Environmental Toxicology and Chemistry, Vol. 2, pp. 343-351, 1983
Printed in the USA. Pergamon Press Ltd.

0730-7268/83 $3.00 + .00

RESPONSE OF A SALT MARSH MICROBIAL COMMUNITY TO INPUTS OF HEAVY METALS: AEROBIC HETEROTROPHIC METABOLISM

ANNE E. GIBLIN* and MICHAEL PIOTROWSKI

Boston University Marine Program
Marine Biological Laboratory
Woods Hole, Massachusetts 02543

BRUCE LEIGHTY

Dartmouth College
Hanover, New Hampshire 03755

IVAN VALIELA

Boston University Marine Program
Marine Biological Laboratory
Woods Hole, Massachusetts 02543

JOHN M. TEAL

Woods Hole Oceanographic Institution
Woods Hole, Massachusetts 02543

(*Received* 28 *September* 1982; *Accepted* 10 *April* 1983)

Abstract — The addition of a sterilized, sludge-based fertilizer to experimental salt marsh plots increased both the nitrogen and the heavy metal content of the surface sediment. Aerobic heterotrophic microorganisms collected from these plots differed from microbes collected from control plots in their oxygen uptake rates on a uniform sediment medium both in the presence and absence of metals. When metals were not present, microbes from fertilized plots took up significantly less oxygen that did microbes from control plots. In the presence of metals, oxygen uptake by microbes from both plots was depressed; however, activity of microbes from the fertilized plots was significantly less inhibited than was that of microbes from the control plots, indicating a greater degree of metal tolerance in the former.

To assess the effect of a metal on the microbial community in the absence of nutrient enrichment, chromium solutions were applied to several 1-m^2 marsh plots. Microbes from these plots developed a tolerance to both copper and chromium within 1 month. The oxygen uptake on sediment media in the absence of metals did not differ from that demonstrated by nonmetal-tolerant microbes.

Keywords — Heavy metals Metal resistance Bacteria Salt marshes Sewage sludge

INTRODUCTION

Changes in the level of decomposer activity are of special interest in salt marsh

*To whom correspondence may be addressed.
This is Contribution No. 4865 of the Woods Hole Oceanographic Institution.

ecosystems, where most of the energy fixed by marsh grasses passes through the decomposer food chain [1]. The majority of consumers in marshes rely on detritus for at least part of their diet. Little is known about the effect of heavy metals on marsh food webs, even though heavy metals are currently entering marshes through sewage, dredging, leaching from adjacent landfills and industrial discharge [2].

As part of a study to examine the fate of metals and nutrients introduced into salt marshes, we have been adding a sewage sludge fertilizer that contains heavy metals to experimental plots in Great Sippewissett Marsh, Massachusetts. The addition of fertilizer to our plots increases grass biomass, and the fertilized grasses contain a greater percentage of nitrogen than do unfertilized grasses [3]. The grasses on the fertilized plots also have significantly higher levels of cadmium, chromium, copper and zinc than do the unfertilized grasses [4,5]. These metals, as well as several others not taken up by the grasses, such as lead and iron, show greatly increased levels in fertilized sediments [5].

Many studies have shown that heavy metal contamination may adversely affect microbial communities. Low levels of cadmium inhibit both microbial colonization and decomposition of leaf material in fresh water [6]. Forest leaf litter decomposition was considerably depressed in soils heavily contaminated with zinc by a smelter in Palmerton, Pennsylvania [7], and the numbers of bacteria, actinomycetes and fungi were also reduced in these soils [8]. Lead has been shown to severely inhibit respiration, dehydrogenase activity and decomposition in soils with low cation exchange capacities [9,10]. These studies indicate that metals may affect long-term mineral cycling and organic matter turnover in soils and sediments [7,11].

Little is known about the effect that metals in sewage sludge would have on the natural population of microorganisms in estuarine environments. Recently, using litter bags, the decomposition rates in fertilized and control salt marsh plots were examined. In spite of the high level of metals present, decomposition of grasses proceeded at least as rapidly, or faster, in the fertilized plots than in the control plots (Valiela et al., unpublished data).

A possible explanation for these results is selection for a community of metal-resistant microbes. Chronic metal addition to laboratory cultures often results in the selection of metal-tolerant microorganisms [12]. Metal-tolerant bacteria and fungi have also been isolated from natural populations, and a greater percentage of metal-tolerant microbes is present in metal-contaminated soils and sediments than is present in similar uncontaminated environments [8,13–15]. Genetic studies on pure cultures have shown that resistance to metals is often mediated by plasmids [16–18], which may also carry genes for resistance to antibiotics [16, 17]. Many metal-tolerant organisms from areas contaminated by sewage and metals also show an increased resistance to antibiotics [15,19,20].

Another possible explanation for the relatively rapid decomposition on the fertilized plots is that stimulation of microbes by nutrients in the fertilizer more than compensates for any inhibition caused by the metals. If stimulation by nutrients were the sole explanation for the apparent lack of inhibition, we would expect microbes from control and fertilized populations to have equal sensitivity to metals when grown on a low-nutrient substrate.

Finally, it is possible that the metals are sequestered in an unavailable form. If this were true, we would not expect to see selection for metal resistance among microorganisms from fertilized plots. The uptake of metals by the animals and grasses on the plots tends to argue against the metals being unavailable [5].

To evaluate these alternatives and to assess the effect of metal tolerance in microorganisms on decomposition rates, we designed a series of experiments in which we measured the effect of metals on the hetero-

trophic activity of microbes from both control and fertilized plots. Heterotrophic activity was measured by inoculating a sterile sediment medium with microbes collected from these plots and measuring oxygen uptake in biological oxygen demand (BOD) bottles in the presence or absence of either copper or cadmium. To measure the effects of metals on an unfertilized marsh community, we established additional plots in the field that received metals several times a week. We then compared the heterotrophic activity of microbes from these plots with that of microbes from control plots. These experiments also allowed us to determine how rapidly metal tolerance could appear.

METHODS

Field plots

The microbes tested for metal tolerance were isolated from control and experimental plots in Great Sippewissett Marsh, Massachusetts. The experimentally fertilized areas were established in April 1974 [3]. The two fertilized plots receive 75.5g/m^2/week of a metal-containing fertilizer made from sewage sludge. The heavy metal content of the fertilizer has varied considerably during the course of the study, ranging from 8 to 425 ppm for copper and from 5 to 200 ppm for cadmium [21]. The cumulative metal loading and the retention of metals by the plots has been reported previously [22].

Preparation of sterile sediment media

We collected surface sediment from Great Sippewissett Marsh in an area well away from the experimental plots. The mud was homogenized and frozen at −20°C. Before each experiment, a portion of the mud was thawed and autoclaved. The seawater used for these experiments was diluted to a salinity of 20 ‰ with distilled water and then filtered through a 0.45-μm Millipore filter. The water was allowed to stand overnight with activated charcoal, which removed most of the dissolved organic material. The water was refiltered once through a Whatman GF/C glass-fiber filter, once through a 0.22-μm Millipore filter and then stored at 4°C. Immediately before use, the water was autoclaved. Sterile sediment medium was then prepared by adding 1 ml of the autoclaved sediment to 300 ml of the sterile seawater in a BOD bottle. Acridine orange–direct counts of bacteria [23] showed that this procedure effectively sterilized the mud and seawater.

Inocula

We prepared the inocula by scraping sediment from the top several millimeters of the marsh surface in several locations in the short *Spartina alterniflora* zone. Sediment was removed from two fertilized and two control plots and placed in separate, acid-washed jars. Equal amounts of sediment from the two fertilized plots were then pooled and mixed well. Five cubic centimeters of this mud was removed, placed in an acid-washed Virtis homogenizing flask with 125 ml of sterile 20 ‰ seawater and homogenized for 5 min. The Virtis flask was immediately placed on a magnetic stirrer, and 1-ml aliquots of the suspension were pipetted into acid-washed 300-ml BOD bottles that contained sediment medium. The same procedure was used to prepare an inoculum from control marsh plots, and this inoculum was added to a second group of bottles. All inoculations were done within 0.5 h of each other and not more than 3 h after collection from the field.

Measurement of metal tolerance by aerobic respiration

We determined metal tolerance by comparing the aerobic respiration of microorganisms growing on sediment medium in the presence and absence of added metals. Aerobic respiration was taken as the rate of decrease of oxygen concentration in the BOD bottles. Metal-treated bottles received 1 ml of a stock metal solution before stoppering. Oxygen concentration was measured every 24 h for up to 6 d with a YSI oxygen electrode. We rinsed the oxygen electrode in sterile seawater between measurements to

prevent cross-contamination. The bottles were stirred by a magnetic stirrer while the oxygen measurements were made. The temperature was maintained at 18°C throughout the experiment.

Four experiments designed to test for metal tolerance were performed. Only copper tolerance was tested in the first experiment. We inoculated 12 BOD bottles with microbes from the fertilized plots, and inoculated 12 with microbes from the control plots. Four bottles from each of these groups received no sediment substrate and served as controls to test whether the inoculum alone accounted for a significant portion of the oxygen uptake. Four bottles from each group received the sediment medium (SED), and four received SED plus a stock solution of copper sulfate (SED + Cu), which resulted in a copper concentration of 2.5 ppm in the bottle. In addition, eight uninoculated bottles were run, four with SED and four with SED + Cu. These bottles were considered blanks, and were used to test for chemical oxygen demand and contamination. In the final three experiments, tolerance to cadmium was tested. Eight additional bottles containing SED were included in the design. Four of these were inoculated with microbes from the control plots and four received inocula from the fertilized plots. These bottles also received a stock solution of cadmium chloride (SED + Cd). The metal tolerance experiments were repeated twice with metal concentrations of 5 ppm Cu and 5 ppm Cd, and once with 10 ppm Cu and 10 ppm Cd.

Sediment analysis

Sediment used for the sediment medium, as well as that used for the inoculations, was analyzed for numbers of bacteria and content of water, nitrogen, carbon, organic matter and metals (Cd, Cu and Cr). Bacteria were enumerated using the acridine orange–direct counting technique [23]. Water content was measured as weight loss after drying for 3 d at 60°C. Organic matter was estimated by measuring the loss on ignition after ashing at 550°C for 24 h. Metals were analyzed by wet-ashing the sediment with nitric acid at 60°C for 6 h. Residual organic matter was oxidized with 30% hydrogen peroxide. The samples were filtered through glass-fiber filters, diluted with distilled water and the metals measured by atomic absorption spectrometry [5]. Carbon and nitrogen were analyzed on a Perkin-Elmer HCN analyzer.

Field metal additions

To test the effect of metals without nutrients on microbial activity, four 1-m^2 plots were set up in a uniform stand of short *Spartina alterniflora.* Two plots served as controls. Four liters of a 10-ppm chromium solution (as $Cr_2(SO_4)_3 \cdot 15H_20$) was applied three times a week at low tide for 4 weeks to the other two plots. Mud for the inocula was collected from the plots before the additions began and on the fourth and tenth days of the experiment. The microorganisms from the plots were tested for heterotrophic activity on sediment medium alone. After 4 weeks, the microbes were tested for tolerance to chromium and copper. This allowed us to see if metal resistance could develop rapidly in natural populations and if the resistances would be specific for the metal added or of a more general nature.

RESULTS

The application of a metal-containing fertilizer to the treated plots has increased both the nitrogen and the heavy metal content of the sediment (Table 1). Fertilized sediment contained one order of magnitude more copper, cadmium and chromium than sediment from control plots. The nitrogen content of control sediments was about 1.1% by weight, compared with 3.5% in sediments from the fertilized plots. The numbers of bacteria and the water content of the fertilized sediment were slightly higher than in control sediments, but the differences were not significant (*t* test,

Table 1. Comparison of the physical and chemical characteristics of the control and fertilized sediments, and the sediment media used for the experiments

	Fertilized sediment		Control sediment		Substrate (autoclaved)
% Organic matter (gdw)	55 ± 5		38 ± 6	*	31 ± 4
% Nitrogen	3.50 ± 0.03		1.06 ± 0.1	*	1.01 ± 0.2
% Water/cc	80 ± 9	*	71 ± 11		—
Carbon/nitrogen	6.6		13.5	*	14.0
Cd (ppm/gdw)	25 ± 5		2.0 ± 0.4	*	1.8 ± 0.3
Cu (ppm/gdw)	680 ± 50		38 ± 10	*	42 ± 8
Cr (ppm/gdw)	500 ± 42		10 ± 4	*	8 ± 3
No. bacteria/gdw	2.1×10^{10}	*	1.8×10^{10}		0

Values are means ± SE, n = 4. An asterisk between columns indicates samples not significantly different ($p \leq 0.05$). gdw, grams/dry weight.

$p \leq 0.05$). The sediment used for the sediment medium in the experiments was not significantly different in metal or nutrient content from sediment in control plots.

When sterile sediment medium was inoculated with microbes from either plot, there was a rapid uptake of the oxygen in the bottle. Sediment media without inocula exhibited no oxygen uptake for the first 48 h, indicating that no chemical oxygen demand was present. Oxygen uptake at the end of the experiment by these sterile controls was normally < 1 to 1.5 ppm. Since these bottles did not exhibit oxygen uptake for the first 24 to 72 h, we believe that oxygen consumption was not due to chemical oxygen demand but rather to contamination that occurred while taking the oxygen measurements. Low numbers of bacteria were present in these bottles at the end of the experiment. Bottles which received the inoculum without sterile sediment medium had oxygen uptakes of < 1 ppm over the course of the experiment. There was no significant difference (*t* test, $p \leq 0.05$) in oxygen uptake of microbes collected from the treated and control plots within these substrate-free controls. We conclude, therefore, that most of oxygen uptake in the experimental bottles was due to microbes growing on the sediment medium.

When growing on sediment medium without metals, microbes from control plots used significantly more oxygen than did those from the fertilized plots (Fig. 1). This result was seen in all four experiments, and the difference in oxygen uptake between microbes from the control and fertilized plots was significant (ANOVA, $p < 0.05$). The addition of 10 ppm Cu to sediment medium reduced the oxygen uptake in both groups; however, there was a greater reduction in uptake by microbes from the control plots (Fig. 1). The inhibition of heterotrophic activity due to metals was expressed as net reduction in oxygen uptake. The net reduction of oxygen uptake due to metals was calculated as the difference between oxygen

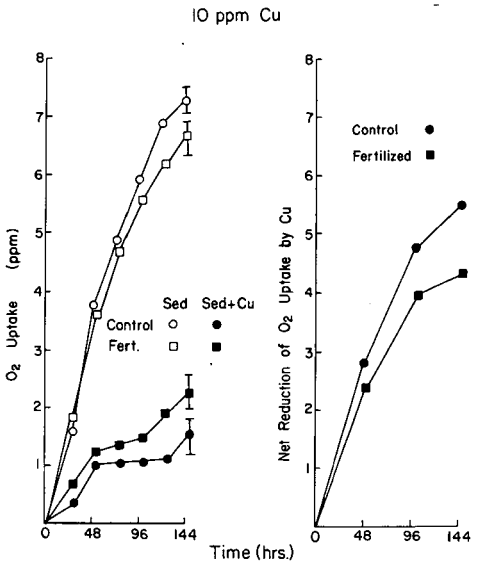

Fig. 1. *Left*: Oxygen uptake by microbes from the control and fertilized plots in the presence and absence of 10 ppm Cu (X ± SE; n = 4). *Right*: Net reduction of oxygen uptake due to the addition of copper for the control and fertilized microbes. Net reduction is calculated by subtracting the oxygen uptake in the presence of copper from the oxygen uptake when no metals are present.

uptake when microbes were grown on sediment media alone and oxygen uptake when metals were added. The net reduction of oxygen uptake caused by copper addition was consistently greater for the control group than for the fertilized group (Fig. 1). The same result was observed in bottles containing 10 ppm Cd (Fig. 2). Microbes from the fertilized and control plots were significantly different in their response to 10 ppm Cd or 10 ppm Cu (ANOVA, $p \leq 0.05$).

At all metal levels tested, microbes from the control plots took up less oxygen than did those from the fertilized plots with metals added. The net reduction in oxygen uptake due to the metals after 144 h of incubation is shown in Figure 3. In the first experiment, only copper was tested, whereas, in subsequent experiments, both copper and cadmium were tested. Copper always proved inhibitory to microorganisms from fertilized and control plots. The control group always showed a greater degree of inhibition than did the microbes from the fertilized plots, although the difference was not significant at the lower copper levels. The degree of inhibition increased with increasing copper concentration for both groups.

Cadmium was always inhibitory to microbes from control plots, but 5 ppm Cd seemed to enhance oxygen uptake in microbes from the fertilized plots (Fig. 3). This result was unexpected, since cadmium has no known physiological function. Sterile controls did not show this effect, so chemical oxygen demand due to cadmium can be ruled out. Ten parts per million cadmium depressed both groups, but microbes from the control plots again showed the greatest inhibition. The results of all four experiments show that aerobic heterotrophic microbes from the fertilized plots have significantly higher rates of oxygen uptake in the presence of metals than do their counterparts in the control areas (Wilcoxon rank sign test for paired observations, $p \leq 0.02$).

Microbes from field plots that received chromium showed no difference in their oxygen uptake on sediment medium, compared with microbes from control groups (Fig. 4). The oxygen uptake of both groups was not significantly different, and did not change over time (ANOVA, $p < 0.05$). When copper or chromium was added to the

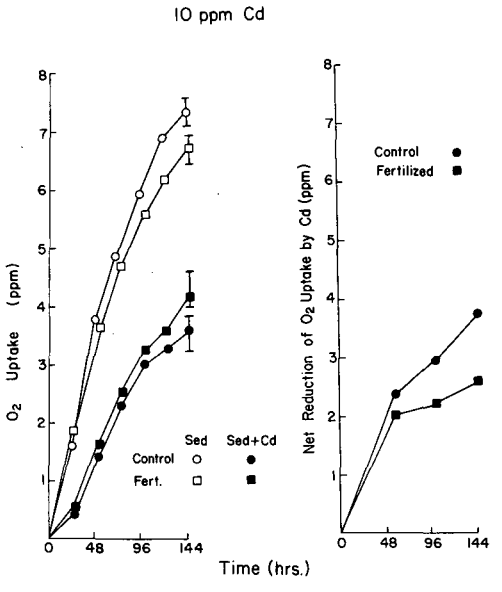

Fig. 2. *Left*: Oxygen uptake by microbes from the control and fertilized plots in the presence and absence of 10 ppm Cd (X ± SE; n = 4). *Right*: Net reduction of oxygen uptake due to addition of cadmium for the control and fertilized microbes. Net reduction is calculated by subtracting the oxygen uptake in the presence of cadmium from the oxgen uptake when no metals are present.

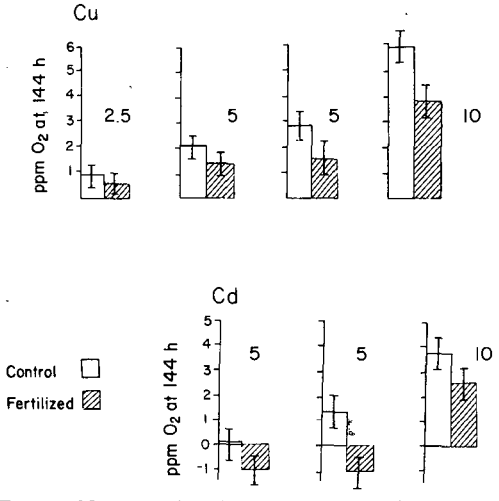

Fig. 3. Net reductions in oxygen uptake after 144 h due to addition of copper or cadmium for the four experiments (X ± 95% confidence limits; n = 4).

sediment medium, microbes from the chromium-treated plots had a greater oxygen uptake than did microbes from the control plots (Fig. 5). Microbes from chromium-treated plots were significantly more tolerant of metals than were those from control plots (ANOVA, $p \leq 0.05$).

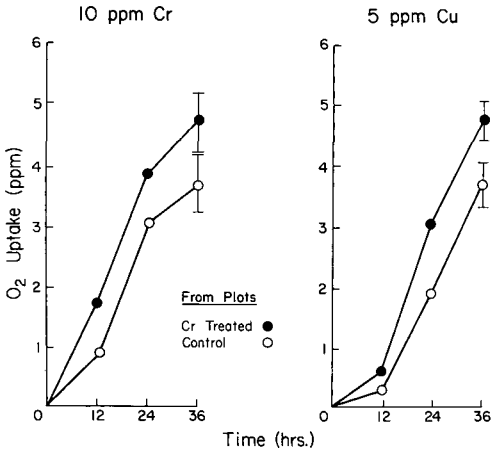

Fig. 5. Oxygen uptake by microbes from the chromium-treated and control plots in the presence of either 10 ppm Cr or 5 ppm Cu after 1 month of chromium treatment (X ± SE; n = 6).

DISCUSSION

Other investigators have reported that the addition of sewage to an area can increase the percentage of metal-tolerant bacteria and fungi in sediments [15]. Since the fertilizer applied to the marsh is dried and sterilized, our work shows that this tolerance can develop *in situ* and need not be the result of differential survivorship of tolerant organisms already present in the sludge. Three mechanisms could be contributing to the metal tolerance of fertilized microbes that we observed in our experiments: greater survival among microbes in the initial inoculum, greater respiration per cell or higher growth rates in the presence of metals. Our experiments did not allow us to distinguish between these mechanisms; however, greater numbers of metal-resistant bacteria can be plated from the fertilized plots [24,25]. This indicates that differential survival of microbes exposed to metals may be important in explaining the differences in oxygen uptake.

When metals were not present, microbes from the fertilized plots had lower oxygen uptake rates on the sediment medium than did microbes from the control plots. It is possible that this effect is directly linked to the increased metal tolerance shown by these organisms. However, if metal tolerance alone were responsible, metal-tolerant microbes from the chromium-treated plots would also show lower oxygen uptake on

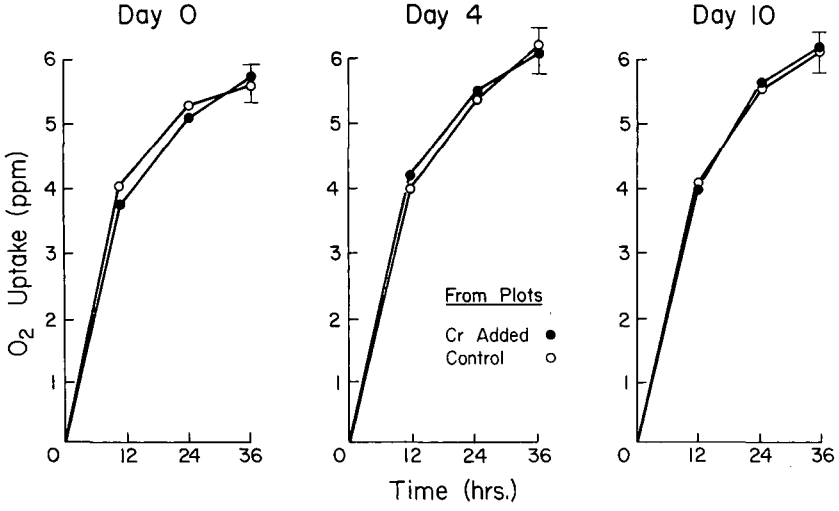

Fig. 4. Oxygen uptake on sediment medium alone by microbes from control and chromium-treated plots before additions of chromium (day 0), after 4 d of chromium treatment and after 10 d of chromium treatment (X ± SE; n = 6).

sediment media when compared to controls. This is clearly not the case (Fig. 4). The sediment medium is similar in nitrogen and carbon content to the sediment from control plots (Table 1). It is probable that the microbes from the fertilized plots have adapted to the more eutrophic conditions found in their habitat, and so cannot use the substrate containing less nitrogen and carbon as effectively as microbes from control plots. Other investigators, using plate counts, have shown that there is a difference in the microbial communities in the two plots [25]. Proteolytic, cytophaga-like bacteria are the predominant mercury-resistant bacteria in fertilized plots, and these may require different carbon and nitrogen sources than do psuedomonads, which are the predominant mercury-resistant bacteria in control plots [25; Hamlett et al., unpublished data].

The microbes from the chromium-treated plots exhibited tolerance to both copper and chromium. This is consistent with other findings that resistances to a number of heavy metals may be linked and need not be specific to the metal stress received [16]. Resistance in the field also developed quickly, within 1 month of the chromium additions. However, the adaptation to the metals may continue for several years. When bacteria were isolated on a nutrient-rich Zobell (2216E) agar that contained 10^{-3}M Cd, more colonies of cadmium-resistant bacteria could be isolated from plots that had received sludge fertilizer for 6 years than from plots that had received sewage fertilizer for only 1 year [24]. No cadmium-resistant colonies were isolated from the control area.

As expected, inhibition of activity occurred at low levels of added soluble metal, compared to the total concentration of metal present in the sediment. This is because the majority of the metals are complexed or precipitated as solids in the sediment, and only a small portion is available to the microorganisms. Samples of interstitial water from our marsh plots showed that, in control areas, soluble copper seldom exceeds 20 ppb and the soluble cadmium never exceeds 1 ppb [21]. Soluble copper and cadmium levels in the fertilized plots were several times greater than those in the control plots, although they were still in the microgram per liter range. The metal concentrations used in our experiments are considerably greater than the pore water metal concentrations; however, most of the added metal becomes complexed [21]. Complexation greatly reduces the toxicity of metals in solution [26,27]. Some of the chemical processes controlling metal availability in the experimental plots have been described previously [21].

In conclusion, we have demonstrated that the addition of a sludge-based fertilizer alters the aerobic heterotrophic microbial community in salt marsh sediments. The microbial communities on the fertilized and control plots differ both in their aerobic respiration rates on control (low nitrogen, low metals) sediment medium and in their respiration rates in the presence of metals. The lower oxygen uptake rates of the microbes from fertilized plots growing on control sediment medium are apparently caused by nutrient limitation, since the addition of metals alone in the field does not select for this difference. The addition of metals to field plots, with or without nutrients, leads to a microbial community that exhibits a greater degree of metal tolerance. These findings are consistent with those of other investigators using plate counts on the same plots [24,25; Hamlett et al., unpublished data].

Acknowledgement — We would like to thank K. Foreman, S. Gutterman, T. Lyons Corliss and M. Jordan for critically reviewing earlier versions of this manuscript and N. Hamlett for sharing her unpublished data with us. We are indebted to the Arnold Gifford family for the use of their land in Great Sippewissett Marsh. Financial support from NSF grant DEB 7905127 BSR 8217040, NOAA Office of Sea Grant, Grant No. 04-8-NOI-149, is gratefully acknowledged.

REFERENCES

1. **Teal J.M.** 1962. Energy flow through a Georgia salt marsh. *Ecology* **43**:614-624.

2. **Galloway J.N.** 1979. Alteration of trace metal geochemical cycles due to the marine discharge of wastewater. *Geochima. Cosmochima. Acta* **43**: 207-218.
3. **Valiela I., J.M. Teal** and **W.J. Sass**. 1975. Production dynamics of salt marsh vegetation and the effects of experimental treatment with sewage sludge. *J. Appl. Ecol.* **12**:973-982.
4. **Banus M.D., I. Valiela** and **J.M. Teal**. 1975. Pb, Zn, and Cd budgets in experimentally enriched salt marsh ecosystems. *Estuarine Coastal Marine Sci.* **3**:421-430.
5. **Giblin A.E., A.E. Bourg, I. Valiela** and **J.M. Teal**. 1980. Uptake and cycling of heavy metals in sewage sludge in a New England salt marsh. *Am. J. Bot.* **67**:1059-1068.
6. **Giesy J.P.** 1978. Cd inhibition of leaf decomposition in an aquatic microcosm. *Chemosphere* **6**:467-475.
7. **Strojan C.L.** 1978. Forest leaf litter decomposition in the vacinity of a Zn smelter. *Oecologia* **32**:203-212.
8. **Jordan M.** and **M. Lechevalier**. 1975. Effects of Zn smelter emissions on forest soil microflora. *Can. J. Microbiol.* **21**:1855-1865.
9. **Doelman P.** and **L. Haanstra**. 1979. Effects of Pb on the decomposition of organic matter. *Soil Biol. Biochem.* **11**:481-485.
10. **Doelman P.** and **L. Haanstra**. 1979. Effect of Pb on soil respiration and dehydrogenase activity. *Soil Biol. Biochem.* **11**:475-479.
11. **Ruhling A.** and **G. Taylor**. 1973. Heavy metal pollution and decomposition of spruce needle litter. *Oikos* **24**:402-416.
12. **Bitton G.** and **U. Freihofer**. 1978. Influence of extracellular polysaccharides on the toxicity of Cu and Cd toward *Klebsiella aerogenes. Microb. Ecol.* **4**:119-125.
13. **Doelman P.** and **L Haanstra**. 1979. Effects of Pb on the soil bacterial microflora. *Soil Biol. Biochem.* **11**:487-491.
14. **Tan T.L.** 1980. Effects of long term Pb exposure on the seawater and sediment bacteria from heterogenous flow cultures. *Microb. Ecol.* **5**:295-311.
15. **Timoney J.F., J. Port, J. Giles** and **J. Spanier**. 1978. Heavy metal and antibiotic resistance of the bacterial flora in the sediment of the N.Y. bight. *Appl. Environ. Micorbiol.* **36**:465-470.
16. **Gadd G.M.** and **A.J. Griffiths**. 1978. Microorganisms and heavy metal toxicity. *Microb. Ecol.* **4**:303-317.
17. **Kondo I., H. Nakahama** and **J. Ishikowa**. 1975. The possibility of a specific plasmid mediating metal resistances. In S. Mitsuhashi and H. Hashimoto, eds., *Microbial Drug Resistance,* Tokyo Press, Tokyo, pp. 145-152.
18. **Novick R.P.** and **C. Roth**. 1968. Plasmid linked resistance to inorganic salts in *Staphlococcus aureus. J. Bacteriol.* **95**:1335-1342.
19. **Grabow W.O.K., D.W. Prozesky** and **L.S. Smith**. 1974. Drug resistant coliforms call for a review of water quality standards. *Water Res.* **8**:1-9.
20. **Olson B.H., T. Barkay** and **R.R. Colwell**. 1979. Role of plasmids in Hg transformations by bacteria issolated from the aquatic environment. *Appl. Environ. Micro.* **38**:478-485.
21. **Giblin A.E.** 1982. Uptake and remobilization of heavy metals in salt marshes. Ph. D. thesis. Boston University, Boston, MA.
22. **Giblin A.E., I. Valiela** and **J.M. Teal**. 1983. The fate of heavy metals introduced into a New England salt marsh. *Water Air Soil Pollut.* **20**:81-98.
23. **Daley R.J.** and **J.E. Hobbie**. 1975. Direct counts of aquatic bacteria by a modified epifluorescence technique. *Limnol. Oceanogr.* **20**:875-882.
24. **Mollura F.** 1980. Cadmium resistance in bacteria from the Great Sippewissett Marsh. *Biol. Bull.* **159**: 463.
25. **Hamlett N.V., J.S. Poindexter** and **W.S. Reznikoff**. 1981. Selective pressures exerted by sewage sludge fertilization on chemoheterotrophic bacterial communities in the Great Sippewissett Marsh. *Biol. Bull.* **161**:326.
26. **Gillispe P.A.** and **R.F. Vaccaro**. 1978. A bacterial bioassay for measuring the copper-chelation capacity of seawater. *Limnol. Oceanogr.* **23**:543-548.
27. **Sunda W.G.** and **J.M. Lewis**. 1978. Effect of complexation by natural organic ligands on toxicity of copper to a unicellular algae, *Monochrysis. Limnol. Oceanogr.* **23**:870-816.