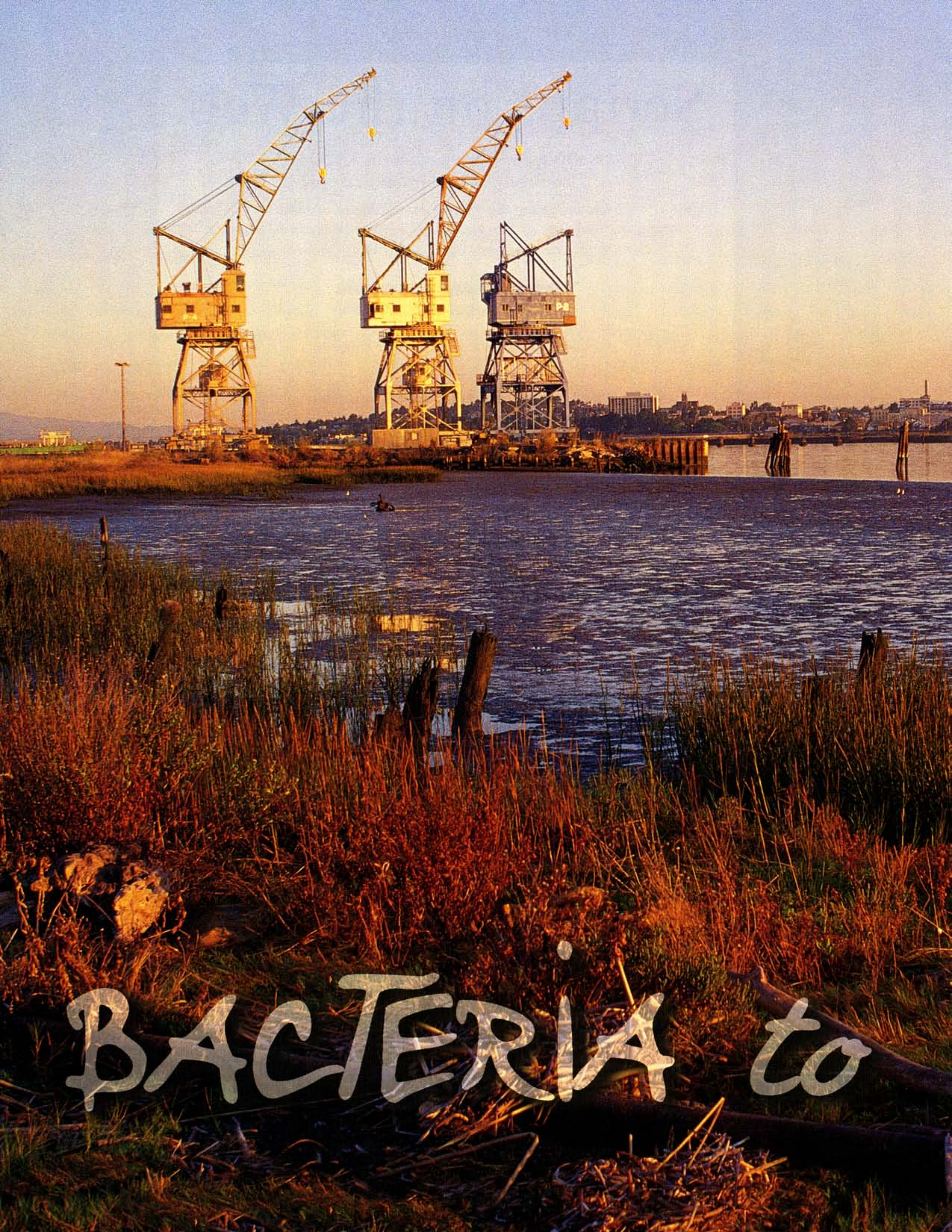

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Marine Microbes Take On Heavy Metals

Bacteria tend to get a bad rap. In the popular view, they are usually associated with infections, bad breath, and dirty drains. In the laboratory of Scripps microbiologist Brad Tebo, however, they may be considered heroes.

"We've begun to think that some bacteria might be useful as a way to clean up metal pollution. In the last five years, we've really been studying this, and it looks as if bacteria can be used for

Marine microbiologist Brad Tebo. The mudflats at Green Sands Beach, Mare Island Naval Shipyard, near San Francisco, California (left).

the RESCUE

BY JOE HLEBICA

Graduate students Chris Francis (standing) and Meriah Arias examine mud samples taken from a polluted field site.

removing toxic metals from the environment, and even recovering those that are valuable," said Tebo.

Processes carried out by some bacteria lead to the precipitation of metals from solution into a solid form. By isolating and then removing these bacteria from a variety of environments and investigating them in the laboratory, Tebo and his associates are studying how and why these reactions take place.

The key appears to be the complementary chemical processes of oxidation and reduction. Oxidation is a chemical reaction in which a compound loses electrons, thereby increasing its ability to bond with other elements. Reduction is a chemical reaction in which an element gains electrons, thereby decreasing its ability to bond with other elements.

...bioremediation—the use of organisms to detoxify and clean up pollution.

Anaerobic chemical solutions, bacterial cultures, and various media are used to test bacterial reduction of toxins.

Tebo thinks that specific bacteria may be able to acquire energy by causing these types of changes in the forms of various metals. Although bacteria may more typically acquire energy by oxidizing organic matter to form carbon dioxide—the process of decay—some may be able to use metals as inorganic energy sources. The economically important metal manganese, which is relatively abundant in the marine environment, is susceptible to bacterial change. The bacteria that oxidize manganese are a focus of Tebo's research.

"You can't destroy pure metals; you can only change their forms," Tebo pointed out. "And it's the soluble forms that are usually toxic. Once precipitated, they're usually nontoxic."

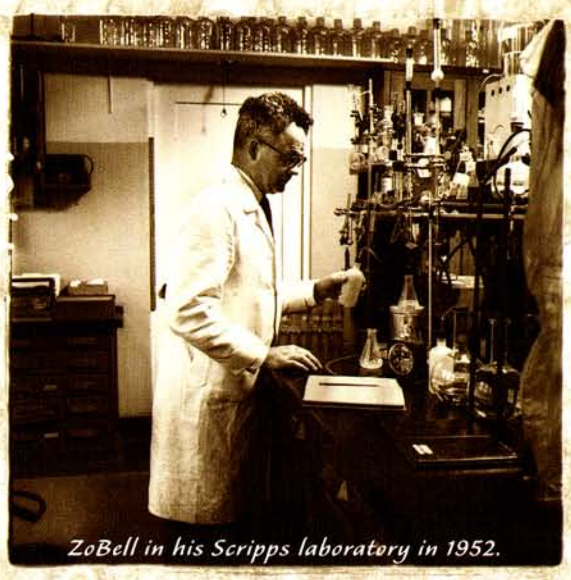
While iron and manganese are not particularly toxic, during the process of their oxidation by bacteria, incidental toxic metals also can be removed from solution. For this reason, manganese-oxidizing bacteria can be exploited to enhance bioremediation—the use of organisms to detoxify and clean up pollution.

Tebo elaborated, "Metal bioremediation might mean changing the metal to another form that's not toxic or not bioavailable. For organic pollutants, the ultimate desirable phase is carbon dioxide; for metals, it's some form that can be precipitated or recovered from the environment."

In the Tebo lab, graduate student Chris Francis is studying the genes and enzymes involved in bacterial manganese oxidation. "In our model bacterium, we have a candidate manganese-oxidizing protein; we also have the gene sequence for this protein. We've made antibodies to the protein, and now we're doing comparative studies with other types of organisms."

Continued on page 26

The Father of Marine Microbiology



ZoBell in his Scripps laboratory in 1952.

When asked about the history of bioremediation, Brad Tebo mentions Scripps microbiologist Claude E. ZoBell. The pioneering efforts of ZoBell and others in investigating hydrocarbon-degrading bacteria ultimately led to industrial applications for cleaning up oily wastes. This and other contributions by ZoBell demonstrated the potential application of bacteria in environmental cleanup and paved the way for a new generation of marine microbiologists.

When ZoBell arrived at Scripps in 1932, marine microbiology as an academic discipline did not exist. By his death in 1989, he had come to be known as "the father of marine microbiology."

After receiving his doctorate in bacteriology from UC Berkeley in 1931, ZoBell joined the Scripps faculty, where he challenged the conventional wisdom that bacteria in the sea have primarily atmospheric and terrestrial origins. He studied bacteria to determine which are active in the open ocean—and to assess their possible importance as geochemical agents. The independent field of marine microbiology was born of his efforts.

Following decades of research, ZoBell concluded, "Bacteria, yeasts, fungi, microflagellates, blue-green algae, and allied microbes are widely distributed in the sea....The domain of the marine microbiologist encompasses more than two-thirds of the earth's surface and 99 percent of the hydrosphere."

ZoBell was credited with several major discoveries including the role of microorganisms in the formation and transformation of petroleum, the relationships of microorganisms to solid surfaces, the identification of 65 species of marine bacteria, and the recovery of living microorganisms from ocean depths greater than 34,000 feet (10,000 m).

Early in his career, ZoBell explored the chemical, geological, and biological effects of microorganisms in the ocean, including the role of bacteria both in the precipitation of calcium carbonate and in the formation of oil in marine sediments. His discovery that bacteria can cause oil to separate from sediments led to his acquiring a patent in 1947 for a process by which old oil fields were inoculated with bacteria to release unrecovered oil. He assigned this patent to the American Petroleum Institute for free public use. His later research shed light on how oil spills are affected by microbial action.

ZoBell published more than 300 papers, established a journal of geomicrobiology, and authored a benchmark textbook on the science of marine microbiology. During much of his career, he conducted research with his colleague and wife, Jean ZoBell, a fellow marine microbiologist.

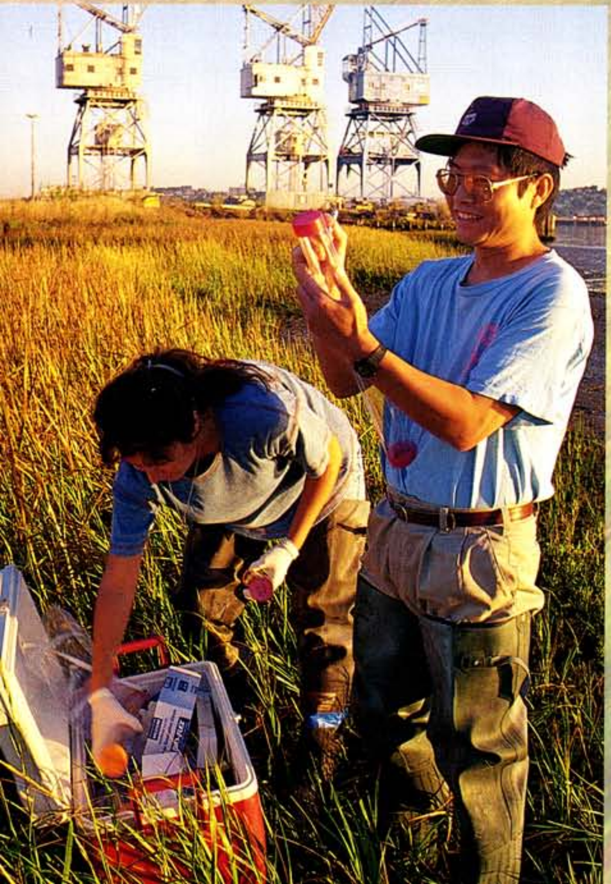
Abandoned Shipyard
Now a Natural Laboratory

EXPLORATIONS
SPRING 1998

Mare Island, near San Francisco, is a valuable field site for Scripps researcher Brad Tebo, his students, and associates. They are interested in the attenuation of toxic substances by bacteria and are investigating methods to mitigate heavy metals in marine habitats. As part of this effort, they are participating in a UC systemwide teaching effort known as the Toxic Substances Research and Training Program.

"Our goal is to rely upon natural processes in bioremediation—not to introduce exotic species of bacteria to the environment," said Scripps graduate student Meriah Arias. The approach, known as natural attenuation, is sometimes faster, cheaper, and more effective than bioengineering methods.

World War II launch of submarine USS *Whale* at Mare Island Naval Shipyard. Polluted mud samples from this site are currently studied by Scripps researchers.



Scripps postdoctoral researcher Lee He and graduate student Meriah Arias record the collection date and origin of mud cores prior to their shipment to the laboratory.

Postdoctoral researcher Lee He elaborated, "We want to look at natural processes in terms of dealing with toxic metals in the environment. The question is: How do bacteria naturally detoxify metals? That's where we've been concentrating our studies in the lab."

At the Mare Island site, the Tebo group is searching for answers to this question. Mare Island, established in the 1850s, was the oldest and largest naval shipyard west of the Mississippi at the time of its recent closure. Throughout the years, many shipbuilding technologies were implemented there, and many toxic substances associated with these technologies remain.

The yard is like a ghost town now, where once-busy cranes stand still, towering over the half-scrapped hulks of obsolete warships at a site where vast quantities of toxic material were bulldozed into the bay.

Arias reported, "We want to establish what is going on at the site. Are the metals chromium and copper, among others, being oxidized or reduced?"

Supervising this group of researchers from the Tebo laboratory at the Mare Island site is UC Davis vertebrate ecologist Mike Johnson, another participant in the Toxic Substances Research and Training Program. On an unseasonably warm autumn morning shortly after dawn, the Tebo group took mud cores on the island's tidal flats. The site, known as Green Sands Beach, is named for the color of the

Lee He and Meriah Arias take mud cores at Mare Island Naval Shipyard.

soil along the shore. The greenish cast is evidence of high concentrations of nickel-zinc slag. The abrasive slag was used to sandblast old paint off hulls and superstructures in preparation for repainting. In this environment the remnants of antifouling paint and other similar materials are a major source of toxic metals such as copper and chromium, known carcinogens.

Watching shore birds peck food on the mud flats, Johnson explained that, surprisingly, the levels of metals in the surface sediments and waters are no higher here than anywhere else on the bay. The heavy metals that have settled in the sediments to depths of a foot or more are the ones that pose the problem.

Tebo suggested that in the anaerobic zone within the site's bottom sediments, specially adapted bacteria may already be rendering these metals relatively nontoxic through natural processes of metal and sulfate reduction.

"Of course, the toxic metals are still sitting there in the sediments in high concentrations and if the sediments are dredged, exposed, or turned over, you have a potential problem. If organisms are burrowing down, they can be increasing the flux of toxic metals out of those sediments even if they can resist the metals themselves," Tebo pointed out.

Before Mare Island and other industrial sites can be declared clean enough to meet public and environmental health standards, these toxic compounds must be neutralized, reduced, or removed. Such a threat might warrant an active cleanup campaign, but the costs—in time, money, and manpower—could prove intractable, as they have in many such situations throughout the industrialized world. As an alternative, Tebo and his research group would like to understand how microorganisms might naturally detoxify the metals.

By monitoring the Mare Island site, the Tebo group may be able to predict whether toxic metals will pose a continued threat, and whether more costly remediation is needed. This is valuable information for any community looking forward to the reclamation and safe use of prime waterfront property.

Wartime construction of a naval vessel at Mare Island.

Background: wetlands near Mare Island.

Using this approach, Francis recently identified an active, manganese-oxidizing protein from a bacterium isolated from sediments collected at Point Loma in San Diego, California.

"We're very excited about having a bacterial protein with which we can do more biochemistry. Having the genes allows us to do genetic manipulations," commented Tebo.

During these manipulations, engineered genes can be reintroduced into the same bacterium in an effort to enhance manganese oxidation. In addition, the metal affinity or the specificity of the genes potentially can be altered so other metals also may be oxidized. Tebo explained that even the rates at which the proteins work and the quantities of metals oxidized can be engineered. "It's opened up a whole new avenue of research that we didn't think would be possible five years ago," he said.

According to Tebo, there are two aspects to bioremediation of metals in the environment. One is biotechnology, in which bacteria transform metals from a soluble form to a precipitated form, selectively removing targeted toxic metals while leaving nontoxic metals in solution. The other is separation and recovery of the precipitated metals. The ultimate goal is not just the removal of toxic metals from a particular environment, but their separation and recovery in more or less pure quantities. "Obviously, if the metal can be recovered then you have the potential to recycle it, and that benefits us all the way around," observed Tebo.

Researchers are exploring various technologies for recovering metal precipitates. One common recovery method uses the same type of filtration technology as in waste-water treatment. Another relies upon the magnetic properties of the targeted metals to selectively remove them from the environment.

"...if the metal can be recovered then you have the potential to recycle it, and that benefits us all the way around."

In addition to the bacterial oxidation of metals, Tebo's group utilizes metal reduction by bacterial processes for bioremediation. Bacteria acquire a metal that is soluble in its oxidized form and reduce it, making it less soluble and therefore less of a threat to the environment. One example is the oxidized form of chromium (chromium VI), a very toxic and highly soluble by-product of industrial electroplating processes. The reduced form of chromium (chromium III) is less toxic and largely insoluble. Tebo states that microorganisms can reduce chromium VI to chromium III and thereby detoxify it. The same is true of uranium VI, used in the nuclear industry. It too is very soluble, but can be reduced by bacteria to uranium IV, which readily precipitates.

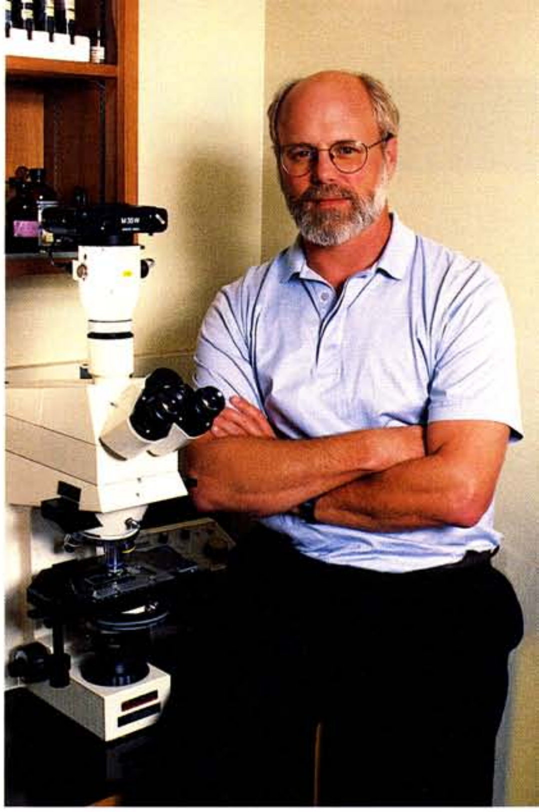
Chromium VI, a carcinogen, is injected into a bacterial culture (above) to test the bacteria's capacity for reducing the toxin to a nontoxic state. Lee He (right) uses a bag purged of air and filled with nitrogen to maintain the anaerobic state found beneath the mud flats from which the core (in bag) was taken.

In the marine environment, many metals occur in anaerobic (oxygen-free) environments such as the sediment layers of bays and estuaries. These coastal areas often are the sites of industrial development, a common source of toxic substances.

In studying bacterial reactions that lead to the formation of nonsoluble metals, the Tebo group is examining the processes of metal precipitation by sulfate-reducing bacteria.

Unlike manganese-oxidizing bacteria, sulfate-reducing bacteria are anaerobic. Thriving in oxygen-free surroundings, sulfate-reducing bacteria enhance the reduction and precipitation, and therefore detoxification, of metals. Hydrogen sulfide, the source of that rotten-egg smell often encountered in tidelands, is a product of sulfate reduction. Hydrogen sulfide is highly reactive with metals, forming insoluble compounds like the iron sulfide that blackens submerged surfaces in bays and estuaries.

Tebo pointed out, "If you can form a metal sulfide from the hydrogen sulfide, that effectively takes the metal out of solution. Iron sulfide tends to be one of the more soluble metal sulfides. Most of the others, such as copper sulfide and zinc sulfide, are even less soluble. Those metals can be removed very effectively through sulfate reduction."



Research by Tebo (above) and colleagues may contribute to bioremediation of polluted wetlands such as Green Sands Beach, on Mare Island (left).

The group's investigations have shown that some bacteria may actually increase their metabolic rates as a result of these anaerobic processes, using certain metals just as humans use oxygen in respiration. According to Tebo, this raises an important question: Do such natural processes contribute significantly to the detoxification of metals in the environment?

How and to what degree bacteria might naturally detoxify metals is now a focus of the group's laboratory research. By observing in the laboratory the extent to which the organisms can carry out metabolic processes, the researchers are determining what to look for in the environment.

"We want an accurate picture. In the lab, you cannot be absolutely sure of the situation in nature, but you can achieve results that are a very reliable estimate," assured postdoctoral researcher Anna Obraztsova.

Tebo elaborated, "We want to understand how the microorganisms might naturally detoxify the metals. Although these organisms occur naturally, they wouldn't be naturally exposed to toxic metals from pollution."

He described a possible scenario. When there is a toxic metal input as a result of pollution, it impacts bacterial populations. Only those bacteria that can resist toxic metals survive in this environment and fulfill their roles as decomposers. If they not only survive, but actually use the toxic metals to generate energy—as laboratory tests have suggested—the benefits to the bacteria may be considerable. If this is the case, then not only do the bacteria benefit from the energy they acquire by detoxifying metals, but their habitat benefits because the toxic metals are reduced.