JA - 12

Chemosphere No. 6, pp 467 - 475.

0045-6535/78/0701-0467$02.00/0

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CADMIUM INHIBITION OF LEAF DECOMPOSITION IN AN AQUATIC MICROCOSM

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(Received in The Netherlands 12 May 1978; received in UK for publication 22 May 1978)

ABSTRACT

The effect of 5 and 10 µg/l Cd on colonization of leaf material by fungi and bacteria and leaf decomposition was studied. Leaf decomposition packs were incubated in stream microcosms for 28 weeks. Decomposition was determined by weight loss and surfaces examined for microbial colonization by scanning electron microscopy. Both 5 and 10 µg/Cd inhibited both microbial colonization of leaf material and leaf decomposition.

INTRODUCTION

Increased releases of heavy metals to the environment due to anthropogenic perturbations pose a threat of undetermined magnitude. Cadmium (Cd) is highly toxic to all components of aquatic communities (8,9,10,23,14,17). Aquatic biota have evolved with very low Cd levels present naturally in the environment so that mobilization of this element into the biosphere has high potential for biological disruption.

Prior to human perturbation, most streams and rivers were densely covered with vegetation. Shielding from direct sunlight and the structure of stream channels fostered the development of a heterotroonic based system. The dominant energy source of small woodland streams is allochthanous input.(21) Only a small portion of the energy contained in leaf material is directly available to aquatic animals.(6) The animal and microbial components of the stream community have evolved to process these inputs, with the animal community relying on microorganisms to degrade recalcitrant plant substances such as lignin and cellulose. The microbial proteins, fats and carbohydrates are then readily available to animals which feed on them.(16) Many stream dwelling invertebrates prefer to eat partly decomposed, or conditioned, rather than freshly fallen leaves and may feed on the leaves to acquire highly nutritious fungal cells.(6,12,20) Because of the importance of fungi and bacteria as intermediaries in leaf litter processing, their inhibition in streams could mean a drastic change in community structure and decreased secondary productivity. While the toxic and inhibitory properties of heavy metals to aquatic microbes have been studied, little is known about the effects of low levels of these toxicants on the colonization and leaf litter decomposition by microbial communities.

This study was part of a program designed to determine the biological effects of low levels of Cd. (drinking water standards and below). Since leaf litter processing is important in lotic aquatic systems, this function was chosen as a critical function to be protected to maintain ecosystem integrity.

MATERIALS AND METHODS

Fresh leaf material was placed in 0.3 cm mesh, 15.2 cm square stainless steel envelopes. These envelopes were tied at the top and sides such that 1.0 cm openings remained on each side. Leaf material was placed into each envelope in the order given in Table 1. Two each of Type I and Type II (Table 1) envelopes were suspended 10 cm above the bottom in each of the tail pools of the U. S. EPA-ERDA stream microcosm facility. This facility is located on the ERDA Savannah River Project, near Aiken, South Carolina and consists of six concrete block channels 91.4 m long, 0.6 m wide and 0.3 m deep with pools 1.5 m wide, 3 m long and 0.91 m deep, located at both ends of each channel. The interior of each channel and pool is lined with black PVC plastic, with a silicon sand substratum over the plastic in the channels and high organic content silt from a local stream in the head and tail pools. Each channel was colonized by aquatic macrophytes, periphyton, micro and macro invertebrates and fish. Water from the Tuscaloosa aquifer was limed to produce water inorganically similar to natural surface water in the area (Table 2). Flow rate to each channel was 94.6 ℓ/min. Flow through the channels is low, approximately 1.3 cm/sec. Cadmium chloride was metered into four of the channels using peristaltic pumps calibrated daily, to maintain constant concentrations of 5 µg/l Cd in two and 10 µg/l in the other two. Background Cd concentration in control channels was 0.02 µg/l. Actual Cd concentrations in the water were monitored by atomic absorption analyses.

Table 1. Initial leaf material in leaf litter packs.

SPECIES		WET WEIGHT ADDED TO EACH ENVELOPE (g)
TYPE I		
Pinus taeda L.		5.0
Sassafras albidum (Nutt.) Nees.		3.0
Quercus nigra L.		3.0
Quercus laurifolia Michx.		2.0
Prunus americana Marsh.		2.0
Acer rubrum L.		2.0
	TOTAL	17.0
TYPE II		
Acer rubrum L.		3.0
Quercus nigra L.		3.0
Prunus americana Marsh.		2.0
	TOTAL	8.0

Table 2. Inorganic constituents of treated well water.

Parameters	Treated Well Water
Alkalinity (mg/l as $CaCO_3$)	9.7
Hardness (mg/l as $CaCO_3$)	10.7
pH	6.5
Specific conductance (μmho)	31.0
Cl (mg/l)	2.8
Total PO_4 (μg/l as P)	3.8
Ca (μg/l)	3,165
Cu (μg/l)	3.4
Co (μg/l)	2.5
Cd (μg/l)	0.023
Cr (μg/l)	0.3
Fe (μg/l)	16.9
K (μg/l)	1,130
Mg (μg/l)	246
Mn (μg/l)	7.0
Na (μg/l)	1,790
Ni (μg/l)	3.2
Pb (μg/l)	2.9

Leaf material was incubated in the Tail Pools for 28 wk between 28 April and 22 November, 1976. Leaf material was removed and examined for macroinvertebrates. Total dry weight biomass was determined after drying at 85°C for 96 hr. Multiple undried samples of each leaf type were fixed in 2% Glutaraldehyde-0.1 M cacodylate buffer. Leaf samples were dehydrated by serially washing 15 min in 70%, 85%, 95% and 100% (twice) ethyl alcohol. Dehydrated material was critical point dried and mounted on aluminium stubs and gold coated for scanning electron microscopy. Each species was examined for fungal and bacterial colonization and permanent records made.

Leaf material was wet ashed with redistilled HNO_3.(2,5) Metal concentrations in water and leaf material were determined by flameless and flame atomization using a Perkin-Elmer model 306 atomic absroption spectrophotometer equipped with an HGA-2100 flameless atomizer and deuterium background corrector. Matrix interferences were evaluated by standard additions and measurements at nonabsorbing wavelengths and comparison to U. S. National Bureau of Standards orchard leaves.

Alkalinity and hardness were measured titrametrically.(2) Hydrogen ion and specific conductance were measured with a Hydrolab Surveyor model 5D. Calcium, K^+ and Na^+ in water were determined by atomic emission.

The experimental design was a randomized nested design with two treatments (5 and 10 μg/l Cd) and a control. There were two replicates of each treatment channel with two replicates of each leaf pack type in each channel resulting in four replicates of each leaf pack type per treatment. Results were analyzed by standard Analysis of Variance Techniques and significance of differences between means tested, using Tukeys'-w procedure.(26)

RESULTS AND DISCUSSION

Both 5 and 10 µg/l Cd significantly reduced leaf decomposition of type I and II leaf packs (Table 3). There was no significant difference in leaf decomposition between Cd treated channels for either litter pack type. The ratio between initial live weights and final dry weights of type I and type II leaf packs were 7.4 and 7.3 respectively.

Visual inspection of leaf material removed from the leaf packs, after 28 wk incubation revealed that leaves in 5 and 10 µg/l Cd had deteriorated much less than those in control water. Leaf material in control packs was brown in color and many of the leaves had only veins and petioles remaining. Leaves in the Cd treatments were green and completely intact. Microscopic examination revealed the intact structure of leaf surfaces, including leaf hairs and stomates. Within the controls, the order of resistance to decomposition, from least to greatest, was: S. albidum, P. americanum, A. rubrum, Q. laurifolia, Q. nigra and P. taeda. Although overall decomposition was reduced by the presence of Cd, S. albidum and P. americana were the most succeptible to decomposition, in the presence of Cd.

Table 3. Effect of Cd on final biomass of leaf material in leaf litter packs. $\bar{X} \pm 2$ SD.

TREATMENT	DRY WEIGHT (g)	
	Type I	Type II
CONTROL	2.3 ± 0.18	$1.1 \pm 0.08_b$
5 µg/L Cd	4.0 ± 0.06^a	1.7 ± 0.19^b
10 µg/L Cd	4.0 ± 0.73^a	1.7 ± 0.11^b

^{a,b}not significantly different from one another, $n = 4$, $\alpha = 0.05$.

Few macroinvertebrates were found in the leaf packs. Two species of Odonata, Erythrodiplax minuscula Rambur and Ishnura sp., one species of snail, Limnea sp. and one species of flatworm were recovered from the leaf packs suspended in control channels. The only macroinvertebrate recovered from leaf packs incubated in treatment channels were flatworms.

Both 5 and 10 µg/l Cd inhibited microbial colonization of leaf surfaces (Figs. 1 and 2). Examination of leaf surfaces, using scanning electron microscopy (SEM) revealed the surfaces of leaves which had been suspended in treatment channels were almost devoid of microbial colonization, while the surfaces of leaves from control channels were well colonized. There were no apparent differences in colonization of the upper and lower leaf surfaces or position along the axes of pine needles.

Relatively little Cd was accumulated by leaf material suspended in the channels (Table 4). Uptake by leaf material was directly proportional to Cd concentration in the water.

Figure 1. Effect of Cd on microbial colonization of P. taeda. A, control; B, 10 µg/l Cd; C, control; D, 10 µg/l Cd; E, control; F, 5 µg/l Cd.

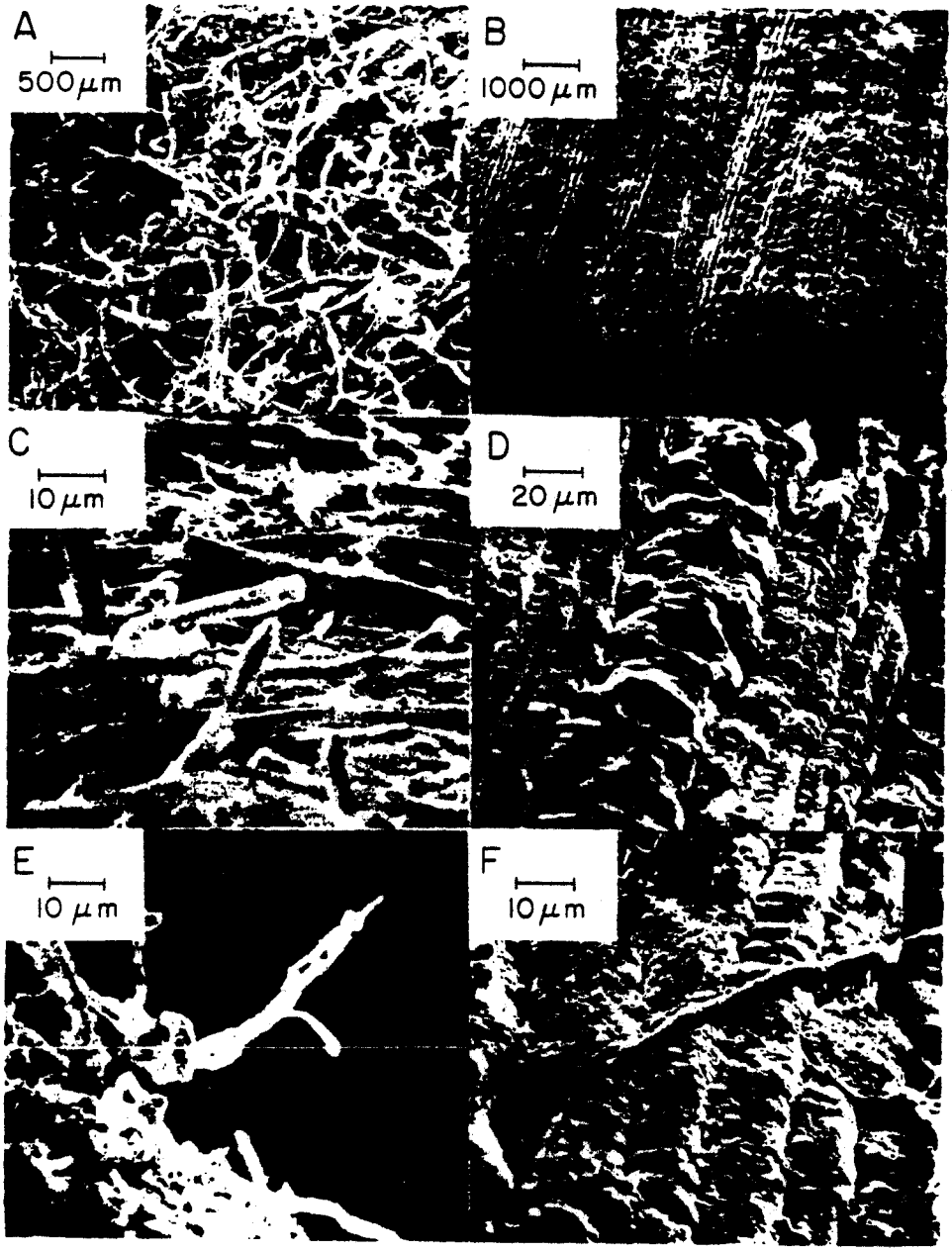

Figure 2. Effect of Cd on microbial colonization of Q. nigra and P. americana leaves. A, Q. nigra control; B, Q. nigra 10 µg/l Cd; C, Q. nigra control; D, Q. nigra 5 µg/l Cd; E, P. americana control; F, P. americana 5 µg/l Cd.

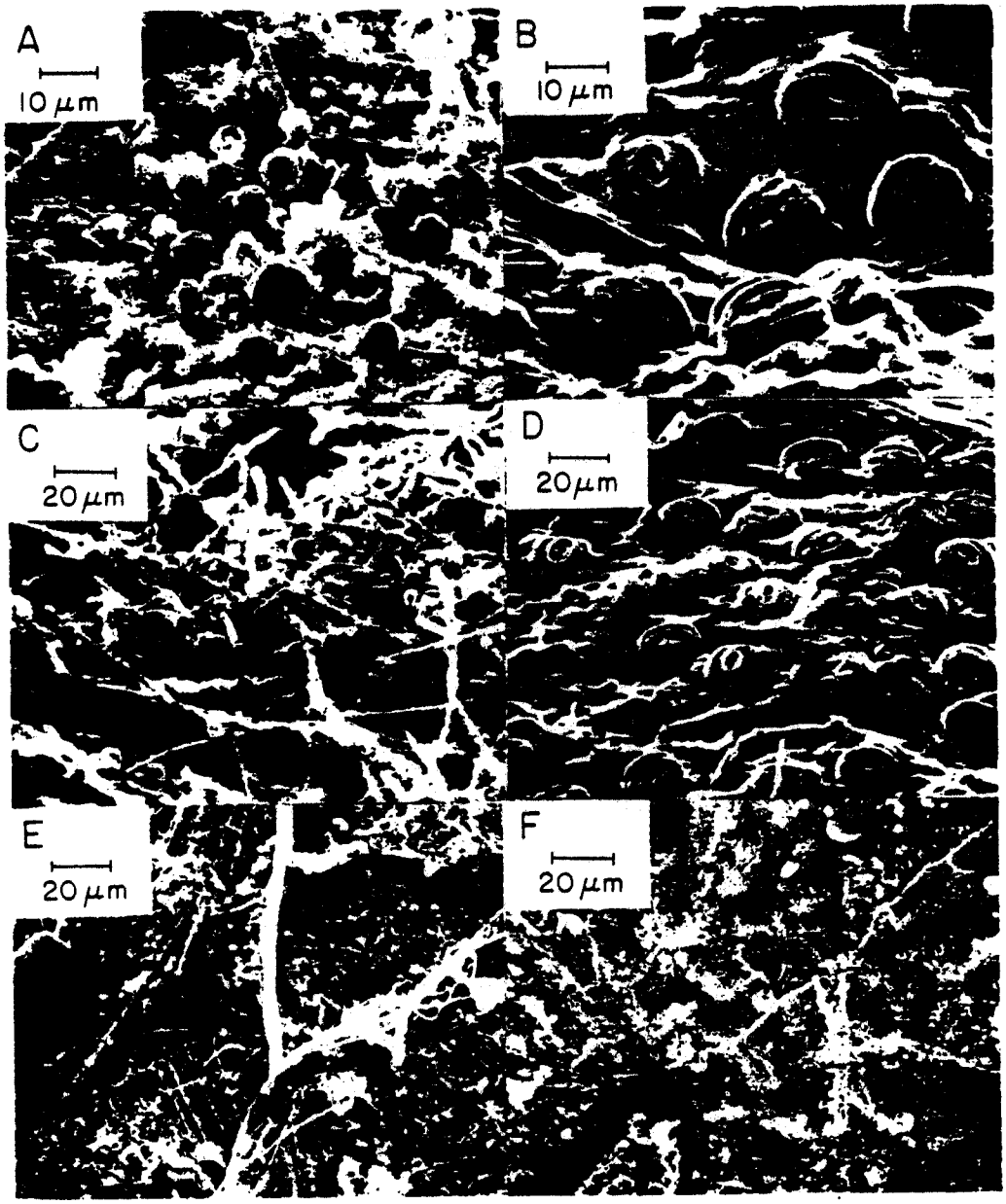

Table 4. Cadmium concentration in leaf matter after 28 weeks incubation. $\bar{X} \pm 2$ SE, n = 4.

Treatment	Cd Concentration (µg/g dry weight) Type I	Type II
Control	2.8 ± 0.04	1.9 ± 0.01
5 µg/L Cd	8.5 ± 2.2	12.2 ± 3.4
10 µg/L Cd	18.4 ± 4.5	23.3 ± 7.8

When assessing the impact of a toxicant on an ecosystem, effects on the most susceptible component of that system should be determined. While particular components may not be of primary economic or aesthetic interest, they may be directly related to the overall desirability or productivity of a stable ecosystem. Such is the case of the aquatic microflora responsible for leaf litter decomposition in streams.

Many microorganisms are adapted to high Cd concentrations.(11,13) A variety of fungi and bacteria have been shown to be tolerant to high concentrations of heavy metals, relative to concentrations which are toxic to other organisms.(3,4) Cadmium does not affect Escherichia coli metabolism of ^{14}C- glucose until a Cd concentration of 6 mg/l is reached (28) and 10 µg/l Cd had no effect on the viability of a natural population of heterotrophic bacteria.(1) Thormann(27) found that the most sensitive estuarine bacteria were inhibited by 100 ppm Cd while the less sensitive species were able to grow in 400 mg/l Cd. Heavy metal resistant actinomycetes and bacteria have been isolated from soil near a zinc smelter which were capable of at least 50% of normal growth of 700 µm Zn.(19)

Leaf surfaces are rapidly colonized by fungi and bacteria under natural conditions.(18) Beech leaves for instance lost 90% of their weight during one year.(18) The results of our study indicate that low Cd concentrations can inhibit the functioning of decomposing microorganisms. Heavy metals such as cooper, zinc and cadmium inhibit fungal spore germination.(24) Metals from a smelter have been found to disrupt microbial processes in terrestrial ecosystems and depress leaf litter decomposition(5) while metals such as Cd may affect the fungi colonizing the phylloplane of leaf surfaces.(15)

Natural microbial communities are more complex than the pure cultures often used to assess toxic effects of metals in laboratory studies.(1) Assessment of toxic and inhibiting effects of low levels of heavy metals should be conducted in more complex situations than pure cultures, and substrates. Ramamoorthy and Kushner(22) suggested that many synthetic media may complex heavy metals which may result in an underestimate of metal toxicity or inhibition which may occur under natural conditions. Batch pure culture bioassays do not represent the complex colonization procedure which may be the critical stage in microbial decomposition of leaf material under natural conditions. Bioassays, to determine toxic or inhibitory effects of compounds on processes as complex as microbial colonization and decomposition of leaf

material must be conducted under conditions which account for the complete colonization process, species interactions and be of sufficient duration to allow for an organismal adaptation to occur.

ACKNOWLEDGEMENTS

Scanning electron photomicrographs were prepared at the University of Georgia electron optics laboratory. C. Kerrick helped with sample preparation and SEM photographs. S. Mashburn, S. Clarkin and L. Briese maintained the microcosm system. D. Adriano, J. Bowling, H. Kania and R. Knight commented on the manuscript. Research supported by Contract E-38-1-819 between the University of Georgia and the U. S. Department of Energy and Interagency agreement EY-76-C-09-0819 between the U. S. Environmental Protection Agency and the U. S. Department of Energy.

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