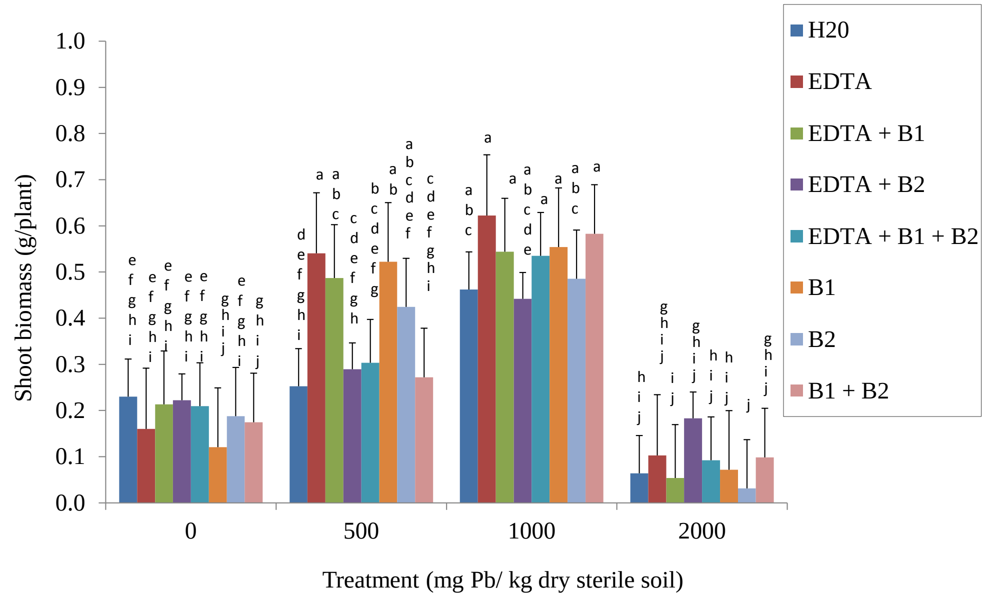

Figure 5. Effects of lead and bacteria on shoot biomass (g/plant) of tall fescue grown in sterile soils. Values and error bars represent means and standard errors of 3 replicates. Treatments with common letters do not differ significantly from other treatments according to Fisher's LSD ($p < 0.05$).

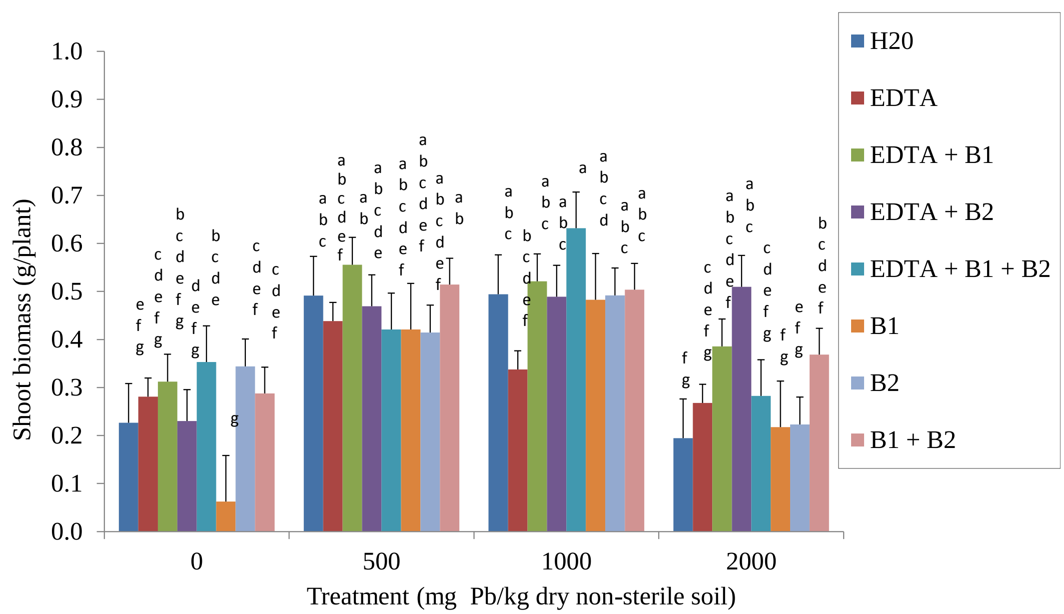

Figure 6. Effects of lead and bacteria on shoot biomass (g /plant) of tall fescue grown in non-sterile soils. Values and error bars represent means and standard errors of 3 replicates. Treatments with common letters do not differ significantly from other treatments according to Fisher's LSD ($p < 0.05$).

Root biomass of tall fescue grown in sterile soil (Figure 7) were higher in treatments of 500 and 1000 mg Pb/kg sterile soil and lowest in treatments of 2000 mg Pb/kg dry sterile soil. In Pb soil treatments of 2000 mg Pb/kg dry sterile soil, root biomass was lower than the control across all treatments. Our research showed that the root biomass of tall fescue grown in non-sterile soil was highest in the control and lowest in treatments of 2000 Pb/kg dry non-sterile soil. Tall fescue grown in soil treated with bacteria had generally higher root biomass than those plants treated with no bacteria (Figure 8).

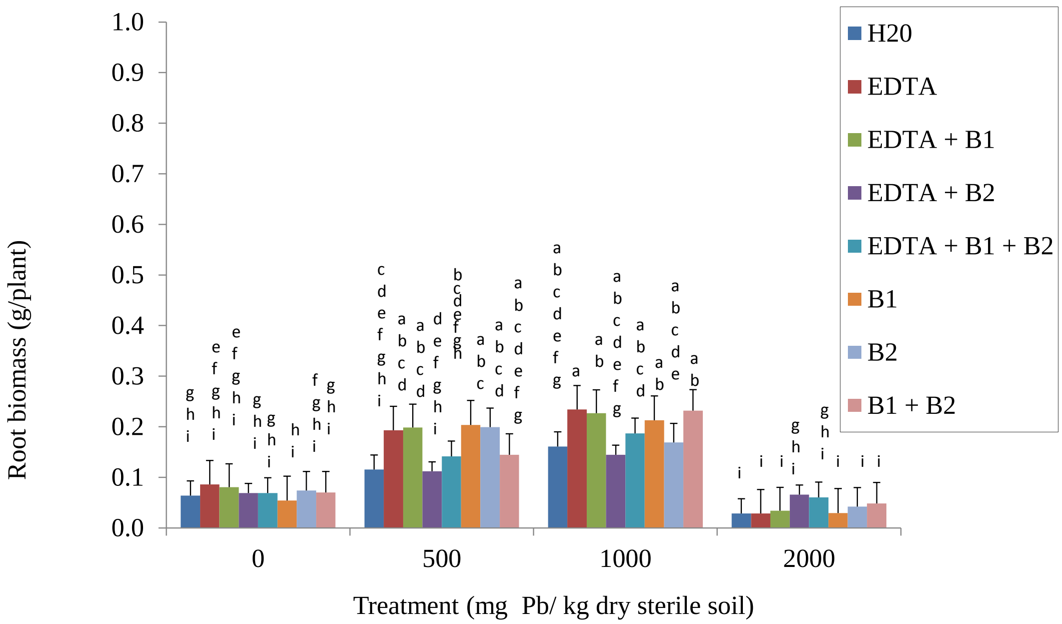

Figure 7. Effects of lead and bacteria on root biomass (g /plant) of tall fescue grown in sterile soils. Values and error bars represent means and standard errors of 3 replicates. Treatments with common letters do not differ significantly from other treatments according to Fisher's LSD ($p < 0.05$).

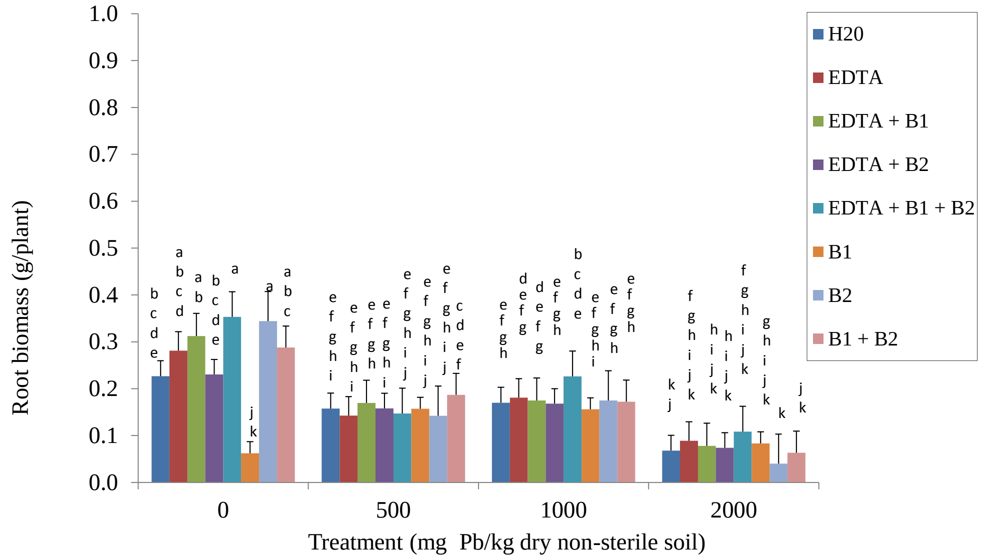

Figure 8. Effects of lead and chelates on root biomass (g /plant) of tall fescue grown in non-sterile soils. Values and error bars represent means and standard errors of 3 replicates. Treatments with common letters do not differ significantly from other treatments according to Fisher's LSD ($p < 0.05$).

DISCUSSION

Soil pH is one of the most influential parameters controlling the mobility and availability of metals in the soils. In our greenhouse study, soil pH before planting was 6.3 and decreased with increasing amounts of soil-applied Pb. After chelate application, the soil pH remained in the range of 5.5 - 6.5 (data not shown), a range not unusual in chelate-assisted phytoextraction [19]. Unlike nitrilotriacetic acid (NTA), another strong complexing agent, which exhibits a significant pH dependency, ethylene diamine tetraacetic acid (EDTA), the chelating agent used in this study, is generally pH-insensitive and can remain relatively constant over a broad pH range (4.9 - 11.3) as demonstrated by Peters and Shem [19].

In general, the chemistry of metal interaction with soil matrix is central to the phytoremediation concept. While the soil used in this study was high in phosphorus, potassium and zinc; and very high in magnesium, these were, nonetheless, essential plant nutrients. Overall, the parameters of the soil used in this study were well within limits for our objectives.

A relevant control soil which accurately represents the polluted soil in its pristine state is rarely available. The polluted soil is often contaminated with more than one metal, and except around point sources of pollution, a gradient of metal concentrations is rarely available. Ideally, the control soil should be the same soil lacking in only the additions of metals, but in reality this is hardly ever achieved [20].

This study was therefore carried out using a laboratory prepared Pb-spiked soil that had been aged for six months. This approach is simplistic in that it bears little relation to most "real-life" contaminated soils where metal concentrations are gradually built up due to atmospheric deposition, fertilizers and contaminated wastes [20].

Although the total Pb concentration in many contaminated soils may be high, the bioavailable Pb fraction (water soluble and exchangeable) is usually very low due to the strong association of Pb with organic matter, Fe-Mn oxides, and clays, and precipitation as carbonates, hydroxides, and phosphates [21].

Miller [22] performed the classical sequential extraction by Tessier and his colleagues [23] on a laboratory prepared Pb-spiked soil that had been prepared using the same method that we used in this study and reported that approximately 17% of the Pb is expected to be bioavailable for plant root uptake. The greater percentage of Pb was concentrated in the residual and exchangeable fractions of this soil type [22, 24]. The residual fraction (fraction 5) can be thought of as what is left over after the first four fractions have been removed. It contains primary and secondary minerals which may hold trace metals within their crystal structure [23]. These metals are not expected to be released under normal environmental conditions and therefore, not bioavailable for plant uptake.

Our results indicate that *Wautersia metallidurans* ATCC 43123 and *Pseudomonas monteilii* ATCC 700476 may have facilitated the growth and Pb tissue uptake of tall fescue (*Festuca arundinacea)* and therefore may increase the phytoremediation efficiency especially at Pb soil concentrations less than 2000 mg Pb/kg dry soil.

Soil rhizobacteria may have also directly influenced metal solubility by changing heavy metal speciation in the rhizosphere of tall fescue, similar to what has been reported earlier by Jing et al. [25]. Study of the roles of mycorrhiza in metal speciation in the rhizosphere and the impact on increasing host plant tolerance against excessive heavy metals in soil showed that speciations of Cu, Zn, and Pb changed significantly in the rhizosphere of arbuscular mycorrhiza.

Tall fescue plants survived the Pb soil treatments of 500, 1000, and 2000 mg Pb/kg dry soil but displayed stunted growth and lower biomass especially at the highest level of soil Pb treatments. This apparently indicated that *Wautersia metallidurans* and *Pseudomonas monteilii* could protect their host plants from the phytotoxicity of excessive Pb. A similar finding was reported by Jing and his colleagues [25] who found that mycorrhiza may have played a part in protecting plants from copper, zinc and lead by changing the speciation from bioavailable to the non-bioavailable form. They further reported that copper and zinc accumulation in the roots and shoots of mycorrhiza-infected plants were significantly lower than those in the non-infected plants, which might also suggest that mycorrhiza efficiently restrict excessive copper and zinc absorptions into the host plants [25]. Our data further suggest that microbial inoculation may have increased biomass of fescue plants especially at lower (500, and 1000 mg Pb/kg dry soil) Pb soil concentrations.

There has been at least one report that EDTA alone was more effective than microbial inoculation in increasing the concentrations of all metals in corn and sunflower plants [26]. We, however, found that EDTA in combination with *Wautersia metallidurans* and *Pseudomonas monteilii* increased lead concentrations in wheat and tall fescue in both sterile and non-sterile soils that were used in this study.

Chemicals such as EDTA and acetic acid applied sequentially can access different soil components which can then bind and/or release lead when subjected to certain conditions [27–29]. While the addition of synthetic chelates has been shown to stimulate the release of metals into soil solution and enhance the potential uptake of metals into roots, it has been documented that the addition of EDTA tends to increase the risk of spreading contamination due to high solubility of Pb-chelate complex [30]. Recent research aims are to eliminate this risk by implementing alternative chelate formulations and innovative agronomic practices.

It has been shown that in plants growing in Pb contaminated soils, Pb translocation from roots to shoots was less than 30% even for the best Pb-translocating variety. Moreover, actively growing roots provide a barrier which restricts the movement of Pb to the above ground parts of the plants [31]. This point was further substantiated by previous findings of Kumar and his colleagues [32] which showed that significant Pb translocation to the shoots of Indian mustard and other species was observed only at relatively high concentrations of Pb in the hydroponic solution and only after the Pb-binding capacity of the roots was partially saturated.

Results from this study showed that the highest Pb translocation index of tall fescue was observed in non-sterile soils and at relatively high concentrations of Pb with the addition of EDTA.

CONCLUSIONS

The success of phytoextraction process depends upon both shoot biomass and shoot metal concentration. Therefore, the potential effectiveness of each plant for phytoremediation was evaluated through determination of the metal accumulation inside the plant i.e. (metal concentration x plant dry weight). The heavy metal removal by plant shoots is an important index which is useful for the practical application of treatments. Our results showed that there were significant differences in the uptake of the heavy metals exhibited by plants exposed to various treatments. Further, we found that the biomass of tall fescue shoots and roots (Figures 5-8) decreased dramatically when grown in soils spiked with 2000 mg Pb/kg dry soil.

We found no indication that the two bacteria species used in this study, *Pseudomonas monteilii* and *Wautersia metallidurans*, had an effect on the growth of tall fescue plants that were grown in sterile and non-sterile soils. While it was generally observed that the highest amounts of Pb uptake was evident in plants treated with a combination of EDTA and rhizobacteria, more research is needed to elucidate the combined effects of rhizobacteria and EDTA on uptake and growth of plants grown especially in soils contaminated with elevated levels of Pb. We conclude that further analyses and technical refinements are needed on the use of rhizobacteria in phytoextraction.

ACKNOWLEDGEMENT

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IMPACT OF ACT COMPOSITE AND ACT SUBSCALE SCORES ON UNDERREPRESENTED MINORITY STUDENT PERSISTENCE IN STEM

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ABSTRACT

The growth and persistence of underrepresented minorities pursuing STEM degrees continue to remain stagnant in the United States. Such an issue keeps the United States STEM workforce at a dismal 5%, with underrepresented minority students representing approximately 1/20th of the total STEM workforce population. Key factors must be identified in the persistence of underrepresented minority students both before and in-situ of their college experiences in order to diversify and strengthen the STEM workforce which accounts for approximately 50% of the United States economic development. A one-way ANOVA statistical analysis was performed on a sample of 161 underrepresented minority students to examine whether or not a variance existed between the ACT composite and ACT subscale scores of students who persisted versus those that did not in STEM fields. Using the .05 alpha level, student persistence as a result of ACT composite score was found to be statistically significant with a reported *p* value of .004. ACT subscale scores math, English and science were also statistically significant with p values of .004, .000 and .007, respectively. Reading on the other hand had a *p* value of .09 which illustrates that there is no statistically significant difference. Underrepresented minority students with higher ACT composite scores and high ACT math, English and science scores persisted more in STEM curricula compared to those who did not.

Keywords: Persistence, STEM, ACT, Underrepresented Minorities

INTRODUCTION

Development of strategic plans to increase the United States STEM workforce remains a complex issue. At the heart of the issue remains the need to increase diversity in STEM along with underrepresented minority student (URMs) success at post-secondary institutions. Fifty percent of the economic development within the United States is driven by the innovation of the STEM workforce which is comprised of approximately 5% of the total workforce, with URMs accounting for approximately 1/20th of this population. Many key factors such as high school cumulative GPA, ACT, SAT, advanced high school math and science AP and honors courses and pre-college exposure programs are currently underway to understand their influences of the persistence of students in STEM disciplines [1]. However, there is not much literature on the ACT and persistence of URMs in STEM. ACT scores are currently being used to indicate whether or not students are college ready. The ACT has developed several benchmarks to indicate college readiness across several disciplines such as English, biology, algebra, and social science courses stated by Philips (2013). Philips also went on to report that students who meet benchmarks set forth by the ACT have a 50% chance of earning a B or better and approximately a 75% chance of earning a C or better in corresponding college courses [2]. Bettinger (2010) found that math was a strong predictor of STEM persistence [3].

BHEF (Business Higher Education Forum, 2010) reported a similar trend to Bettinger and went on to note that approximately one in every five twelfth graders possess a high interest in majoring in a STEM discipline and are proficient in mathematics [4]. In 2015, Meyer employed t-tests to show that the effects of the math ACT composite and ACT math subscale scores were significant (p=.001) for students who persisted in STEM disciplines [5]. This paper aims to examine the persistence of URMs in STEM via ACT composite and ACT subscale scores by employing the question "Are URMs with higher ACT Composite and ACT subscale scores more likely to persist in STEM?"

METHODS AND DATA

Descriptive statistics was used to gather the mean and variance in the ACT composite and ACT subscale scores of 232 URMs in Summer Bridge Programs for the years of 2008 to 2014 at a predominately white institution (PWI). Within this study, persistence was defined as students who matriculated through their junior year of college. The demographics of the participants for this study consisted of 84 females (36%) and 148 (64%) males. Ninety-three percent of the population was 18 years old, while 94% were African Americans and the other 6% consisted of Asians, Hispanics, Latinos, and Multiracial students. Of the 232 students involved in the Summer Bridge program, data for the ACT composite and ACT subscale scores were recorded for 161 of those students since the URMs from the 2013 and 2014 cohort groups had not complete their junior year. The number of participants in each cohort group is shown in Table 1.

This paper aims to examine the persistence of URMs in STEM via ACT composite and ACT subscale scores by employing the question "Are URMs with higher ACT Composite and ACT subscale scores more likely to persist in STEM?" A one-way ANOVA statistical analysis was employed in Table 2 and Table 3 to compare ACT composite score to ACT English, math, reading and science subscale scores. Any statistically differences or similarities in the means of each of the groups are easily shown using a one-way ANOVA.

RESULTS AND DISCUSSION

Within this section, results of the students' ACT composite and ACT subscale scores are presented and discussed. Mississippi State University's Summer Bridge Program accepted students that had an ACT score that ranged from 15 to 36. The average ACT score for the 2008 through 2014 Summer Bridge cohorts was 23 which was 2 points higher than the national average of 21 during this time span. The average ACT subscale scores for the population of students by category are 25, 23, 24 and 24 for English, mathematics, reading and science, respectively.

Table 1 *Number of Participants in Each Cohort Group*

Cohort	*Number*
2008	20
2009	18
2010	48
2011	44
2012	31
2013	37
2014	34
Total	232

A series of one-way ANOVAs were performed. A descriptive analysis of the results are shown in Table 2 and Table 3. The results of the analysis of ACT composite scores revealed a statistically significant difference, $F(1,159) = 8.76$, $p = .004$, $\eta = .05$, between students who persisted in a STEM major versus those who did not. Students who persisted ($n = 80$, $M = 24.10$, $SD = 3.04$) had a higher mean ACT composite score in comparison to students that did not persist in a STEM major (n = 81, M = 22.59, SD = 3.04). Likewise, the results of analyses for ACT subscale scores revealed three statistically significant differences. Statistically significant differences between the mean ACT subscale scores for mathematics (F (1, 153) = 8.61, p = 004, η = .05), English (F (1, 153) = 14.14, p = .000, η=.09) and science (F (1, 153) = 7.41, p = .007, η= .05) of students who persisted and students who did not persist were observed. In each case, the mean subscale scores of the students who persisted were higher than the mean subscale scores of students who did not persist. The difference in the reading mean subscale score of the two groups was not statistically significant, F(1, 153) = 2.96, p =.09. Accordingly, to and the research question posed, a statistically significant difference does exist in the mean composite, math, English, and science ACT scores between students who persisted in STEM majors and students who did not persist in STEM majors. However, the difference in the mean reading subscale scores between the two groups was not statistically significantly different. Table 3 displays the descriptive statistics of the composite and subscale scores for the students who persisted and the students who did not persist.

Table 2 *ACT Descriptive Statistics by Population and Cohort*

Group	ACT Descriptive Statistics									
	Composite		English		Mathematics		Reading		Science	
	M	SD	M	SD	M	SD	M	SD	M	SD
Population	23.47	3.30	24.80	4.32	23.22	3.71	24.05	4.65	23.76	3.06
2008	25.30	3.20	27.40	3.68	25.50	3.41	26.05	3.82	24.95	2.82
2009	23.83	2.38	25.06	3.73	24.61	2.28	24.11	3.53	23.56	2.36
2010	23.41	3.71	24.96	4.88	22.74	4.31	23.74	4.98	23.44	3.61
2011	22.50	3.03	23.35	3.29	22.54	3.72	22.98	4.04	23.61	3.32
2012	22.87	3.13	23.57	3.84	22.39	3.84	23.82	4.62	23.61	2.74
2013	23.62	3.23	25.28	4.35	23.17	2.90	24.71	5.49	23.83	3.16
2014	23.97	3.38	25.32	5.07	23.39	3.82	24.71	4.85	23.84	2.49

Table 3

ACT Composite and Subscale Score Descriptive Statistics

		ACT Scores				
Group		Composite	English	Mathematics	Reading	Science
Persisted	Mean	24.10	25.81	24.09	24.49	24.40
	N	80	77	77	77	77
	Std. Deviation	3.42	4.38	3.84	4.32	3.08
Did Not Persist	Mean	22.59	23.39	22.31	23.28	23.05
	N	81	78	78	78	78
	Std. Deviation	3.04	3.60	3.73	4.45	3.10
Total	Mean	23.34	24.59	23.19	23.88	23.72
	N	161	155	155	155	155
	Std. Deviation	3.31	4.17	3.87	4.41	3.15

CONCLUSIONS

In conclusion, it was shown that URMs who persisted had ACT composite and ACT subscale scores higher than students who did not persist. However, the ACT reading subscale was the only subscale score that did not have a statistical impact on URM student persistence in STEM. Results are quite similar to the findings of Bettinger (2010) and Philips (2013). Although the national average continued to hover around 21 for 2008 to 2014, the 2014 ACT Profile Report illustrated that minorities had achieved a mean ACT composite score less than 20 during this that time span. According to the 2014 ACT Profile Report, URMs have scored less than 20 in all of the subscale scores during that time frame as well. In contrast, this report has found that participants of the Summer Bridge Program during the 2008 to 2014-time frame had a higher mean ACT Composite score of 23, with mean ACT subscale scores of 25, 23, 24 and 24 for English, math, reading and science, respectively.

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MECHANICAL PROPERTY IMPROVEMENT OF CARBON FIBER REINFORCED POLYMERIC COMPOSITES BY FILLER DISPERSION: A REVIEW

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ABSTRACT

Fiber reinforced composites are vital to the development of low cost, strong lightweight materials. Due to widespread usage and safety impacts on the automotive and aerospace industries, it is important to understand how these materials age and perform over time. Despite the advantages, there are concerns regarding the cost and long-term durability of these composites especially when it comes to performances under critical and varying conditions. With the implementation of magnetostrictive particles to the system, internal flaws can be sensed while the net strength of the composite is increased. This paper will provide a preliminary study on how particles affect the overall mechanical properties of the polymeric composite with embedded particles.

INTRODUCTION

Due to high specific strength ratios, high stiffness to weight ratios, high creep resistance, high toughness, corrosion resistance and tailorable properties, composites are ideal materials for aerospace and automotive structural applications [1]. Researchers are currently investigating additive materials such as silica particles, alumina particles, nanotubes, nanofibers and many other additives to see how they impact the mechanical properties of composites. However, one material that is commonly used today is Terfenol-D, a magnetostrictive material. Terfenol-D is an alloy composed of terbium, dysprosium and iron developed by the Naval Ordnance Laboratory in 1971. The magnetostrictive material is currently being embedded at the interface of lamina layups and within polymer resins as second phase particles. The material has the ability to change its shape in the presence of a magnetic field. It has been found that Terfenol-D can strain up to 2000 microstrain in a field of approximately 160 ampere per meter. This makes the material a powerful tool for developing composites that are capable of performing as transducers [2]. The Villari Effect governs the shape change of the particles in the presence of magnetic fields. As a magnetic field is applied, particles expand due to a mechanical strain imposed by the field. This makes the material a great actuator. The mechanical strain also induces a voltage in the material, in turn, forcing the particles to become exceptional sensing materials [3-5].

Advanced composites such as the carbon fiber reinforced polymeric composites (CFRP) with embedded Terfenol-D are termed hybrid composites. Hybrid composites are often referred to as composites containing more than one type of filler and/or more than one type of matrix. They are commonly used for improving the properties and/or lowering the cost of conventional composites. Particles have been used as additional fillers in continuous CFRP's in order to increase the strength, stiffness, toughness, and/or fatigue resistance. As the fiber reinforced content is increased so is the net strength of the composite system. The primary energy-absorbing mechanism for these composites is a crack-pinning process [6]. Hybrid composites perform better than aluminum and steel in applications where cyclic loads are encountered leading to potential fatigue failure. With CFRP's, the continuous fiber design has been widely used for structural applications due to their excellent mechanical properties. However, their matrix dominant properties are much weaker than their fiber-dominated properties, thus limiting the benefits of conventional composites. In addition, it is known that composites such as the CRFP exhibits lower longitudinal compressive strength, a matrix-dominated property, than tensile strength. Thus, it is desirable to enhance the potential of structural applications of composites [7].

RESULTS AND DISCUSSION

A. Loading, Young's modulus, Flexural Strength, Rule of Mixtures (ROM)

In-plane stress has been the focus in the development of fiber composites. In numerous practical applications, high stress may be applied on the FRP resulting in crack propagation through fiber matrix interfaces. Therefore, a strong adhesion between fiber and matrix, high strength and high toughened matrix are desired. The main purpose of hybridization is to optimize the properties of the matrix for high performance fiber reinforced composites. [8]. The entire composite, along with its reinforced material is governed by the rule of mixtures (ROM). The equations for the ROM is shown in Fig. 1 (a) and (b) above. For longitudinal loading, isostrain conditions are assumed where deformation of the fibers and matrix are identical. In this assumption, there are stiff and strong mechanical properties. For transverse loading, isostress conditions are assumed where the matrix and fibers are under the same stress ($\sigma_c = \sigma_f = \sigma_m$). In this assumption, the mechanical properties are considered soft and weak. Fig. 1 (c.) represents a typical unidirectional stress-strain curve for a unidirectional composite where Poisson's ratio and Young's modulus are calculated from ϵ_T/ϵ_L and σ/ϵ_L, respectively [8].

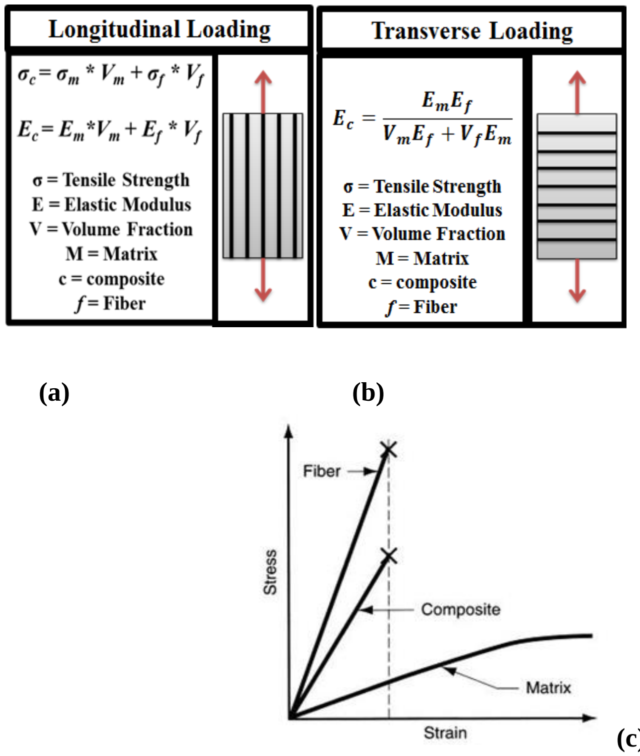

Fig. 1—(a) Longitudinal and (b.) transverse loading on fiber reinforced polymers (FRP) composites. Transverse (c.) Stress-strain diagram for composite material.

Fig. 2 and Fig. 3 show that Young's modulus and flexural strength for both increase as a result of particle reduction for the CFRP and CFHRP. At higher volume fractions of the overall composite, it is evident that Young's modulus falls below results obtained from the ROM. This phenomenon is due to the overlapping fiber-fiber interactions that reduce the interfacial surface area between the fibers and the matrix, resulting in an effective fiber volume fraction.

In Fig. 3, the flexural strength also has an effective fiber volume fraction according to the ROM. Improvement of the flexural strength by the addition of particle filler into the matrix were suggested for the following reasons. (a) Young's modulus of the second phase dispersed particles is higher than that of the matrix and thus stress transfer from the matrix to the particles will take place. (b) Strong interfacial bonding between the fiber and matrix also contributes to higher flexural strength. (c) Dispersed filler particles act as mechanical interlocking between fiber and epoxy matrix which creates a high friction coefficient. Finally, (d) a mixed mode of fracture (flexural and shear) occurred under the bending-load conditions. As a result, the strength of the composites is increased. In Fig. 4, the load-displacement curve shows a sharp increase and then falls off abruptly thereafter. However, it is evident that the initial load and crack arrest are higher in CFHRP composites which are suggested for high energy absorbing mechanisms. The interlaminar shear strength (ILSS) in Fig. 5 is used to determine the matrix/fiber adhesion. The ILSS is improved due to an increase in the vol% of the fiber content as shown in the figure. In Fig. 4, it is evident that there is also an effective vol% of fiber content.

Fig. 6 shows a continuous increase in the fracture toughness of both the CFRP and CFHRP with increasing vol% of fiber content along with a 40% increase in the toughness with the addition of the micro- and nanoparticle fillers. This work was in agreement with the work done previously by Srivastava and Harris in 1994. The increase in fracture toughness is a direct result of various crack growth processes as investigated by Deborah D.L. Chung [6] and K. Asano et. al. [10]. The primary crack might move around the filler particles or the crack tips become less sharp, thus increasing the fracture toughness [8,9].

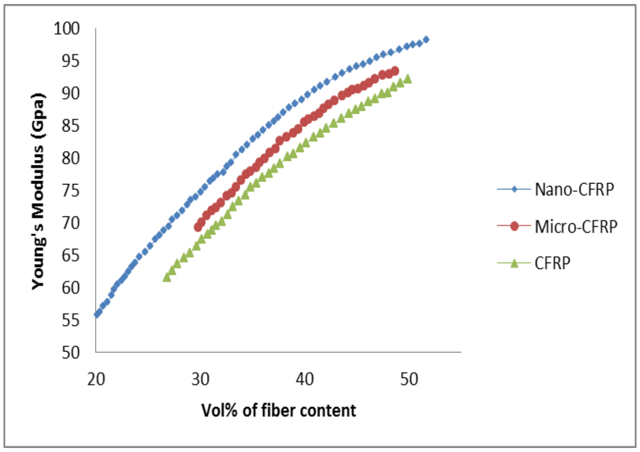

Fig. 2—Young's modulus vs. vol% fiber content [8].

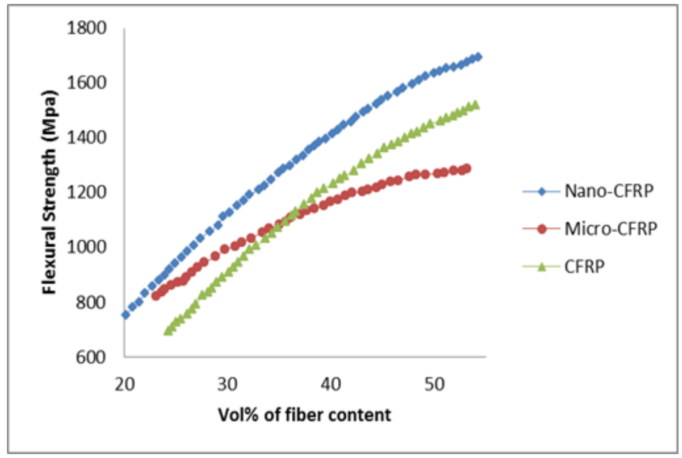

Fig. 3—Flexural strength vs. vol% of fiber content [8].

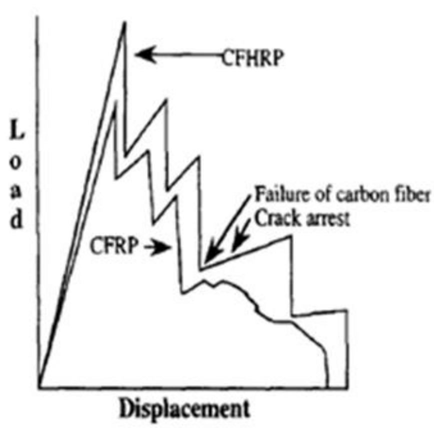

Fig. 4—Typical load deflection pattern of CFRP and carbon fiber hybrid reinforced polymers (CFHRP) [8].

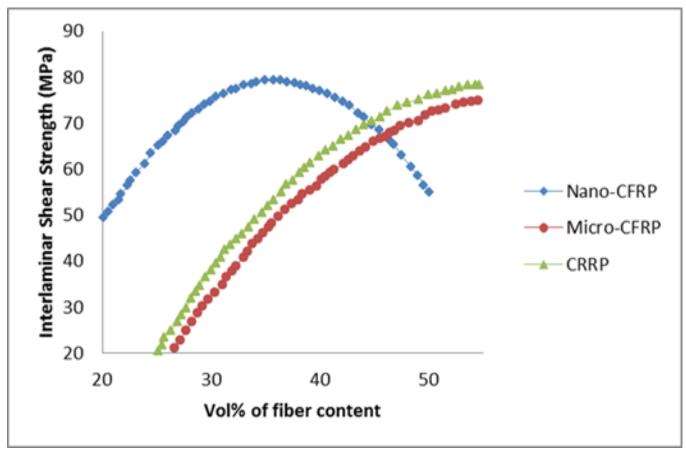

Fig. 5—Interlaminar shear strength vs. Vol% fiber content [8].

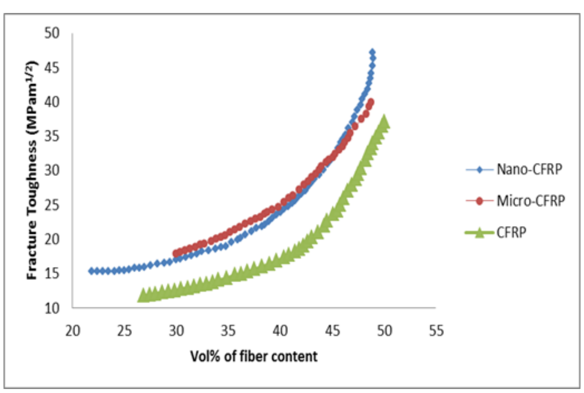

Fig. 6—Fracture toughness vs. Vol% of fiber content [8].

B. Crack Propagation

When second phase particles in the form of very small uniformly distributed particles are added to a matrix, the strength of the matrix is increased due to the particles blocking dislocation motion extremely well, thus strengthening the material. This phenomenon can be examined in Fig. 7 (b) and Fig. 8 (b.).

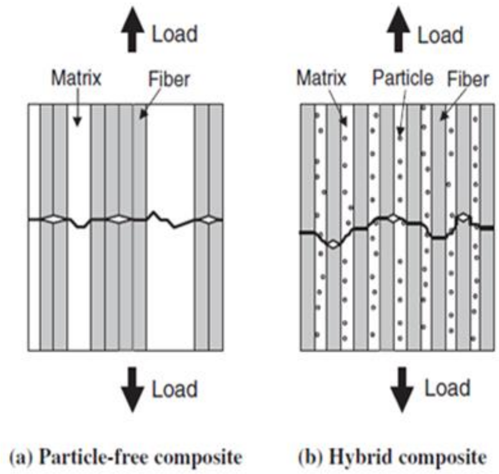

Fig. 7—Longitudinal fracture in composites [10].

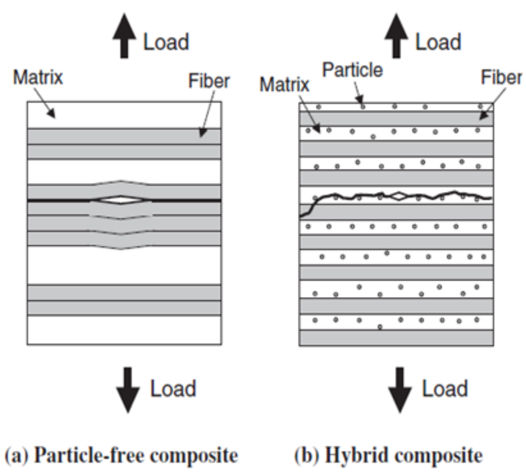

Fig. 8—Transverse fracture in composites [10].

In longitudinal fracture surfaces post tensile testing in Fig. 9 below, no fiber pullout can be seen at 293K. This indicated that the interfacial bond between the fibers and the matrix was relatively strong. Observations of the longitudinal surfaces post tensile testing at 623 K in Fig. 10 exemplified the same SEM morphology as Fig. 9. It is evident in Fig. 7 (b.) that the crack propagates around the particles in tensile loading where the fibers are parallel to the epoxy. When a fiber ruptured, neighboring fibers in close contact with that fiber were broken easily due to the stress concentration occurring in neighboring fibers. When the touching fibers ruptured, the fracture surface was assumed to be flat. Under this longitudinal fracture morphology, the strengthening effects of the particle-free composite could not be fully displayed. In contrast, the matrix of the hybrid composites with uniformly distributed fibers, relieved the stress concentration after the rupture of a single fiber. Thus the stress concentration does not easily occur, and each individual fiber is subject is subject to the stress. As a consequence, irregular surfaces are formed in the composite as can be seen in Fig. 11 (b.). Such irregular surfaces are a direct result of the particles reducing the contact between fiber-fiber interactions, making an easy stress transmission between the fiber and the matrix. This is why the longitudinal strength of the composite is much higher than that of the particle-free composite at any temperature as can be seen in Fig. 13 (a.) and (b.)[8-11].

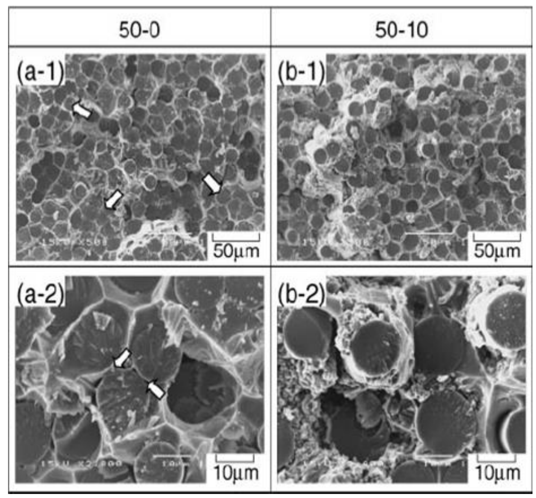

Fig. 9—Scanning electron micrographs (SEM) of longitudinal fracture surfaces of composites after tensile test at 293 K (50-0: 50% fibers and 0% particles, 50-10 refers to 50% fibers and 10% Particles) [10].

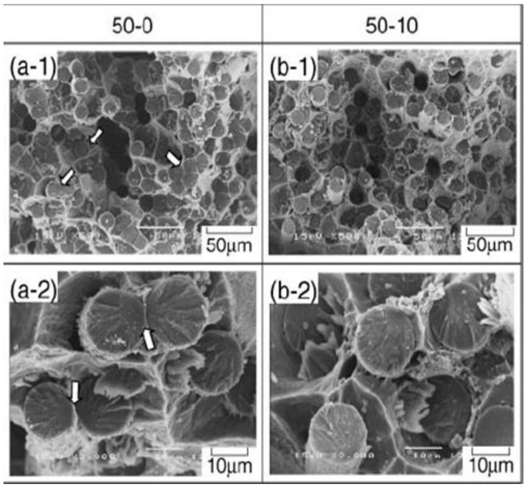

Fig. 10—SEM photographs of longitudinal fracture surfaces of composites at 623 K [10].

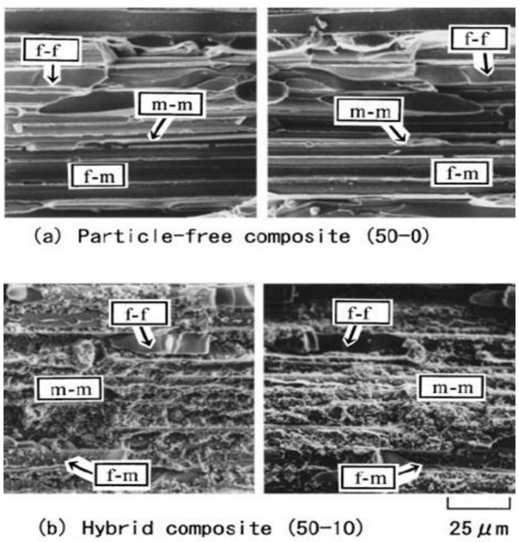

Fig. 11—SEM photographs of transverse fracture surfaces of composites after tensile test at 293 K (f-f: Fracture at fiber, f-m: Fracture at fiber-matrix interface, m-m: Fracture at matrix) [10].

The transverse tensile strength of the particle-free composite decreased as v_f increased. The composite was also improved by the introduction of the particles. The increase of the strength by the introduction of particles was particularly large up to about 423 K, above which the increase was small due to the low strength of the matrix. However, the strength of the hybrid composite was slightly larger than that of the particle-free composite at 623 K [10].

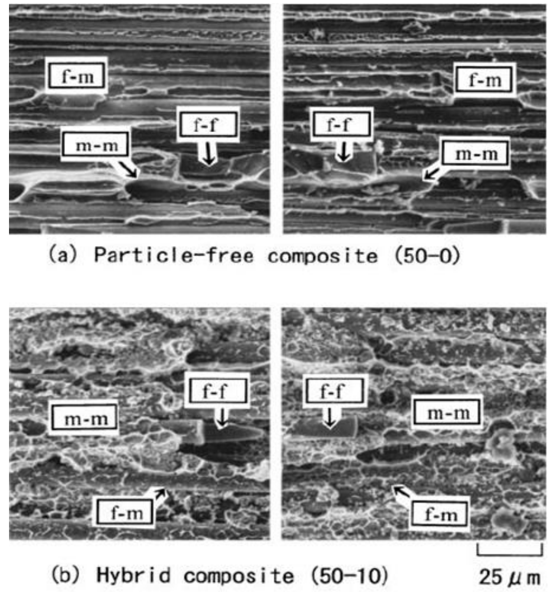

Fig. 12—SEM photographs of transverse fracture surfaces of composites after tensile test at 623 K [10].

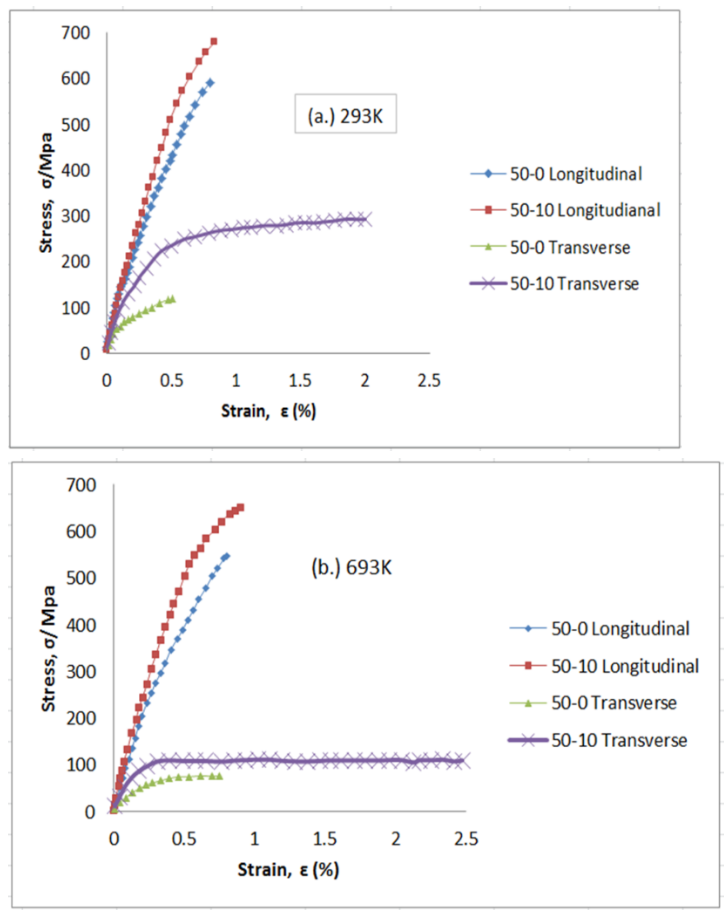

Fig. 13—Stress-strain curves of Alumina Fiber-Reinforced

composites (v_f = 50%) tested at (a.) 293 K and (b.) 623 K [10].

C. Compression Strength

Matrix material properties of continuous fiber reinforced composites have a primary effect on both composite strength and intra- and interlaminar cracking resistance was seen previously in this paper. However, matrix stiffness is essential variable affecting composite compression strength due to the fact that fiber microbuckling, a major compression failure mechanism, depends on the amount of support provided by the matrix to the fibers. The compression strength of the composite was measured according to the suppliers of Advanced Composite Materials Association used an SRM 1R-94 "Recommended Test Method for Comporessive Properties of Oriented Fiber-Resin Composites." It is evident from Table 1 that the average strength of the composite uniformly increased with and increasing volume fraction of nanosilica particles. The authors concluded from the table that in Fig. 13 the variations in the fiber volume fraction are likely to have a significant effect on the measured compression strengths. Additional considerations are due to the sensitivity of this compression test methods to changes in the matrix modulus. J. Cho et al. stated, "under in-plane shear loading, the fiber reinforced composite can be analyzed as a series model with constant average shear stress in the fibers and matrix." The matrix-dominated to the shear strength, τ_{12}, can be related to the neat matrix, τ_{nm} and the stress concentration factor by the following equation:

$$\tau_{12} = \frac{\tau_{neat\ matrix}}{k_\tau} \qquad (eq.1)$$

The stress intensity factor, k_τ, is expressed as:

$$k_\tau = \frac{1 - V_f * (1 - \frac{G_m}{G_f})}{1 - \left(\frac{4 * V_f}{\pi}\right)^{\frac{1}{2}} * (1 - \frac{G_m}{G_f})} \qquad (eq.2)$$

where V_f is the fiber volume ratio, G_m and G_f are the shear moduli of the matrix and fiber." The stress concentration factor decreases as the particle loading increases as can be seen in Fig. 15. As a result, the in-plane shear strength of the fiber reinforced composite is increased. This does agree with the work done by Hackett et al [11,12].

Table 1

Nanosilica particle influence on CFRP properties [12].

Silica (wt%)	In-plane Shear		SRM-1R94 Compression		0° Flexure		
	Modulus (GPa)	FV (%)	Strength (GPa)	FV (%)	Modulus (GPa)	Strength (GPa)	Strain (%)
0	4.74	62.9	1.779	62.5	126.9	1.53	1.21
15	5.18	61.5	1.841	61.2	128.2	1.68	1.32
25	5.44	60.6	1.889	60.3	126.0	1.71	1.37
35	5.96	61.1	1.903	60.3	126.9	1.83	1.49
45	6.63	60.0	1.979	59.2	124.7	1.93	1.56

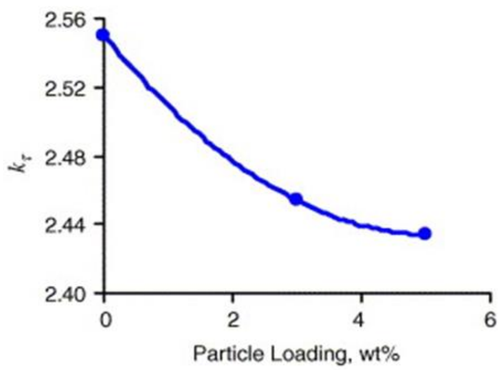

Fig. 15—Shear stress concentration factor in fiber-reinforced composite [7].

CONCLUSIONS

1. Young's modulus, flexural strength, ILSS and fracture toughness are all increased as a result of particle filler into a matrix.

$$\Delta\tau = \frac{G * b}{vol\%} \qquad (eq.3)$$

$$\sigma_{yield} = \tau_o + \Delta\tau \qquad (eq.4)$$

$$Vol\% = \frac{4 * (1 + vol\%) * r}{3 * vol\%} \qquad (eq.5)$$

τ— represents shear, G represents the shear modulus, b represents the burger's vector and r represents the radius of the particle.

2. As can be examined from the equations above, particle shape, particle size, distribution and volume fraction all influence second phase particle strengthening. It is evident from the study that as the radius r of the particles decreased, so did the interparticle spacing since the volume fraction ***Vol%*** and ***r*** are proportional. With those two factors decreasing, both $\boldsymbol{\Delta\tau}$ and $\boldsymbol{\tau_{yield}}$ increased. The closely-spaced small particles are more favorable in increasing the microscopic yield strength for the overall hybrid epoxy. Overall, the strength of the composite depends on the % content, arrangement and type of fiber or particle reinforcement filler in the resin.

3. The tensile resistance to deformation in the transverse direction was smaller than that in the longitudinal direction and decreased as the temperature increased. The resistance

increases with the introduction of the particles as well as in the longitudinal direction [10].

4. This preliminary study has provided great insight on the mechanical properties of composites with particles embedded in the matrix. Placing particles such as Terfenol-D in a polymeric resin will a magnetostrictive effect and at the same time reduce the disadvantages of the monolithic material. As the brittleness of the particles are reduced, toughness is improved which allows for tensile loading and the weight of the composite is decreases with a much lower density [13,14].

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Research papers are reports of original research. Submission of a manuscript implies that the paper has not been published and is currently at the time of submission being considered for publication elsewhere. At least one of the authors must be a member of the Academy, and all authors are encouraged to join.

Manuscripts. Submit the manuscript electronically to the Mississippi Academy of Sciences under your profile in the member location of the website. Please also provide a cover letter to the Editor of the Journal. The cover letter should authorize publication: give the full names, contact information, for all authors; and indicate to whom the proofs and correspondence should be sent. Please notify the Editor on any changes prior to publication.

Manuscripts must adhere to the following format:

- One inch margins on 8.5 x 11 inch paper;
- Text should be left-justified using twelve point type;
- Double spaced throughout, including the title and abstract;
- Arabic numerals should be used in preference to words when the number designates anything that can be counted or measured (7 samples, 43 species) with 2 exceptions:
- To begin a sentence (Twenty-one species were found in…)
- When 2 numeric expressions are adjacent in a sentence. The number easiest to express in words should be spelled out and the other left in numeric form (The sections were divided into eight 4-acre plots.).
- Measurements and physical symbols or units shall follow the International System of Units (SI *Le Système international d'unités*) with metric units stated first, optionally followed by United States units in parentheses. *E.g.*: xx grams (xx ounces); and
- Avoid personal pronouns.

Format

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

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

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

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

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

Acknowledgments. Colleagues and/or sources of financial support to whom thanks are due for assistance rendered in completion of the research or preparation of the manuscript should be recognized in this section rather than in the body of the text.

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

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

Pearson HA, Sahukhal GS, **Elasri** MO, Urban MW. Phage-bacterium war on polymeric surfaces: can surface-anchored bacteriophages eliminate microbial infections? Biomacromolecules. 2013 May 13;14(5):1257-61.

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

Web-page

- name of author(s) -if known
- title of the work - in quotes, if known
- title of the Web page - in italics, if applicable
- date of last revision
- URL
- Date accessed

Example:

Ackermann, Ernest. "Writing Your Own Web Pages." *Creating Web Pages.* 23 Oct. 1996. http://people.umw.edu/~ernie/writeweb/writeweb.html 10 Feb. 1997.

File available by anonymous FTP

- name of author(s) -if known
- title of the work - in quotes, if known
- date of last revision
- URL
- Date accessed

Example:
American Civil Liberties Union. "Briefing paper Number 5, Drug Testing in the Work Place." 19 Nov. 1992. ftp://ftp.eff.org/pub/Privacy/Medical/aclu_drug_testing_workplace.faq
13 Feb. 1997.

Please Tables and Figures at the end of the manuscript submitted.

Tables. Tables must be typed double spaced, one table to a page, numbered consecutively, and placed at the end of the manuscript. Since tables must be individually typeset, consolidation of data into the smallest number of tables is encouraged. A horizontal double underline should be made beneath the title of the table, and single underlines should be made the width of the table below the column headings and at the bottom of the table. Do not use vertical lines, and do not place horizontal lines in the interior of the table. Use footnotes, to clarify possible questions within the table, should be noted by asterisks, daggers, or other symbols to avoid confusion with numerical data. Tables should be referred to parenthetically in the text, for example (Table 1).

Figures and illustrations. Figures may be photographs, computer -generated drawings, or graphs and should be placed at the end of the manuscript and referenced in the appropriate place.. All illustrations are referred to as “Figures” and must be numbered consecutively. Illustrations other than those generated by the author(s) must include permission for use and credit to the originator. Each figure must have a complete legend that is typed, double-spaced, on a separate sheet which precedes the figures in the manuscript. Figures should be referred to parenthetically in the text, for example (Fig. 1).

Footnotes. Text footnotes **should not be used**

Submission Preparation Checklist

As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

1. The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).
2. The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.
3. I acknowledge that if my manuscript is peer-reviewed and accepted for publication, there will be a paper charge fee of $50/page for **non-Academy members**.
4. The manuscript file is in Microsoft Word format.

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