BROWARD COUNTY BEACH DEMONSTRATION PROJECT:

BIOLOGICAL ANALYSIS OF RECYCLED GLASS CULLET

Submitted to:

Broward County Waste and Recycling Services
Broward County Department of Planning & Environmental Protection
Florida Fish & Wildlife Conservation Commission

Submitted by:

Coastal Planning & Engineering, Inc.

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1. INTRODUCTION

Since the early 1960's, Broward County, Florida has conducted beach nourishment using offshore sand sources to address critical shoreline erosion. Critically eroded shoreline areas pose a myriad of environmental concerns (e.g., limited nesting habitat for sea turtles and shorebirds, increased risk of inundation of sea turtle nests, destruction of threatened beach flora). Significantly, 21 of the 24 miles of Broward County beaches have been defined as critically eroded (FDEP, 2000).

Potential sources of beach compatible sand include offshore inter-reef sedimentary infills, upland dune, inland sources, and aragonite sand from the Bahama Banks. Coastal Planning & Engineering, Inc. (CPE), in conjunction with the Broward County Waste and Recycling Services (WRS) and Malcolm Pirnie, Inc. (MPI), have proposed using recycled glass cullet as a potential alternative source material for beach fill (Edge, 2002; Malcolm Pirnie, 2003; Thomson *et al.*, 2004). Recycled glass cullet has physical properties similar to natural silica sands, making it a viable alternative beach fill material along critically eroded shorelines (Finkl, 1996; Thomson *et al.*, 2004). Recycled glass cullet has many advantages as a potential alternative beach fill material including:

1. The ability to mechanically select grain sizes comparable to the native beach characteristics;
2. Chemical, mechanical, geologic, and engineering properties of appropriately sized recycled glass cullet are similar to natural sand;
3. The ability to utilize a readily available recycled product;
4. Elimination of expensive transportation and disposal fees of recycling material;
5. Proactive implementation of a comprehensive recycling program;
6. Utilization of an environmentally safe, inert material.

A concern associated with the use of recycled glass on beaches relates to the assimilation of cullet by beach sediments. Based on general physical characteristics, Kerwin (1997) summarized the compatibility of recycled glass cullet with natural beach sand and found that the two materials are compatible with each other (Tables 1 - 3). In addition, CPE performed contaminant tests on beach sand and recycled glass cullet for lead (Pb), mercury (Hg), total coliform, fecal coliform, enterococci bacteria, semi volatile organics, total recoverable petroleum hydrocarbons (TRPH), and total salt content (Thomson *et al.*, 2004). The contaminant levels of the beach sand and recycled glass cullet samples were either undetectable or below the Florida Department of Environmental Protection (FDEP) Reuse Target Levels for direct residential exposure (Thomson *et al.*, 2004; Table 4). Therefore, based on the geotechnical and contaminant analysis performed for this study, recycled glass cullet is a viable source for beach fill material.

Table 1.
Kerwin's (1997) compatibility summary of recycled glass cullet with natural sand.

Test	Compatible with Sand	Comments
Mean grain size	Yes	Processed to a wide range of specified sizes.
Sorting	Yes	Varies, dependent on grain size criteria.
Sphericity	Yes	Low, flat, oblate, and bladed shapes provide stability as beach fill material.
Roundness	Yes	Angular shape quickly abraded by littoral action.
Color beach	Yes	Varies with source (like sands) pale yellow-light brown.
Specific Gravity (Relative Density)	Yes	Cullet=2.49 to 2.65; natural aggregate = 2.68; silica-silicon dioxide = 2.21 to 2.32; quartz = 2.65.
Gradation	Yes	Shows little or no gradation.
Particle Shape (Workability)	Yes	Suitable for mechanical handling, low potential for punctures and cuts.
Durability	Yes	Less than crushed rock but close to normal limits for roadway aggregate.
Compaction	Yes	Little or no gradation changes, compaction curves are relatively flat, material insensitive to moisture content.
Permeability	Yes	Medium to high permeability corresponding to medium sand and gravel aggregate.
Thermal Conductance	Yes	Slightly lower than natural aggregates, cullet conducts heat somewhat more slowly.
Shear strength	Yes	Cullet exhibits good to excellent CBR values; cullet has resilience moduli >29 ksi at a bulk stress of 25 psi.

Table 2.
Kerwin's (1997) physical characteristic performance results of recycled glass cullet with natural sand from Broward County.

Test Conducted	Study Site Sand	Glass Cullet	67% Sand, 33% Glass Cullet	91% Sand, 9% Glass Cullet
Sphericity	Oblate low	Oblate-bladed low	Oblate low	Oblate low
Roundness	Round	Angular/sub-round	Round	Round
Mean grain size	0.49	0.78	0.46	0.44
Sorting	mw	m	mw	mw
Munsell Color Notation				
Dry	7.5YR 7/4 to 10YR 8/3	5Y 8/3	10YR 7/2	2.5Y 7/2 to 5Y 7/2
Moist	7.5YR 7/2 to 10YR 7/3	5Y 7/3	10YR 6/2 to 5Y 7/2	2.5Y 7/4

*m=moderately sorted, mw=moderately well sorted

Table 3.
Kerwin's (1997) FDEP regulation compliance summary of recycled glass cullet.

FDEP Regulation	Glass Cullet Properties	Compliance Status
Grain size < 5% fines	Ground to specified grain sizes < 5% fines	Yes
Grain size of <5% rock and gravel	Ground to specified grain sizes <5% gravel	Yes
Similar composition	Amorphous silica Similar to 50% quartz component	Yes
Similar coloration	Varies with source Pale yellow-light brown	Yes

Table 4.
Summary of contaminant exposure limits and test results of recycled glass cullet.

Parameter	Detection Limits & Units	Glass Cullet Samples			Existing Beach Samples			Limit for Direct Exposure Residential [1]
		Andela Products	Glass Aggregate Systems	Recycle America Alliance	Sample 1	Sample 2	Sample 3	
Lead	10 mg/kg	71	U [2]	U	U	U	U	400
Sodium	10mg/kg	1300	850	U	2700	3300	2500	N/A
Mercury	0.05 mg/kg	0.11	U	0.099	U	U	U	3.4
Chloride	20 mg/kg	480	U	160	U	U	200	7800
Enterococci Bacteria	1 CFU/1g	U	U	U	U	U	U	N/A
Fecal Coliform Bacteria	1 CFU/1g	U	U	U	U	U	U	N/A
Total Coliform Bacteria	1 CFU/1g	U	U	U	U	U	U	N/A
Benzyl butyl phthalate	100µg/kg	340	U	240	U	U	U	15,000,000
Bis(2-ethylhexyl)phthalate	100µg/kg	1800	U	680	U	U	U	76,000
Di-n-butyl phthalate	100µg/kg	250	U	250	U	U	U	7,300,000
Fluoranthene	100µg/kg	170	U	U	U	U	U	2,900,000
Pyrene	100µg/kg	130	U	U	U	U	U	2,200,000
Petroleum Range Organics	10µg/kg	280	190	350 [3]	U	U	U	340
Hydrocarbon Range	-	C_{18}-C_{36}	C_{18}-C_{36}	C_{18}-C_{38}	-	-	-	-

1. FDEP, 2001.
2. U=Analyte not detected.
3. Within the soil cleanup target level for the C_{18}-C_{38} carbon chain range.

In response to a directive from the FFWCC and FDEP, the CPE team developed a phased scientific investigative program to evaluate material effects on invertebrate and vertebrate (fish) biotic communities assemblage. Other studies have demonstrated that experimental bioassays can accurately detect either adverse or benign effects on marine organisms (Peters *et al.*, 2002; Ringwood and Keppler, 2002). In addition, State resource protection agency representatives have suggested that the habitation and colonization of lower invertebrates may serve as a good indicator of the affect recycled glass cullet will have on higher vertebrates. These investigations were undertaken to validate the hypothesis that recycled glass cullet is a viable alternative to more traditional sand sources.

The objective of this project was to monitor the colonization and survivability of lower invertebrates and fish, through experimental bioassays, in various cullet mixtures proposed for use in the Broward County Demonstration Project. A saltwater flow system was utilized to simulate natural beach conditions within each test matrix. Direct observations and supplemental chemical analyses were conducted to determine whether recycled glass cullet has an affect on biological communities.

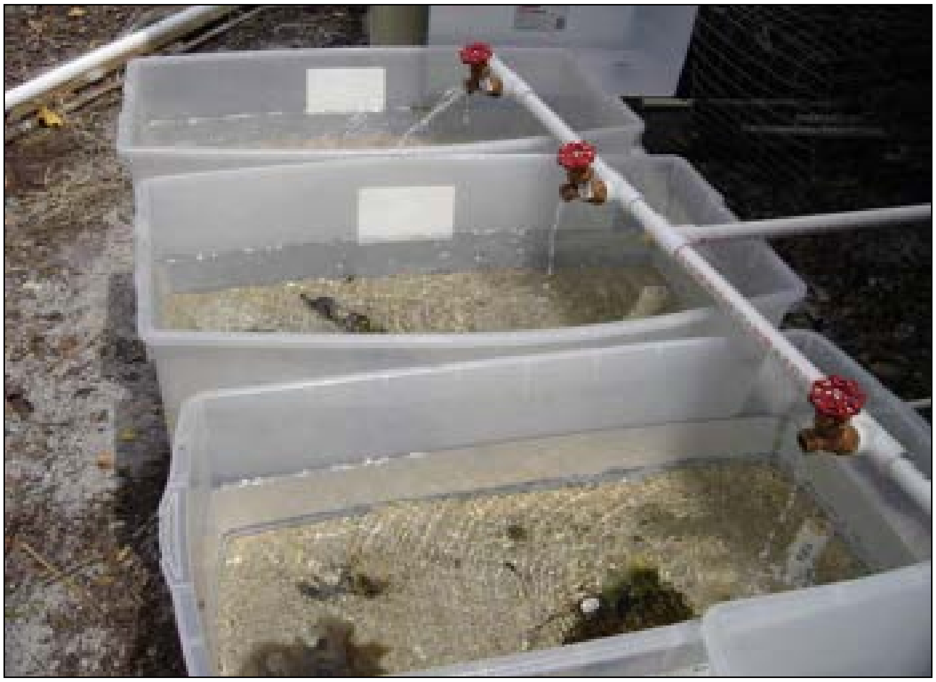
2. METHODS

Experimental Design

A biological infaunal assay program was developed to monitor invertebrate survivability and colonization within specific proportions of natural sand and glass cullet. Each bioassay was performed within a Rubbermaid® 24 gallon clear plastic container (84.3 x 43.3 x 34.4 cm), with each container containing approximately 50 lbs net dry weight of a specific natural sand/glass cullet mixture. Natural sand was collected from the mid-berm native beaches in southern Broward County. Recycled glass cullet was provided to the project team by Glass Aggregate Systems. Each test mixture of natural sand/glass cullet was manually proportioned, weighed, and mixed by hand to obtain uniform composition.

Experiments were arranged in a single row, and consisted of five, three-container series (for a total of 15 containers) that each tested a specific natural sand/glass cullet matrix (Photographs 1 and 2). The first series served as a control with 100% natural sand, while the second series of containers tested a matrix of 75% natural sand and 25% glass cullet. The third series of containers tested a 50% natural sand/50% glass cullet mixture, and the fourth series of containers tested a mixture of 25% natural sand and 75% glass cullet. The fifth series of experiments tested 100% glass cullet. Saltwater, obtained through the Gumbo Limbo Nature Center oceanic intake system, was delivered through a network of PVC pipes to each test container and provided a constant supply of fresh saltwater (approximately nine water changes over a 24 hr period) to the marine organisms and sediments (Photograph 2). Using a combination of bulkheads and control valves, a drainage outlet system was constructed on each test container in order to maintain optimum water volume (approximately 10 gallons). Shade was provided to shield the test containers from overexposure to UV radiation.

Photograph 1. Experimental design with tandem series of bioassays.



Photograph 2. Top-view of bioassays.

Species introduced into each test matrix were collected or supplied by the Gumbo Limbo Nature Center of Boca Raton, Florida, and included local representative microfauna (i.e., copepods, ostracods, nematodes) and macrofauna organisms (i.e., crustaceans, mollusks, fish). All testing and recorded observations were conducted from May 1st to July 1st, 2005, at the Gumbo Limbo Nature Center.

Biological Monitoring

a) Macrofauna Organisms

Macrofaunal organisms were either collected within the Atlantic Intracoastal Waterway and Atlantic Ocean (Photograph 3), or were taken from established reef organism display tanks located at the Gumbo Limbo Nature Center. Organisms were immediately introduced to their respective bioassay test container after collection. A 24-hour acclimation period was allowed before monitoring was initiated. Table 5 lists the number of macrofauna test organisms included in each bioassay series. Live marine shrimp were added as a source of food.

Biological monitoring of macrofauna test organisms were conducted through direct visual observations performed at least twice per week for the duration of the study. Active status of macrofauna was digitally recorded using a Sony Cybershot P900 camera, and a complete census of all individuals was taken on a weekly basis.

b) Microfauna Organisms

Microfauna organisms were introduced to each test container by taking a five pound sample of ‘live’ sand from established (nitrogen cycled) reef display tanks located at the Gumbo Limbo Nature Center. A one-week acclimation and colonization period was allowed before monitoring was initiated.

Photograph 3. Macrofauna collection within the Atlantic Intracoastal Waterway.

Table 5.
Glass Cullet Bioassay Macrofauna Test Organisms
Number of individual species introduced into each experimental bioassay series.

Macrofauna Species	100% Natural Sand	75% Natural / 25% Cullet	50% Natural / 50% Cullet	75% Natural / 25% Cullet	100% Cullet
Sea Urchin *(Lytechinus variegates)*	8 (8)	8 (8)	8 (8)	8 (8)	8 (8)
File Fish *(Monacanthus tuckeri)*	6 (5)	6 (6)	6 (5)	6 (6)	6 (4)
Sargassum Fish *(Histrio histrio)*	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
Checkered Puffer Fish (*Sphoeroides testudineus*)	1 (1)	0	0	1 (1)	1 (1)
Sargassum Crab (*Portunus sayi*)	8 (5)	8 (7)	8 (6)	8 (5)	8 (6)
Lightning Whelk (*Busycon sinistrum*)	1 (1)	1 (1)	1 (1)	1 (1)	2 (2)
Blue Crab (*Callinectes sp.*)	1 (1)	1 (1)	1 (1)	1 (1)	2 (2)
Sargassum Nudibranch (*Scyllaea pelagica*)	4 (0)	4 (0)	4 (0)	4 (0)	4 (0)
Red-striped Hermit Crab (*Phimochirus holthuisi*)	0	0	0	0	1 (1)
Total Individuals	30 (22)	29 (24)	29 (22)	30 (23)	33 (25)

*Number of survived individuals is shown in parenthesis

Biological monitoring of microfauna test organisms was conducted through interstitial core analysis. A 50.10 gram interstitial core sample was extracted from each test matrix using a 50 centimeter long piece of ½ inch PVC pipe and a stopcock. Core samples were taken on a weekly basis and analyzed for the presence of microfauna under a trinocular video microscopy system at a total magnification of 400x (Figure 3). Identification of microorganisms was through external characterization, and active status of individuals was digitally documented using a Sony Cybershot P900 camera.

Photograph 4. Microfauna analysis using a trinocular video microscopy system.

c) Organic Content

Organic content of each test matrix was analyzed weekly by using two methods. The first method, as described in Mikutta *et al.* (2005), included (1) taking an interstitial core sediment sample; (2) air drying the sample and weighing it; (3) adding an excess of 6% hydrogen peroxide (H_2O_2) solution; (4) and allowing the sample to dry overnight. The dry sample was then reweighed and subtracted from the initial sample weight to determine the amount of organic content within the sediment (Mikutta *et al*., 2005). The following equation was used to determine the percentage of organic content in each sample:

[(Pre-treat Weight – Post-treat Weight)/Pre-treat Weight)] X 100

The second method involved combusting the organic content at a high temperature. An interstitial core sample was extracted and dried in an oven at 70°C to evaporate all moisture. The sample was then weighed and placed in a Humboldt MFG Co. combustion oven at 475°C for approximately 4 hours. After 4 hours, the oven was turned off and the sample was allowed to cool down to room temperature. Once the sample had cooled, it was weighed again. The measure weight loss was assumed to be the amount of organic material present in the original sample.

Abiotic Parameters
A series of chemical analyses of the water column were performed weekly in each test container to determine abiotic factors associated with biological colonization performance. Dissolved oxygen (O_2), pH, ammonia (NH_3), nitrites (NO_2), nitrates (NO_3), and organic phosphates (PO_4) were all tested weekly using Salifert Marine Test Kits. In addition, hydrogen sulfide (H_2S) levels were measured using a Lamotte Hydrogen Sulfide Test Kit in the second month of experimentation. Water temperature was measured and recorded at least twice a week.

Statistical Analysis

A paired two-sample T-test was performed on H_2O_2 treated organic content data vs. combustion oven organic content data from each test series to determine if the data sets differed significantly from each other. A two-way analysis of variance (ANOVA) was conducted to determine if temperature, dissolved oxygen concentrations, pH, and organic content percentages differed significantly in the experimental bioassays when compared to the 100% natural sand control samples.

Analyses were conducted with SPSS software (SPSS Inc.; Chicago, Illinois) with significance based on $p \leq 0.01$. Mean values are followed by standard deviations (± SD), unless otherwise noted.

3. RESULTS

The following results (Table 6) were obtained during the biological analysis phase of the Broward County Beach Demonstration Project conducted between May 1 and July 1, 2005.

Control Bioassays

The series of control bioassays recorded an average water temperature in the sample containers of 25.9°C ±0.4 (78.6°F ±0.7) over the two-month testing period. Average pH was 8.2, while dissolved oxygen (O_2) averaged 6.9 mg/L ±0.1. Ammonia (NH_3), nitrites (NO_2), nitrates (NO_3), and organic phosphates (PO_4) were all recorded levels at the lowest detectable limits using applied methods. Average concentrations of 0.01 ppm (NH_3), 0.02 ppm (NO_2), 0.2 ppm (NO_3), and 0.02 ppm (PO_4) were documented over the study period. Hydrogen sulfide (H_2S) concentrations were never recorded above 0.2 ppm.

Within the series of 100% natural sand control bins, an average 3.9% ±0.2 of the interstitial sediments were compromised of organic matter. Both methods (H_2O_2 treatment & combustion oven) measured data with insignificant differences (t = 1.49; p = 0.14). Upon microscopic investigation of the control sediments, the following microorganisms were identified and documented during the duration of the two-month testing period: Amphipods, copepods, nematodes, and ostracods. Introduced macrofauna documented a mean success rate of 73% (Photographs 5 & 6). Three sargassum crabs, four sargassum nudibranches, and one file fish either died, or fell prey to other individuals, over the two-month testing interval.

Table 6. Bioassay summary table for each series.

Experimental Parameters	Control	Test Matrices			
	100% Natural Sand	**75% Natural / 25% Cullet**	**50% Natural / 50% Cullet**	**25% Natural / 75% Cullet**	**100% Cullet**
Temperature (°C)	25.9 ±0.4	25.9 ±0.4	25.9 ±0.4	25.8 ±0.4	25.9 ±0.4
pH	8.2	8.2	8.2	8.2	8.2
Dissolved O_2 (mg/L)	6.9 ±0.1	6.9 ±0.1	6.8 ±0.1	6.9 ±0.1	6.8 ±0.1
Ammonia (ppm)	0.01	0.01	0.01	0.01	0.01
Nitrites (ppm)	0.02	0.02	0.02	0.02	0.02
Nitrates (ppm)	0.2	0.2	0.2	0.2	0.2
Organic Phosphates (ppm)	0.02	0.02	0.02	0.02	0.02
Hydrogen Sulfide (ppm)	0.02	0.02	0.02	0.03	0.03
Organic Content	3.9% ±0.2	3.7% ±0.3	3.8% ±0.4	3.7% ±0.3	3.7% ±0.3
Macrofauna Mean Success Rate	73%	83%	76%	77%	76%

Photograph 5. Bioassays containing 100% natural sand.

Photograph 6. Sea urchin (*Lytechinus variegates*) in control bin.

25% Cullet / 75% Natural Sand Bioassays

The 25% cullet / 75% natural sand matrix series recorded an average water temperature of 25.9°C ±0.4 (78.6°F ±0.7). O_2 concentrations averaged 6.9 mg/L ±0.1 and pH averaged 8.2. NH_3, NO_2, NO_3, and PO_4 all recorded levels at the lowest detectable limits with average concentrations of 0.01 ppm, 0.02 ppm, 0.2 ppm, and 0.02 ppm, respectively. H_2S concentrations were never recorded above 0.2 ppm.

Photograph 7. Sea urchin (*Lytechinus variegates*) in a 25% cullet / 75% natural sand matrix.

The 25% cullet / 75% natural sand matrix series recorded an average organic content percentage of 3.7% ±0.3 within the interstitial sediments. Both methods (H_2O_2 treatment & combustion oven) measured data that did not differ significantly (t = 1.34; p = 0.18). Upon microscopic investigation of the 25% cullet / 75% natural sand test matrix, the following microorganisms were identified and documented during the duration of the two-month testing period: Amphipods, copepods, nematodes, and ostracods. Introduced macrofauna documented a mean success rate of 83% (Photographs 7 & 8). One sargassum crab and four sargassum nudibranches either died, or fell prey to other individuals, over the two-month testing interval.

50% Cullet / 50% Natural Sand Bioassays

The experimental series of 50% cullet / 50% natural sand recorded water temperatures that averaged 25.9°C ±0.4 (78.6°F ±0.7). pH levels averaged 8.2 and recorded concentrations of O_2 averaged 6.8 mg/L ±0.1. NH_3, NO_2, NO_3, and PO_4 all recorded levels at the lowest detectable limits with average concentrations of 0.01 ppm, 0.02 ppm, 0.2 ppm, and 0.02 ppm, respectively. H_2S concentrations were never recorded above 0.2 ppm.

Photograph 8. Sargassum crab (*Portunus sayi*) in 25% cullet experimental bin.

Photograph 9. Blue crab (*Callinectes sp.*) in the 50% cullet / 50% natural sand substrate.

The experimental series of 50% cullet / 50% natural sand recorded a 3.8% ±0.4 average organic content of the interstitial sediments. Both methods (H_2O_2 treatment & combustion oven) measured data that were not significantly different (t = 0.92; p = 0.36). Upon microscopic investigation of the 50% cullet / 50% natural sand test matrix, the following microorganisms were identified and documented during the duration of the two-month testing period: Amphipods, copepods, nematodes, and ostracods. Introduced macrofauna documented a mean success rate of 76% (Photographs 9 & 10). Two sargassum crabs, four sargassum nudibranches, and one file fish either died, or fell prey to other individuals, over the two-month testing interval.

Photograph 10. File fish (*Monacanthus tuckeri*) swimming in 50% cullet experimental bin.

75% Cullet / 25% Natural Sand Bioassays

The 75% cullet / 25% natural sand matrix series of bioassays recorded an average water temperature of 25.8°C ±0.4 (78.5°F ±0.7). O_2 concentrations averaged 6.9 mg/L ±0.1 and pH averaged 8.2. NH_3, NO_2, NO_3, and PO_4 all recorded levels at the lowest detectable limits with average concentrations of 0.01 ppm, 0.03 ppm, 0.3 ppm, and 0.02 ppm, respectively. H_2S concentrations averaged 0.3 ppm, and ranged between 0.2 – 0.4 ppm.

The 75% cullet / 25% natural sand matrix series of bioassays recorded an average organic content of 3.7% ±0.3 within the interstitial sediments. Both methods (H_2O_2 treatment & combustion oven) measured data that did not differ significantly ($t = 0.40$; $p = 0.69$). Upon microscopic investigation of the 75% cullet / 25% natural sand test matrix, the following microorganisms were identified and documented during the duration of the two-month testing period: Amphipods, copepods, nematodes, and ostracods. Introduced macrofauna documented a mean success rate of 77% (Photographs 11 & 12). Three sargassum crabs and four sargassum nudibranches either died, or fell prey to other individuals, over the two-month testing interval.

Photograph 11. Sargassum crab (*Portunus sayi*) in a 75% cullet / 25% natural sand substrate test bin.

100% Cullet Bioassays

The series of bioassays containing 100% recycled glass cullet recorded an average water temperature of 25.9°C ±0.4 (78.6°F ±0.7), with an average pH of 8.2 and an average O_2 concentration of 6.8 mg/L ±0.1. NH_3, NO_2, NO_3, and PO_4 all recorded levels at the lowest detectable limits with average concentrations of 0.01 ppm, 0.03 ppm, 0.3 ppm, and 0.02 ppm, respectively. H_2S concentrations averaged 0.3 ppm, and ranged between 0.2 – 0.5 ppm.

Photograph 12. Sargassum fish (*Histrio histrio*) in 75% cullet experimental bin.

Photograph 13. Lightning whelk (*Busycon sinistrum*) in a bioassay containing 100% recycled glass cullet.

Photograph 14. Blue crab (*Callinectes sp.*) on 100% cullet.

The series of bioassays containing 100% recycled glass cullet recorded an average of 3.7% ±0.3 organic content within the interstitial sediments. Both methods (H_2O_2 treatment & combustion oven) measured data that were not significantly different (t = 1.32; p = 0.19). Upon microscopic investigation of the 100% recycle glass cullet, the following microorganisms were identified and documented during the duration of the two-month testing period: Amphipods, copepods, nematodes, and ostracods. Introduced macrofauna documented a mean success rate of 76% (Photographs 13, 14, & 15). Two sargassum crabs, four sargassum nudibranches, and two file fish either died, or fell prey to other individuals, over the two-month testing interval.

Photograph 15. Checkered puffer fish (*Sphoeroides testudineus*) swimming in 100% cullet bin.

4. DISCUSSION

This study is the first to document the biological effects of recycled glass cullet on macrofaunal and microfaunal communities to ascertain the environmental suitability as an alternative sand source to offset critical beach erosion. Over a two-month testing period at the Gumbo Limbo Nature Center of Boca Raton, five series of bioassays, each containing a different sediment matrix, were analyzed to record how newly introduced organisms (macrofaunal and microfaunal) were affected by the cullet grain substrates. In addition, chemical parameters (toxic and non-toxic) were monitored in conjunction with the living organism observations.

Experimental Bioassays

The contamination of marine sediments on biological processes goes beyond digestive uptake and chemical solubility; it is rather a complex result of compound-particle interaction. Bioavailability of particle-associated contaminants has been shown to be influenced by sediment specific properties such as organic matter and surface area (Chung and Alexander, 1998) and can impact different organisms on varying exposure routes (Harkley *et al.*, 1994; Liss and Ahlf, 1997). Due to this complexity, attention has been increasingly given to bioassays which examine the effect of sediments directly exposed to the test organisms (Ahlf *et al.*, 2002; Heise and Ahlf, 2005). Our experimental bioassays performed in this study were designed to allow for the compound-particle interaction between representative local fauna and varying glass cullet concentrations to identify whether recycled glass cullet exposes organisms to particle-adhered contaminants, or whether it would be an environmentally benign substrate.

Biological Analysis

After a two-month exposure to recycled glass cullet sediments, it has been determined that an artificial cullet substrate does not adversely affect macro-organism habitation or micro-organism colonization. This was evident with the uniform lack of mortality observed in the introduced individuals and the active presence of interstitial microfauna. Previous studies have shown that marine sediment bioassays reveal different patterns of toxicity through mass mortality on mollusks, crustaceans, echinoderms, and fish (Carr and Chapman, 1992; Long *et al.*, 1995). However, in the recycled glass cullet bioassays, the experimental macrofauna showed a mean success rate of 78% ±3. Species that were most hearty and exhibited no mortality included *Lytechinus variegates*, *Histrio histrio*, *Busycon sinistrum*, and *Callinectes sp*. All specimens of *Scyllaea pelagica* showed mortality, suggesting that this species of nudibranch was not an appropriate test subject due to the spatial limitations of the experimental containers or other unidentified factors. When sargassum nudibranches were eliminated from the mortality totals, the mean success rate for the cullet bioassays increased to 91% ±3. Minimal mortality (i.e., never more than four individuals per series) was seen in *Portunus sayi* and *Monacanthus tuckeri*, however it is believed that this is a not a function of sediment contamination, but rather the result of an encounter with a larger individual or from pre-existing compromised physiology.

Individuals displayed normal active behavior within the cullet matrices and showed no adverse signs of physical stress. Of particular note, was the use of the cullet as defensive camouflage by the test crustaceans and mollusks. In order to shield themselves from predators or direct UV light, the sargassum and blue crabs would thrust their carapaces backward into the cullet until they were completely covered (Photograph 16). This behavior was shown repeatedly over the testing period with no harm to the crabs. Similarly, the conchs would burrow deep into the cullet as a protective measure, and would do so with no observed injury to their foot or mantle.

Photograph 16. Sargassum crab (*Portunus sayi*) using recycled glass cullet as camouflage.

Microscopic analyses revealed that colonization of interstitial microorganisms were successful within the cullet mixtures (Photograph 17). All the same classes of microorganisms were observed in the control as well as the experimental replicates. Interstitial movements were documented and showed that microorganisms were able to actively transport without adverse effects. Uniform colonization of the sediment mixtures was also reaffirmed by the consistent organic content readings in all the test series. Even though initial rates of organic concentration within the cullet took significantly longer than the natural sand (ANOVA $p < 0.01$; $\alpha = 0.05$), all the test series contained similar sediment organic content at the end of the testing interval with a mean of 38% ±1.

Photograph 17. Interstitial colonization of microorganisms.

Abiotic Parameters

Abiotic parameter testing revealed that marine water chemistry was not negatively altered when in contact with recycled glass cullet. Water temperature steadily increased over the testing interval and was not significantly different among the five series (mean = 25.9°C ±4; ANOVA $p > 0.01$; $\alpha = 0.05$). Dissolved oxygen was readily available for all the test series organisms, and did not significantly deplete in the presence of the cullet (mean = 6.9 mg/L ±1; ANOVA $p > 0.01$; $\alpha = 0.05$). Similarly, pH readings were consistent and did not significantly differ among series (mean = 8.2 ±0; ANOVA $p > 0.01$; $\alpha = 0.05$).
Of the initial toxic chemicals monitored, ammonia, nitrites, nitrates, and organic phosphates, all recorded low risk factor levels.

An important and unexpected observation during the study was the formation of a blackish film that began to develop on the different cullet sediment mixtures after three weeks of testing, first starting on the 100% cullet and then appearing on the other experimental series. Further analysis determined that the black film was a precipitation of hydrogen sulfide (Photograph 18). This anaerobic sulfide-reducing process has been documented in stagnant coastal waterway systems that lack influential wave action (Carlson *et al.*, 1994). However, when sediments containing the cullet were mixed and allowed to aerate (wave simulation), the visible hydrogen sulfide accumulations disappeared (Photograph 19). Hydrogen sulfide test readings also showed that levels were decreased after the wave simulation and aeration was initiated.

Photograph 18. Red-striped hermit crab (*Phimochirus holthuisi*) walking on precipitated H_2S atop recycled glass cullet.

Photograph 19. Aerated cullet became void of visible sulfide accumulations.

The researchers believe that the hydrogen sulfide was more apt to accumulate on the glass cullet due to the nature of its grain shape. Natural sand grains are primarily spherical in shape, whereas the cullet has a slightly more flattened, angular shape. It is suspected that this flattened cullet shape limits the aeration of inundated sediment in the absence of wave action. Therefore, it is recommended that the application of recycled glass cullet for alternative beach erosion control would best be served if placed directly in the active surf zone where wave action will facilitate aeration and mixing of the material into the native beach sands.

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