

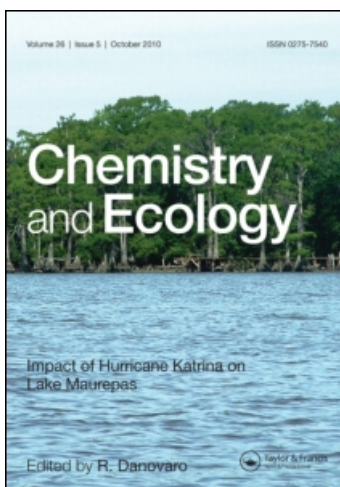
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### **Preliminary Evaluation of the Use of Phosphogypsum for Reef Substrate. I. A Laboratory Study of Bioaccumulation of Radium and Six Heavy Metals in an Aquatic Food Chain**

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## PRELIMINARY EVALUATION OF THE USE OF PHOSPHOGYPSUM FOR REEF SUBSTRATE. I. A LABORATORY STUDY OF BIOACCUMULATION OF RADIUM AND SIX HEAVY METALS IN AN AQUATIC FOOD CHAIN

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Phosphogypsum (PG), a solid by-product of phosphoric acid production, contains radionuclides and trace metals in concentrations which may pose a potential hazard to human health and the environment. To investigate the possibility of bioaccumulation of radium and six heavy metals over time when aquatic organisms experience both trophic and environmental exposure to PG, we designed a laboratory experiment representing three levels of an aquatic food chain. During the 135 day experiment, a meiobenthic copepod species (*Amphiascoides atopus*) was cultured in the presence of PG. The copepods were subsequently fed to grass shrimp (*Palaemonetes vulgaris* and *P. pugio*) which were in turn fed to gulf killifish (*Fundulus grandis*); both the grass shrimp and the killifish also experienced an environmental PG exposure. Other than elevated radium levels in the experimental grass shrimp, the experiment demonstrated little effect of environmental or trophic exposure to PG on microinvertebrates, macroinvertebrates, or fishes that could be attributed to PG. In all cases where increased concentrations were indicated within the experimental group, roughly equivalent increases in metal concentrations also occurred in the control group.

*Keywords:* Phosphogypsum; radium; copper; zinc; arsenic; lead; cadmium; chromium

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## INTRODUCTION

Phosphogypsum (PG,  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ), a solid by-product of phosphoric acid production, contains radionuclides and some trace metals in concentrations which may pose a potential hazard to human health and the environment. The primary disposal method, on site stockpiling, has resulted in at least 33 PG stacks (average area of 90 ha per stack) distributed among all Gulf States (except Alabama), and has created a tremendous management problem. Environmental concerns associated with PG disposal, coupled with increasing land costs for stockpiles, has prompted research on alternative beneficial uses of this solid waste that will result in applications considered protective of public health and the environment.

A sound alternative to current disposal practices must address the issue of airborne radioactive contact with the public, while providing evidence that PG reuse or recycling is more economical than the long term cost of stockpiling. This issue has prompted researchers in Louisiana, Florida, and Texas to investigate alternatives for the utilization of this material. Commercial utilization is the best long term, economical solution to reducing current and future PG inventories. Four broad categories for alternative uses of PG have been identified: 1) agriculture, 2) building construction, 3) road construction and 4) other applications including artificial reefs, rip rap, retaining wall back-fill, coastal erosion barriers, and jetty stone. In addition to the above mentioned coastal applications, the use of PG as an artificial substrate for oyster settlement has been suggested to complement the declining supply of natural oyster shell substrate in the Gulf region (Soniati *et al.*, 1991; Chen *et al.*, 1995).

The utilization of PG for underwater applications provides a means for minimizing public exposure because the airborne vector of transmission is eliminated or, at least, significantly diminished. Most other alternatives, while proposing economic solutions to the growing PG inventories (agriculture, road bed aggregate, building material) do not address the fundamental issue: the high potential for human exposure to radium (Ra) and its decay products (principally radon). An initial uncontrolled pilot demonstration study and a follow up two year study conducted at Louisiana State University (LSU) showed that PG/cement test blocks placed in the Gulf of Mexico supported a

diverse population of surface attached and burrowing organisms, indicating the potential of using PG for offshore artificial reefs (Malone *et al.*, 1994).

In addition to radionuclides, PG contains various trace elements in concentrations which the United States Environmental Protection Agency (EPA, 1990) reports may pose a potential hazard to human health and the environment. The EPA has identified a number of potential constituents in PG that could, under the appropriate conditions, cause adverse health effects or restrict the potential uses of nearby surface or groundwater resources. Elements identified include arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr), fluorine (F), zinc (Zn), antimony (Sb), and copper (Cu) (EPA, 1990). Among these elements, only chromium and arsenic were identified in the PG produced at certain facilities at concentrations that may pose significant health risks (EPA, 1992).

The leachability of potentially toxic substances from PG stacks has previously been investigated (May and Sweeney, 1982). No significant leaching of copper and nickel was detected among nine PG stacks located in Florida. More importantly, all core samples contained toxic element concentrations lower than the EPA definition of toxicity, with the exception of arsenic. However, analyses indicated that arsenic could not be leached and thus would not be available to impact the environment. In leaching tests performed on compressed PG/cement (70%/30%) and sand/cement (70%/30%) blocks (Chen *et al.*, 1995), all elements measured (Cd, Pb, Cr, and As) were below EPA drinking water quality standards. And quite unexpectedly, lead and chromium concentrations were higher in leachates from the sand/cement blocks than in those from the PG/cement composites. In a related study, Shieh and Duedall (1994) investigated the chemical interactions of cement stabilized oil ash and coal fly ash artificial reef blocks with sea water. Loss, no change, or enrichment of nine elements was found to be confined to the outer surface layer (1 cm) of the blocks. Also, recovery of all nine elements was greater than 95% after 2.5 years of submersion. It was concluded that elements of environmental concern were essentially stabilized within the blocks.

A stable, abundant source of low-profile material for use in the construction of inshore artificial reefs has become imperative in many states bordering the Gulf of Mexico. Such reefs both provide

additional suitable habitat for fishes dependent on hard substrates and sustained growth of oysters. With Louisiana as the example, the economic significance of these fisheries can be seen. An examination of the impact of recreational fishing on the Louisiana economy estimated that participants spent over \$200,000,000, not including multipliers (Bertrand, 1986). Additionally, the Louisiana oyster industry has been valued at over \$20,000,000 per year (Keithly and Roberts, 1988). Most of this fishing activity occurs in the shallow, inshore and nearshore coastal waters. However, before large-scale use of PG in the fabrication of artificial reef materials can proceed, it must be demonstrated that its potentially harmful trace element contaminants are not assimilated into the aquatic food chain and ultimately passed along to the consuming public.

To investigate the possibility of bioaccumulation and biomagnification of  $^{226}$ radium and selected heavy metals when organisms are cultured in the presence of PG, we designed a laboratory experiment consisting of three components representing increasing levels of an aquatic food chain: a copepod (*Amphiascoides atopus*), grass shrimp (*Palaemonetes vulgaris* and *P. pugio*), and the gulf killifish (*Fundulus grandis*).

## MATERIALS AND METHODS

*Chaetoceros muelleri*, a planktonic diatom, was cultured in a two chamber turbidostat system housed in a temperature and light controlled room. The cultures were grown at 30°C in 25 ppt artificial sea water with continuous lighting from one 250 watt metal halide light per chamber. Algae were harvested from the system with a continuous duty centrifuge which consolidated the algal suspension into paste of 80% moisture content. The paste was refrigerated until needed and resuspended immediately prior to feeding to the copepods.

A mass culture system (System II described by Sun and Fleeger, 1995) was constructed to provide *Amphiascoides atopus* for the experiment. Copepods were grown at 24°C in 32 ppt artificial sea water with a 12 h light, 12 h dark light cycle. Crushed limestone supplemented with 1 kg of unconsolidated PG powder was provided as substrate material. Because the nauplii larvae of *A. atopus* are

benthic, they should have come into contact with larger particles of PG in the substrate. All life stages may also have ingested fine particulate PG during feeding. The system was provided *Chaetoceros muelleri* at a rate of >1 million cells per ml every other day. When supplies of the alga were occasionally insufficient to maintain copepod growth, the diet was augmented with fish flake food. An average of two to four million copepods were produced per day, a quantity well in excess of that necessary to maintain grass shrimp in the cultures described below.

Grass shrimp (*Palaemonetes vulgaris* and *P. pugio*) were obtained as needed from emergent vegetation in Caminada Pass at the west end of Grand Isle, Jefferson Parish, Louisiana. After transport to the laboratory, they were maintained in an array of four 40 litre aquaria: three dedicated to producing PG exposed shrimp and one for control shrimp. All four aquaria were kept at approximately 24°C, 20 ppt salinity, and 12 h light, 12 h dark. The three PG aquaria provided both a trophic exposure (PG copepods from above fed at approximately 500,000 per day per aquarium) and an environmental exposure (three 5 cm diameter and 10 cm length cylindrical blocks composed of 70% PG and 30% cement) to the grass shrimp. These were stocked weekly on a rotating basis with approximately 150 grass shrimp allowing a minimum 2 weeks and a maximum 3 weeks feeding and exposure prior to their being fed to the gulf killifish. The single control aquarium was provided with three 5 cm diameter and 10 cm length cylindrical blocks composed of 70% sand and 30% cement; the grass shrimp there were fed commercial shrimp pellets.

Gulf killifish (*Fundulus grandis*) were obtained from stocks under culture at the LSU Aquaculture Research Facility. Twelve of these were frozen at the beginning of the experiment to provide baseline data. Four killifish were placed into each of six 80 litre aquaria held at approximately 24°C, 10 ppt salinity, and 12 h light, 12 h dark. Three of these were designated as experimental aquaria to which six 5 cm length by 10 cm diameter, 70% : 30% (PG : cement) cylindrical blocks were added. The killifish in these were fed an average of one PG exposed grass shrimp per fish per day. The remaining three aquaria, designated as controls, were supplied with six 5 cm length by 10 cm diameter, 70% : 30% (sand : cement) cylindrical blocks and were provided with an average of one non-PG exposed grass shrimp per fish per day.

Forty-five days in the experiment, a maximum of two killifish were removed from each aquarium and sacrificed for analysis. These were replaced at two killifish per aquarium with individuals whose right pelvic fin had been clipped for later identification. Ninety days later, all remaining individuals were sacrificed for analysis. This methodology should have yielded six killifish from each treatment which had been exposed to PG and control conditions for 45, 90 and 135 days, or a total of 36 individuals available for analysis. However, this number was reduced due to nine mortalities of unknown cause.

Samples for analysis consisted of pooled aliquots (3.5–0.5 g wet weight) of experimental ( $N = 5$ ) and control ( $N = 4$ ) copepods, pooled aliquots (3.0–3.5 g wet weight) of experimental ( $N = 10$ ) and control ( $N = 15$ ) grass shrimp, and the individual headless bodies (1.3–16.1 g wet weight) of experimental ( $N = 14$ ) and control ( $N = 26$ ) killifish. The heads were removed from the killifish for subsequent removal of the otoliths and microchemical analyses of same (not presented here). Control copepods were from a stock of *Amphiascooides atopus* harvested from a culture maintained with limestone cobble, but without PG. Grass shrimp were analyzed whole, *i.e.*, the exoskeletons were not removed. Each sample was digested with nitric acid for analysis of seven elements: radium, copper, zinc, cadmium, lead, chromium, and arsenic. Metals concentrations were measured with the Inductively Coupled Argon Plasma Emissions Spectroscopy (ICAP) system and were expressed as milligrams per gram wet weight. Radium analyses were conducted at the LSU Nuclear Science Center with the following techniques: radium in the digested tissue samples was coprecipitated with a barium carrier in sulphate. Extracted radium was allowed to come to equilibrium with radon daughters which then were measured by quantification of alpha particle emission in a photomultiplier. Baseline data for radiation analyses of grass shrimp and gulf killifish were derived from digestion and assessment of 1 kg samples of wild caught specimens of each. All radium concentrations are given as picocuries per gram fresh weight.

Statistical analyses of the elemental concentration data were carried out with the GLM procedure (SAS Institute Inc., 1985) on 11 main-effect means defined by species (copepod, grass shrimp, killifish), treatment (PG or control), and time of exposure (0, 45, 90 and 135 days) for killifish. In addition to analysis of variance (ANOVA) for

unbalanced data, main-effect means of elemental concentrations were further compared with pairwise *t* tests and Duncan's multiple range tests.

## RESULTS

All components of both the control and experimental food chains which we established appear to have functioned well. Sufficient copepods were produced to continually nourish the experimental grass shrimp; little or no mortality was experienced. Among the nine killifish (five control and four experimental) which were lost during the course of the experiment, most died shortly after their transfer from the supply stock tanks to the aquaria. These mortalities were due to the likely shock of handling or to changes in water salinity or temperature. The 27 surviving killifish all appeared to be healthy and to have noticeable growth.

The grass shrimp which received both trophic and environmental exposure to PG were found to have a minimum seven-fold greater mean concentration of radium than the remaining ten groups. Analysis of variance ( $P < 0.05$ ) and both the *t* test and Duncan's comparisons of means for this element (Tab. I) suggest that

TABLE I Comparisons of mean values (as picocuries per gram fresh weight) of radium in control and experimental copepods (*Amphiascoides atopus*), grass shrimp (*Palaemonetes* sp.), and gulf killifish (*Fundulus grandis*). C = control, E = experimental, SD = standard deviation. Means sharing the same letters under *t* test and Duncan's multiple range test were not shown to be significantly different

Category	<i>N</i>	Mean $\pm$ 1 SD (pCi/g)	<i>t</i> test (LSD)	Duncan's Multiple range test
C copepods	4	-0.0043 $\pm$ 0.0071	A	A
C killifish-90 d	4	0.0100 $\pm$ 0.0069	A B	A
C killifish-135 d	5	0.0102 $\pm$ 0.01123	A B	A
C killifish-45 d	3	0.0290 $\pm$ 0.0185	A B	A
E copepods	5	0.0302 $\pm$ 0.0132	A B	A
C grass shrimp	15	0.0383 $\pm$ 0.0211	A B	A
E killifish-135 d	5	0.0386 $\pm$ 0.0200	A B	A
C killifish-0 d	12	0.0391 $\pm$ 0.0556	A B	A
E killifish-45 d	6	0.0445 $\pm$ 0.0322	A B	A
E killifish-90 d	3	0.0540 $\pm$ 0.0512	B	A
E grass shrimp	10	0.3583 $\pm$ 0.0671	C	B



bioaccumulation of radium occurred either from the ingestion of PG exposed copepods or from leachate of the PG blocks, or both. The radium, a calcium analogue, could have been accumulated in either the soft tissues or the exoskeleton as has been shown in molluscs (Van der Borght, 1963; Jeffree and Simpson, 1984). However, this increase in corporal radium concentration evidently was not passed along to the killifish. Despite slightly elevated radium concentrations among the killifish PG exposure groups, they were not found to be different significantly from those of the control killifish groups.

Both the control and experimental grass shrimp exhibited elevated concentrations of copper compared to the other nine groups (Tab. II). However, this is most probably not related to any factors associated with PG exposure. A copper based oxygen transport molecule (haemocyanin) found in the haemolymph of grass shrimp and many other crustaceans of the subclass Malacostraca (Mangum, 1985) is thought to be responsible largely for the increased concentrations. In copepods and other small crustaceans, gas exchange is achieved by diffusion over the general body surface without the aid of an oxygen carrier (Barnes, 1974).

Apparent increases in the concentrations of zinc from copepods to grass shrimp to gulf killifish within the experimental group could be indicative of bioaccumulation (Tab. III). However, equivalent or near

TABLE II Comparisons of mean values (as micrograms per gram fresh weight) of copper in control and experimental copepods (*Amphiascoides atopus*), grass shrimp (*Palaemonetes* sp.), and gulf killifish (*Fundulus grandis*). C = control, E = experimental, SD = standard deviation. Means sharing the same letters under *t* test and Duncan's multiple range test were not shown to be significantly different

Category	N	Mean $\pm$ 1 SD ( $\mu\text{g/g}$ )	<i>t</i> test (LSD)	Duncan's Multiple range test
C copepods	4	0.501 $\pm$ 0.166	A	A
C killifish-135 d	5	1.194 $\pm$ 0.169	A B	A B
E copepods	5	2.087 $\pm$ 0.233	A B	A B
C killifish-45 d	3	2.453 $\pm$ 0.671	A B	A B
C killifish-90 d	5	2.696 $\pm$ 0.466	A B	A B
E killifish-135 d	5	2.870 $\pm$ 1.389	B	A B
E killifish-45 d	6	3.028 $\pm$ 0.247	B	A B
E killifish-90 d	3	3.133 $\pm$ 1.476	B	A B
C killifish-0 d	10	3.220 $\pm$ 1.219	B	A B
E grass shrimp	10	13.041 $\pm$ 2.366	C	C
C grass shrimp	15	13.615 $\pm$ 2.971	C	C

TABLE III Comparisons of mean values (as micrograms per gram fresh weight) of zinc in control and experimental copepods (*Amphiascoides atopus*), grass shrimp (*Palaemonetes* sp.), and gulf killifish (*Fundulus grandis*). C = control, E = experimental, SD = standard deviation. Means sharing the same letters under *t* test and Duncan's multiple range test were not shown to be significantly different

Category	N	Mean $\pm$ 1 SD ( $\mu\text{g/g}$ )	<i>t</i> test (LSD)	Duncan's Multiple range test
C copepods	4	3.885 $\pm$ 0.957	A	A
E copepods	5	4.689 $\pm$ 0.357	A	A
E grass shrimp	10	8.992 $\pm$ 2.011	A	A
C grass shrimp	15	9.416 $\pm$ 2.080	A	A
C killifish-135 d	5	26.700 $\pm$ 8.804	B	B
C killifish-45 d	3	33.467 $\pm$ 5.550	B C	B C
E killifish-90 d	3	37.467 $\pm$ 6.152	C D	C D
E killifish-45 d	6	39.233 $\pm$ 6.383	C D	C D
C killifish-0 d	10	39.440 $\pm$ 10.130	C D	C D
E killifish-135 d	5	40.360 $\pm$ 8.487	C D	C D
C killifish-90 d	5	42.080 $\pm$ 1.942	D	D

equivalent increases in zinc are shown within the control group. The concentrations shown in the copepods and grass shrimp are not different significantly from one another. Further, the zinc concentrations found at all levels of exposure in both the experimental and control killifish are roughly equal to or less than the day 0 killifish. If bioaccumulation is occurring, it is occurring equally within both the control and experimental food chains. Conversely, the differences in zinc concentrations among copepods, grass shrimp, and killifish may be due to unique physiological requirements for zinc in maintaining stasis.

The analyses for cadmium were complicated by many concentrations shown as zero. This does not necessarily indicate that cadmium was not present, rather that it may have been present at concentrations below the level of detectability ( $0.001 \mu\text{g g}^{-1}$ ) of the ICAP system. Among the experimental group, there is evidence of bioaccumulation of cadmium from the copepods to the grass shrimp (Tab. IV). However, the increased levels of this element were not passed along as further increased concentrations to the killifish. The highest concentration of cadmium in the control copepods cannot be explained.

Among all elements surveyed, lead showed the greatest degree of within group variability. All mean concentrations of lead were less than or equal to about  $1 \mu\text{g g}^{-1}$  and showed little statistical variation

TABLE IV Comparisons of mean values (as micrograms per gram fresh weight) of cadmium in control and experimental copepods (*Amphiascoides atopus*), grass shrimp (*Palaemonetes* sp.), and gulf killifish (*Fundulus grandis*). C = control, E = experimental, SD = standard deviation. Means sharing the same letters under *t* test and Duncan's multiple range test were not shown to be significantly different

Category	N	Mean $\pm$ 1 SD ( $\mu\text{g/g}$ )	<i>t</i> test (LSD)	Duncan's Multiple range test
C killifish-0 d	10	0 $\pm$ 0	A	A
C grass shrimp	15	0 $\pm$ 0	A	A
E killifish-45 d	6	0 $\pm$ 0	A	A
C killifish-90 d	5	0 $\pm$ 0	A	A
C killifish-45 d	3	0 $\pm$ 0	A	A
C killifish-135 d	5	0.0200 $\pm$ 0.0447	A	A
E killifish-135 d	5	0.0460 $\pm$ 0.1029	A B	A B
E killifish-90 d	3	0.1967 $\pm$ 0.0651	B C	B C
E copepods	5	0.3038 $\pm$ 0.0282	C	C
E grass shrimp	10	1.0392 $\pm$ 0.1693	D	D
C copepods	4	1.8632 $\pm$ 0.4697	E	E

(Tab. V). Only the concentration of this element in killifish at 90 days PG exposure was consistently shown to be greater than the other groups. However, among 135 day killifish the concentrations of lead are equivalent for both the control and PG exposed groups; thus, bioaccumulation of lead with PG as its source is unlikely to occur.

TABLE V Comparisons of mean values (as micrograms per gram fresh weight) of lead in control and experimental copepods (*Amphiascoides atopus*), grass shrimp (*Palaemonetes* sp.), and gulf killifish (*Fundulus grandis*). C = control, E = experimental, SD = standard deviation. Means sharing the same letters under *t* test and Duncan's multiple range test were not shown to be significantly different

Category	N	Mean $\pm$ 1 SD ( $\mu\text{g/g}$ )	<i>t</i> test (LSD)	Duncan's Multiple range test
C copepods	4	0 $\pm$ 0	A	A
E killifish-45 d	6	0 $\pm$ 0	A	A
C killifish-45 d	3	0 $\pm$ 0	A	A
C grass shrimp	15	0.0596 $\pm$ 0.1592	A B	A
E grass shrimp	10	0.1009 $\pm$ 0.3191	A B	A
E copepods	5	0.1644 $\pm$ 0.2257	A B	A
C killifish-0 d	10	0.3860 $\pm$ 0.6333	A B	A
C killifish-90 d	5	0.4960 $\pm$ 0.4675	A B	A
C killifish-135 d	5	0.5200 $\pm$ 0.5454	B	A
E killifish-135 d	5	0.5360 $\pm$ 0.6267	B	A
E killifish-90 d	3	1.1600 $\pm$ 0.6251	C	B

Concentrations of both chromium (Tab. VI) and arsenic (Tab. VII) showed a high degree of variability not only in the concentrations measured, but also significant statistical variation. A weak argument for bioaccumulation of each could be made if one considers only the experimental data. However, largely equivalent increases in chromium and arsenic concentrations within both food chains suggest sources other than PG as being responsible.

TABLE VI Comparisons of mean values (as micrograms per gram fresh weight) of chromium in control and experimental copepods (*Amphiascoides atopus*), grass shrimp (*Palaemonetes* sp.), and gulf killifish (*Fundulus grandis*). C = control, E = experimental, SD = standard deviation. Means sharing the same letters under *t* test and Duncan's multiple range test were not shown to be significantly different

Category	N	Mean $\pm$ 1 SD ( $\mu\text{g/g}$ )	<i>t</i> test (LSD)	Duncan's Multiple range test
C copepods	4	0.0183 $\pm$ 0.0365	A	A
E copepods	5	0.0338 $\pm$ 0.0463	A	A
E grass shrimp	10	0.0932 $\pm$ 0.1173	A	A
E killifish-45 d	6	0.1333 $\pm$ 0.2273	A	A
C grass shrimp	15	0.1504 $\pm$ 0.0779	A	A
C killifish-0 d	10	0.2280 $\pm$ 0.3010	A	A
C killifish-45 d	3	0.2500 $\pm$ 0.2261	A	A
C killifish-135 d	5	0.8100 $\pm$ 0.5611	B	B
E killifish-135 d	5	1.2240 $\pm$ 0.2868	B C	B C
C killifish-90 d	5	1.6640 $\pm$ 0.8470	C D	C D
E killifish-90 d	3	1.9067 $\pm$ 1.3349	D	D

TABLE VII Comparisons of mean values (as micrograms per gram fresh weight) of arsenic in control and experimental copepods (*Amphiascoides atopus*), grass shrimp (*Palaemonetes* sp.), and gulf killifish (*Fundulus grandis*). C = control, E = experimental, SD = standard deviation. Means sharing the same letters under *t* test and Duncan's multiple range test were not shown to be significantly different

Category	N	Mean $\pm$ 1 SD ( $\mu\text{g/g}$ )	<i>t</i> test (LSD)	Duncan's Multiple range test
E copepods	5	0 $\pm$ 0	A	A
C copepods	4	0 $\pm$ 0	A	A
C killifish-0 d	10	0.135 $\pm$ 0.4269	A	A
C grass shrimp	15	2.404 $\pm$ 0.7457	B	B
E killifish-45 d	6	2.577 $\pm$ 2.0958	B	B
C killifish-45 d	3	3.030 $\pm$ 2.7537	B C	B C
E grass shrimp	10	3.503 $\pm$ 0.5800	B C	B C
C killifish-135 d	5	4.132 $\pm$ 1.9330	B C D	B C D
C killifish-90 d	5	4.900 $\pm$ 3.0147	C D	C D
E killifish-135 d	5	5.570 $\pm$ 3.3306	D	D
E killifish-90 d	2	8.355 $\pm$ 1.6856	E	E

## DISCUSSION

We know of neither similar bioaccumulation studies nor pond studies involving PG which can be used in comparisons with our findings. Quite a large literature of research involving waste-to-energy ash does exist; however, due to differences in source materials and their constituents, comparisons between waste-to-energy studies and our data would be tenuous at best. A factor which would complicate comparisons among PG studies is the well documented heterogeneity of PG based both on the source of the mother ore and the length of time the PG has been stockpiled (Luther *et al.*, 1993; Rutherford *et al.*, 1994; Arocena *et al.*, 1995; Rutherford *et al.*, 1995a; Rutherford *et al.*, 1995b).

This experiment demonstrated little effect of environmental or trophic exposure to PG on microinvertebrates, macroinvertebrates, or fishes which can be solely attributed to PG. No unequivocal evidence of bioaccumulation of radium or of six heavy metals could be discerned among the various trophic stages. In all cases where increased concentrations were indicated within the experimental group, roughly equivalent increases in metal concentrations also occurred in the control group. We suspect that this phenomenon is largely due to uptake from the leachate of the cement used to consolidate the sand and PG. Also, concentrations of all elements considered herein are exceedingly low and fall well below the Food and Drug Administration standards for crustaceans and bivalve molluscs used as foodstuffs (USFDA, 1996).

## CONCLUSIONS

Among the analyses of five experimental and six control groups for radium and six heavy metals, the results are equivocal and show little evidence of bioaccumulation through the food chain. Analysis of variance revealed significant differences among means for all main-effects with elements. However, if bioaccumulation were occurring, one would expect the experimental concentrations to be larger than the control concentrations within species and exposure groups. Also, among those groups receiving environmental, trophic, or both,

exposure to PG, one would assume further incremental increases both in the concentrations among the trophic levels and over time of exposure within the killifish groups. Given our data, the case for bioaccumulation is difficult to make.

## CONSIDERATIONS FOR THE FUTURE

The ultimate decision for using PG as colonizing substrate for oysters, reef material, or other aquatic application will depend upon expansion of this line of research and an active dialogue with regulatory agencies. Assuredly, results to date warrant continuing laboratory trials and field testing of the ecological safety of PG in pursuit of this goal. Among the proposed tasks are: developing a procedure for the manufacture of PG composites which will maximize their strength with a minimum of effort, determining the affinity of larval marine epifauna for PG composites, investigating the suitability of PG briquettes for settlement and growout of oysters, ascertaining the diffusion coefficients of PG leachates at different salinities, and producing a risk assessment model which will be used to predict possible long term bioaccumulation of trace toxic materials through the food chain.

Should our research show that the use of PG composites in marine environments poses little danger to the associated fauna and, ultimately, to public health, several benefits may be realized. The use of PG composites in a variety of marine applications would reduce current and future stockpiles of PG and decrease industry's economic and legal liabilities resulting from on site stockpiling. Further, the manufacture of PG composites for artificial reefs or substrate for oyster settlement could provide long term economic benefit to the Gulf Coast by creating new industries or rejuvenating commercial ventures which have been impacted by damaged or lost aquatic habitats.

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## References

- Arocena, J. M., Rutherford, P. M. and Dudas, M. J. (1995) Heterogenous distribution of trace elements and fluorine in phosphogypsum by-product. *The Science of the Total Environment*, **162**, 149–160.
- Barnes, R. D. (1974) *Invertebrate Zoology*. W. B. Saunders, Philadelphia, p. 870.
- Bertrand, A. L. (1986) Marine recreational finfishermen in Louisiana: A socioeconomic study of licensed recreational finfishermen in *Coastal Study Area IV*, Technical Series Report Number TS-3, Coastal Fisheries Institute, Louisiana State University, pp. 36.
- Chen, S., Rusch, K. A., Malone, R. F., Seals, R. K., Wilson, C. A. and Fleeger, J. (1995) Preliminary evaluation of stabilized phosphogypsum for use within the aquatic environment. *Proceedings of the World Environmental Federation Special Workshop on Food Chain Toxicity, Toxic Substances in Water Environments: Assessment and Control*, Cincinnati, OH, 14–17 May 1995. pp. 8–73–8–84.
- EPA (United States Environmental Protection Agency) (1990) Report to Congress on Special Wastes from Mineral Processing, Solid Waste and Emergency Response. EPA/530-SW-90-070C, Washington, D. C.
- EPA (United States Environmental Protection Agency) (1992) Final Rule, National Emission Standards for Radon Emissions from Phosphogypsum Stacks, 40 CFR part 61, Subpart R. FRL-4103–2, Washington, D. C.
- Jeffrey, R. A. and Simpson, R. D. (1984) Radium-226 is accumulated in calcium granules in the tissues of the freshwater mussel, *Velesunio angasi*: support for a metabolic analogue hypothesis? *Comparative Biochemistry and Physiology*, **79A**, 61–72.
- Keithly, W. R. and Roberts, K. J. (1988) The Louisiana oyster industry: economic status and expansion prospects. *Journal of Shellfish Research*, **7**, 515–525.
- Luther, S. M., Dudas, M. J. and Rutherford, P. M. (1983) Radioactivity and chemical characteristics of Alberta phosphogypsum. *Water, Air, and Soil Pollution*, **69**, 277–290.
- Malone, R. F., Wilson, C. A. and Fleeger, J. (1994) Substrate suitability of phosphogypsum composites for artificial reef construction. *Final Report*, Institute for Recyclable Resources, Louisiana State University, pp. 24.
- Mangum, C. P. (1985) Oxygen transport in invertebrates. *American Journal of Physiology*, **248**, 505–514.
- May, A., and Sweeney, J. W. (1982) Evaluation of radium and toxic element leaching characteristics of Florida phosphogypsum stockpiles. BuMines RI 8776, United States Bureau of Mines, Washington, D. C.
- Rutherford, P. M., Dudas, M. J. and Arocena, J. M. (1995a) Radioactivity and elemental composition of phosphogypsum produced from three phosphate rock sources. *Waste Management and Research*, **13**, 407–423.

- Rutherford, P. M., Dudas, M. J. and Arocena, J. M. (1995b) Radium in phosphogypsum leachates. *Journal of Environmental Quality*, **24**, 307–314.
- Rutherford, P.M., Dudas, M. J. and Samek, R. A. (1994) Environmental impacts of phosphogypsum. *The Science of the Total Environment*, **149**, 1–38.
- SAS Institute Inc. (1985), *SAS User's Guide: Statistics, Version 5 Edition*. SAS Institute Inc., Cary, North Carolina, pp. 965.
- Shieh, C. S. and Duedall, I. W. (1994) Chemical behavior of stabilized oil ash artificial reef at sea. *Bulletin of Marine Science*, **55**, 1295–1302.
- Soniat, T., Broadhurst, R. C. and Haywood, E. L. (1991) Alternatives to clamshell as cultch for oysters, and the use of gypsum for the production of cultchless oysters. *Journal of Shellfish Research*, **10**, 403–410.
- Sun, B. and Fleeger, J. W. (1995) Sustained mass culture of *Amphiascoides atopus*, a marine harpacticoid copepod in a recirculating system. *Aquaculture*, **136**, 313–321.
- USFDA (United States Food and Drug Administration) (1996) The fish and fisheries products hazards and controls guide, June 1996 draft. USFDA, Washington, D. C.
- Van der Borght, O. (1963) Accumulation of radium-226 by the freshwater gastropod, *Lymnaea stagnalis* L. *Nature*, **197**, 612–613.