

planning field work in connection with these projects, much valuable information and material for examination in the laboratory will be gained, and will thus bolster our laboratory results. Moreover, by participating in these projects, we are able to render services by giving advice and information to the control operators.

An important part of the program will include cooperative work with entomologists and members of the chemical industries to try to develop more specific and less toxic pesticides to help bring about effective pest control but with minimum hazards to fish.

We feel that this projected program, carried out carefully and vigorously, will be a major contribution to sport fishery management in the United States, and, indeed, in other parts of the world where the use of pesticides is increasing. The research program is aimed toward securing the answers needed by management personnel in the control of fish populations endangered by pesticides. Research of this type will equip the fishery manager with the means for accurately predicting the effects on fish of specific pesticide treatments. We look toward the day when the biologist can make a scientific appraisal of the fish population and the chemical and physical characteristics of the water before any treatment and calculate the amount of damage that will result. We are confident that fishery science is beginning the research that will bring this about, and in not too many years.

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## **Clay-Mineral Sediments As A Reservoir For Radioactive Materials In The Sea<sup>1</sup>**

K. M. RAE AND R. G. BADER

*Department of Oceanography and Meteorology  
Texas A. & M. College, College Station, Texas*

WE ARE ALL TOO aware that any title which has in juxtaposition the expressions "radioactive materials" and "in the sea" automatically becomes controversial in this day and age. It is not our intention here to side either with the British who pump free liquid containing upwards of 10,000 curies each month into the Irish Sea only three miles from their coastline or with public opinion in parts of America which appears apprehensive about the suggestion that some 20 curies a month might be deposited safely in selected inshore areas if contained in concrete and iron drums.

Whatever one's views may be about sea-disposal of low level wastes, the fate of radio-chemicals which may find their way into the sea is a most important problem. Nuclear powered submarines and surface ships are things of the present and nuclear powered aircraft of the near future. Nuclear weapons may well be tested further, if not used in anger. Undoubtedly from now on, every year will see a steep increase in the amount of radioactive material created by man and in the variety of his uses of nuclear power. So the chance of fission products finding their way into the sea by accident or intent will grow correspondingly and inevitably.

In the simplest—but quite unrealistic—model, one can visualize complete

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dispersion of all radioactivity introduced into the sea over the total volume of sea water, some  $360 \times 10^6$  cubic miles. In such a situation the specific activity could be raised until it reached some upper level compatible to man's safety. Thereafter the permissible addition could be no more than would offset the natural loss of activity by decay to stable isotopes.

In any more realistic model, one finds factors tending towards this simplest solution and others tending to prevent it. Amongst the first category are all the physical processes associated with water movement and diffusion. Amongst the latter are the reverse processes leading to concentration and uneven distribution; prominent amongst these is, of course, the effect of particulate matter in the sea, both animate and inanimate. Clearly, plants and animals by involving radioisotopes in their metabolism may concentrate them well above the dilution in the surrounding sea water, and then redistribute them in increasing concentrations through complicated food chains. Migrations, swarming and schooling habits may also tend to redistribute radioactivity that becomes involved in living animals.

Inanimate particles have an analogous, if less obvious, function resulting from the process of "sorption." Clay-mineral particles are highly efficient scavengers of organic molecules and thus of any cations which may become complexed with these molecules. A good deal of work has been done on this by soil-chemists but relatively little is known quantitatively about the process in the marine environment. We do know, however, that marine sediments often contain a considerable organic fraction and that sediments when in suspension can remove a high percentage of dissolved organic substances from a liquid medium by some sorptive process. From the practical aspect, for example, the sediments immediately around the outfall from the Windscale reactor in Britain have become relatively hot through this process. And shaking with clay-minerals has often been proposed as a technique for decontamination of radioactive solutions.

As part of a program sponsored by the U. S. Atomic Energy Commission, we have been investigating these scavenging properties of sedimentary particles. So far emphasis has been on simple systems—the capacity of various minerals to take various organic substances out of solution. As examples, we can consider the ability of montmorillonite, kaolinite, illite and quartz to accumulate glycine, succinic acid, sucrose and fructose. In general terms the experimental techniques have been as follows.

$C^{14}$  labeled solutions of glycine, succinic acid, sucrose and fructose were made up in 4 concentrations ranging from 0.1 to 1.2 gm/100 ml. and duplicate 500  $\lambda$  samples were pipetted from each. Each sample was then dried in a planchet containing lens paper and counted in a Nuclear Chicago automatic sample changing Gas Flow Proportional Counter or alternatively the sample was pipetted into a scintillating liquid in 22 ml. K-40 free vials and counted in an Automatic Packard Tri-Carb Liquid Scintillation Spectrometer. A known weight of clean mineral was placed in a 40 ml. tube and wetted with 1 ml. of deionized water. Ten ml. of the  $C^{14}$  labeled organic solution was then pipetted into each 40 ml. tube containing the wetted mineral and the tubes were shaken for 20 minutes. After settling, two 500  $\lambda$  samples of the supernatant were taken off and counted in the manner described above. The difference in radioactivity between the original solution and the supernatant gave a measure of the organic material removed from the solution by the mineral.

**TABLE 1**  
**PERCENTAGE RETENTION OF ORGANIC COMPOUNDS**  
**BY 20 MG. OF MINERAL**

5°C.				
	Montmorillonite	Illite	Kaolinite	Quartz
Sucrose	71	30	9	2.5
Fructose	55	19	7	2
Succinic Acid	44	15	5	2
Glycine	36	15	3	1
10°C.				
	Montmorillonite	Illite	Kaolinite	Quartz
Sucrose	71	31	8	2.5
Fructose	40	11	5	1
Succinic Acid	17	4	1	0.5
Glycine	10	4	1	0.5
15°C.				
	Montmorillonite	Illite	Kaolinite	Quartz
Sucrose	30	10	3	1
Fructose	66	23	7	2
Succinic Acid	20	8	2	0.4
Glycine				

Data obtained at temperatures of 5°, 10° and 15°C. are given in Table 1. From this it can be seen that montmorillonite removes the greatest percentage of each organic compound from solution, illite next, then kaolinite and last quartz. This can be explained on the basis of active surface area.

Over the entire concentration range there is a linear relationship between the solution concentration and the organic uptake. It should also be noted that montmorillonite is capable of removing from solution 3.5 times its own weight of organic compound. For example, 20 mg. of montmorillonite can retain 70 mg. of fructose or sucrose at solution concentrations of 100-110 mg/10 ml. This can hardly be explained on the basis of adsorption. For the present this system can be best visualized as a complex clay-organic gel.

Apparently adsorption does occur at low solution concentrations. In one instance kaolinite-fructose concentrations ranging from 0 to 1.0 mg/10 ml were investigated and normal adsorption isotherms were observed.

Let us suppose, as is almost certainly true, that the natural sediments and detritus which are in temporary suspension in the sea scavenge out by sorption a proportion of the dissolved organic material and with it a proportion of the more hazardous isotopes that happen to be there. What effect will this have on the distribution of radioactivity in the sea and its potential danger to man?

On the one hand, it may be argued that this process is a factor tending towards safety. It has been suggested that as the sediments sink to the bottom and form successive layers, they will remove radioactive elements from the water and contain them in such a way that they are no longer available to the biota. In other words, we have in the sea a natural scavenging and burying system which will be particularly efficient in inshore waters where run-off

insures a plentiful supply of sorptive particles. There might be a counter danger of such hot sediments being transported to beaches and so interfering with recreational facilities, but the process would mitigate against the primary hazard of the radio-nuclides re-appearing in man's diet incorporated in seafood. To put this argument into perspective, one can quote from the recent publication (No. 655) of the National Academy of Sciences-National Research Council, which contains a very vivid appreciation of the practicability of "Radio-active Waste Disposal into Atlantic and Gulf Coastal Waters."

"Because we are unable to make a quantitative estimate of the magnitude of uptake on suspended and deposited solids, we have neglected this factor in our evaluation of the quantities of wastes likely to be found in the water and in marine food products for various rates of disposal of radioactive wastes. We believe that by neglecting this factor our recommendations concerning disposal rates include a safety factor of at least 10, and possibly more."

As a counter argument, however, it can be proposed that this very process may increase the over-all hazard. It may be feared that certain benthic animals can strip the sorbed materials from sediments despite its bonding. Then two dangers arise. Firstly, filter feeding animals, including those exploited commercially, may themselves become radioactive or pass radioactivity on to their predators. (The authors of the NAS-NRC publication No. 655 noted this point and wisely postulated that no disposal should be permitted near areas exploited by commercial fisheries.) Secondly, seasonal fluctuations in the abundance of such animals may lead to a more or less sudden release of the radioisotopes which have accumulated in the sediments over several months of sorption.

In considering such possibilities, one immediately thinks of the potential role of bacteria in recycling sorbed radioactive materials either by direct release or by making them more available to those animals which may pass the sediments through their digestive systems. It is well known that bacteria proliferate rapidly in the presence of discrete particles that provide a suitable substrate. Sedimentary particles certainly provide a suitable diffuse substrate but can the bacteria utilize the potential source of nutrients that is bonded to it?

Quite recently we have started some experiments to try to find out. We emphasize, however, that this project, also sponsored by the U. S. Atomic Energy Commission, is only in its preliminary stages. We are now only looking for suitable experimental areas and possible techniques. The preliminary results, if suggestive, cannot be considered convincing until more rigorous experiments have been conducted.

For the first trials we needed labeled organic material typical of that naturally occurring in sea water. To obtain an approximation to this, unicellular marine algae, *Platymonas* and *Phaeodactylum*, were grown in mass culture in a medium in which a high proportion of the carbon was the  $C^{14}$  isotope. These cultures were then killed, macerated, and run through a Millipore filter. The filtrate was shaken with montmorillonite. The clay was then removed and washed several times. A considerable amount of the organic material and its associated radioactivity remained on the clay.

To get a general idea of whether marine bacteria could utilize or release the organic material from such clay, twenty-one flasks were set up in seven sets of three. Each flask contained 40 ml. of ZoBell's culture medium #2216, modified to the extent that the peptone enrichment was reduced from 5 gm/L to 2 gm/L.

To each flask was added 1 gm. of the labeled montmorillonite which had an activity of about 2,000 counts per min/gm in a gas flow counter. The pH was then adjusted to 7.9. Next, six sets of three flasks were inoculated with marine bacteria using a different strain in each set. The seventh set was not inoculated and although not necessarily completely sterile, presumably contained relatively few bacteria and none of marine origin. The whole battery of flasks was allowed to incubate for seven days. The supernatant was then drawn off, and the clays were washed and counted. The results are shown in Table 2.

TABLE 2  
ACTIVITY OF ABOUT ONE GRAM OF MONTMORILLONITE AFTER INCUBATION  
WITH VARIOUS STRAINS OF BACTERIA FOR ONE WEEK.

Bacterium Strain	Cultures			Average cpm*
	A	B	C	
(1) 10 BGP	489	495	423	469
(2) 14 TG	559	560	483	536
(3) 18 TGA	618	697	586	639
(4) 19 TG	653	750	600	668
(5) 16 TG	748	794	718	753
(6) 16 TGB	650	744	(1,100)	831
	Clay: 1261	880	1125	1089
(7) Control Supernatant:	903	1302	1163	1122

\*Counts per minute.

Rather surprisingly, about half the radio-activity found its way off the clay in the control flasks but it is most unlikely that this was entirely due to leaching or washing. Almost certainly the supernatant contained an appreciable fraction of the montmorillonite which very readily goes back into suspension.

However, be this as it may, it is obvious that the removal of activity from the clay was about twice as much in the flasks that had been inoculated with bacteria.

Counting the clays, rather than the supernatant, presents some technical problems. Firstly montmorillonite after exposure to salt water and organics does not readily dry in the planchets to a consistent geometry. Secondly, the particles, although previously exposed to the labeled algal material, take up a considerable added load from the organic enrichment of the culture medium. This added uptake varies under different experimental conditions, so it becomes difficult to refer the counted activity back to a unit weight of clay. Further, the distribution of organic material and dried salt in the sample will vary back-scattering and self-absorption, and so preclude any standard correction for thickness. In view of this, and the fact that it is not easy to ensure equal inoculations of bacteria in every flask, it was encouraging to find how well the counts agreed within replicate series. It was presumed that in the one really anomalous result, flask 6C, the bacteria failed to grow.

Subsequent experiments have shown that bacteria will grow for a time in a system comprising unenriched synthetic sea water in which the only organic source is the algal material sorbed on the clay. More recently too, we have been trapping and counting the respired  $C^{14}O_2$  from each flask. Such further results are substantially the same as those given in Table 2. There is thus every indica-

TABLE 3  
 AVERAGE COUNTS PER MINUTE PER GRAM DRY WEIGHT OBTAINED FROM  
 HOMOGENIZED TISSUE OF SMALL FISH 96 HOURS AFTER FORCE-FEEDING  
 WITH LABELED CLAY.

Tissue	<i>F. similis</i>	<i>G. felix</i>
Gill	8,900	14,795
Digestive tract	4,030	2,520
Rest of body	610	430

tion that marine bacteria can hasten the release of sorbed organic materials from the sediments.

Other preliminary trials were made to see whether the sorbed organic materials were available to benthic animals. In each case, labeled clay slurries were prepared in the manner described above by suspending montmorillonite in a solution of disintegrated algae that had been grown in a C<sup>14</sup> medium.

In one series small fish, mud-puppies (*Fundulus similis*) and sea cats (*Galeichthys similis*), were force-fed by pumping labeled clay directly into the gut through the mouth by means of a syringe. Each fish was then put in clean sea water for thirty minutes to wash off any slurry which might be regurgitated, washed again, and finally transferred to solitary confinement in 1,500 ml. of 28 p.p.t. sea water. After 96 hours the fish were killed, washed in dilute HCl, and dissected into three parts: the gills, the alimentary canal and the rest of the body-tissue. Each of these fractions was homogenized in 3 ml. of distilled water in a Serval Omni Mixer for fifteen minutes, dried in a planchet, weighed and counted for five minutes in a gas flow counter. The counts are shown in Table 3.

In other experiments, mussels (*Modiolus modiolus*) and oysters (*Crassostrea virginica*), were kept in bowls containing 1,500 ml. of filtered sea water to which labeled clay had been added and allowed to settle for twenty-four hours. After certain periods, individual animals were sacrificed and their entire soft parts were removed, washed, homogenized, dried, weighed and counted in the same manner as used for the fish. Average counts are given in Table 4. Two white shrimp (*Penaeus setiferus*) were treated in essentially the same manner (Table 5).

It must be emphasized that the counts listed in Tables 3, 4 and 5 have little quantitative value; strictly speaking, they are not even inter-comparable. As mentioned above, the object was first to obtain qualitative observations that might demonstrate the ability of the animals to remove the organic material from the clay. At this stage no corrections were available for self-absorption, which may well vary between different tissues. The samples counted were small, so the counts must reflect sample weight which also varied considerably.

TABLE 4  
 AVERAGE COUNTS PER MINUTE PER GRAM DRY WEIGHT OBTAINED FROM  
 HOMOGENIZED SOFT PARTS OF MOLLUSKS AFTER EXPOSURE TO 2 ML. OF  
 LABELED CLAY IN 1500 ML. OF SEA WATER.

Exposure time	<i>M. modiolus</i>	<i>C. virginica</i>
24 hr.	no data	1960
96 hr.	562	1390
14 days	210	75

We have not yet adequate evidence to say with confidence that the animals were able to utilize the clay-sorbed material in their metabolism. It is, however, clear that a proportion of the radioactivity found its way off the clay and became associated with the animal tissues. There remains the possibility that the labeled organics were transferred from the montmorillonite to the animals by solution and re-adsorption; the high activity of the shed exoskeleton of *P. setiferus* demonstrates that this may be an important factor. However, the fact that the soft parts of the mollusks were radioactive during the first four days and some activity was detected in the internal tissues of the shrimp and fish suggests that surface adsorption is not the only process involved. The very high counts from the gills of the fish may also imply metabolic excretion of the C<sup>14</sup> placed in the gut.

It now remains to repeat experiments in a quantitative basis measuring in absolute units of activity. This is being done.

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TABLE 5  
COUNTS PER MINUTE PER GRAM DRY WEIGHT OBTAINED FROM HOMOGENIZED PARTS OF *Penaeus setiferus* AFTER EXPOSURE TO 2 ML. OF LABELED CLAY IN 1500 ML. OF SEA WATER.

Tissue	After 48 hrs.	After 72 hrs.
Whole animal	449	394
Cephalothorax	560	376
Abdomen	165	96
Exoskeleton	672	*

\*This specimen ecdysed during the first 48 hours. The shed skin showed relatively high activity at 1944 counts per minute per gram.

## Commercial and Biological Uses of the Maryland Soft Clam Dredge<sup>1</sup>

J. H. MANNING

*Chesapeake Biological Laboratory,  
Department of Research and Education, Solomons, Maryland*

FILES OF THE UNITED STATES PATENT OFFICE contain drawings of dozens of mechanized shellfish harvesting devices, some more or less resembling the Maryland soft clam dredge. The Maryland gear, however, is unique in its demonstrated capability of harvesting sub-tidal stocks of deeply burrowing shellfish on a commercial basis. Developed in the early 1950's and patented by Mr. Fletcher Hanks of Oxford, it has been used in Maryland exclusively for digging soft shell clams (*Mya arenaria*), with a degree of success which has

<sup>1</sup>Contribution No. 131, Maryland Department of Research and Education, Solomons, Maryland.