The Effect of Clay Minerals on Cadmium Toxicity to Bacterial Production

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ABSTRACT

The effect of clays on cadmium toxicity to bacterial production and specific production was investigated in Lake Brazos, McLennan County, Texas. Bacterial production calculated from ^{3}H-thymidine incorporation rates in samples without added cadmium ranged from 5.3×10^{-4} to 2.13×10^{-2} µg C l^{-1} hr^{-1}. Specific production varied between 8.1×10^{-6} and 3.32×10^{-4} µg C µg C^{-1} hr^{-1}.

Cadmium significantly reduced bacterial productivity at low levels (1 mg Cd l^{-1}). The response was non-linear. Reduction ranged from 79% at 1 mg Cd l^{-1} to 98.4% at 20 mg l^{-1}. Reduction at 5 and 10 mg Cd l^{-1} was 62.4% and 65.1%, respectively. Specific production dropped by 70.5% at 1 mg Cd l^{-1}, 82.2% at 20 mg l^{-1}, 55% at 5 mg l^{-1}, and 50.4% at 10 mg Cd l^{-1}.

Added clays increased cadmium toxicity to bacteria. Bacterial production and specific productivity were reduced by 90.7% and 85.9%, respectively, at 1 mg Cd l^{-1} in samples amended with 20 mg l^{-1} clay. Cadmium toxicity decreased with further additions of clay, approaching that observed in the untreated samples.

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INTRODUCTION

Environmental variables modify pollutant effects on organisms. The objective of this study was to determine if clay turbidity affected cadmium toxicity to aquatic bacteria. Little information exists on such triple interactions of the pollutant, organism, and environment, especially in aquatic systems.

Bacterial nutrient cycling in aquatic systems has long been recognized. Bacterial decomposition of organic matter provides essential nutrients to phytoplankton and macrophytes. Recently ecologists have recognized bacteria as important secondary producers. Bacteria convert dissolved organic matter into biomass that is consumed by filter-feeding zooplankton. Thus, bacterial production makes dissolved organic carbon available to higher trophic levels (Rheinheimer, 1980). The conversion of dissolved carbon into biomass serves as a significant input of carbon into aquatic food webs (Pomeroy, 1974; Kirchman and Mitchell, 1982).

Bacteria may be free-floating or associated with detrital and inorganic particles such as clays. Suspended particles adsorb and concentrate organic nutrients, providing a favorable growth substrate for bacteria (Stotzky, 1966a;

Pomeroy, 1984; Jeffery and Paul, 1986). While numerically less predominant in most waters than free-living bacteria, attached bacteria contribute significantly to carbon dynamics. Attached bacteria account for 45% of the bacterial carbon in coastal waters (Ferguson and Rublee, 1976).

Assessment of pollutant toxicity on microbial communities is more applicable than traditional single species evaluations. First, the combined responses of several populations can be evaluated. Second, toxicants that may threaten ecosystem function can be screened since bacteria are a complete functional group. Third, results can be compared across ecosystem boundaries since most bacterial processes are common to all ecosystems (Babich and Stotzky, 1983). Duxbury (1985), reviewed data on the effect of heavy metals on such processes as nitrogen fixation, litter decomposition, and methanogenesis. Because investigations on bacterial secondary production are relatively new, the effects of metal contamination on this process are unknown.

Investigation of an element's toxicity must consider abiotic environmental parameters such as clay concentration (Wong, 1987). Variation in the physical/chemical environment affects a compound's toxicity by altering its speciation, mobility, and biological availability. Clays have a large surface area on which many materials, including heavy metals, may concentrate. Bacteria attached to particles bind,

incorporate, and accumulate these metals, leading to biomagnification in higher trophic levels (Duxbury, 1985). In addition, the presence of toxic metal ions on particle surfaces could reduce the quantitatively important production of attached bacteria.

Locally high concentrations of heavy metals in lakes and streams originate from both natural and anthropogenic sources and may limit the bacterial productivity in contaminated systems. Cadmium deposition in lakes results from mineral weathering, industrial waste disposal, and allochthonous inputs of phosphate fertilizers and detergents. Cadmium is the most toxic heavy metal to microorganisms (Babich and Stotzky, 1980). Cadmium is considered a Type 2 metal, meaning it is highly toxic and readily available to organisms (Duxbury, 1985). Abundant literature exists on cadmium toxicity to aquatic plants and animals (Friberg et al., 1974; Fleisher et al., 1974); however, relatively little information exists on cadmium toxicity to bacteria.

Clay minerals reduce cadmium toxicity to bacteria and fungi in soils by immobilizing the metal ions through cation exchange reactions (Babich and Stotzky, 1977,1980). However, this relationship has not been established in aquatic environments. Given the importance of bacterial production to aquatic food web dynamics, evaluation of pollutant effects on this process and environmental variables that may modify

these effects is essential. This investigation examined the effect of clay suspensoids on cadmium toxicity to bacterial production in Lake Brazos, Waco, Texas. I hypothesised that clays would bind cadmium and decrease the toxic effects on the bacteria.

LITERATURE REVIEW

Bacteria convert significant amounts of organic carbon to particulate forms in aquatic systems. Bacterial production is modified by environmental parameters such as clay concentration and the presence of toxic substances. The interactive effects of clay and cadmium on bacterial production remain unclear. Evaluation of these effects requires measurement of bacterial growth rates and biomass at various combinations of these variables.

The Role of Bacteria in Aquatic Food Webs

Bacterial transformation of dissolved organic substances into particulate material is an important process in all aquatic ecosystems. Bacterial utilization of dilute organic compounds brings these otherwise unavailable carbon sources into the food web.

Dissolved organic compounds are supplied by several sources. Phytoplankton and macrophytes release photosynthetically fixed carbon (Fenchel and Jorgensen, 1977). Wetzel et al. (1972) reported that algae and macrophytes released 5.6% and 4%, respectively, of their annual primary production in a hard water lake. As much as 10% to 30% of primary production is released by marine

phytoplankton (Riley, 1970). Wolter (1982) found that an average of 15% of phytoplankton production was released as exudates in Kiel Bight. Release rates vary with season, locality, species composition, and the physiological condition of the algae.

The breakdown of dead plant material, algal cell lysis, and the feeding and excretion activities of animals also release dissolved organic carbon (VanEs and Meyer-Reil, 1982). As much as 33% of the food assimilated by animals may be excreted (Fenchel and Jorgensen, 1977).

Williams (1981) considered algal exudates as the most important carbon and energy source for marine bacteria. Approximately 50% of bacterial production in marine ecosystems results from the utilization of algal exudates (Larsson and Hagstrom, 1982). In freshwater systems, these values can range from 13% (Reimann and Sondergard, 1984) to 100% (Bell and Kuparinen, 1984). Algal cell lysis controls bacterial production during periods of nutrient stress or low phytoplankton productivity. During periods of heavy phytoplankton grazing, the relative importance of zooplanktonic release increases.

Bacteria are important food for a variety of aquatic invertebrates. In waters with high bacterial productivity, some animals depend entirely on bacteria for protein

(Reinheimer, 1980). Approximately 10% of the nitrogen required by benthic organisms in the Caspian Sea is supplied by bacteria (Zhucova, 1963).

Protozoans are the most important bacteriovores. Seventy-five percent of bacterial grazing in arctic tundra ponds was done by protozoa (Fenchel, 1975). Many flagellates and ciliates have specially adapted feeding mechanisms that concentrate bacterial cells (Fenchel and Jorgensen, 1977). Tetrahymena pyriformes consumed 100-200 bacteria individual^{-1} hr^{-1} during stable growth phases (Barsdate et al., 1974). Many metazoans such as sponges, corals, and oysters also are bactiverous. Sorokin (1973) estimated that coral polyps may consume 10%-20% of their carbon content daily as bacteria. Annelid worms and insect larvae also consume bacteria. Bacteria constitute half of the food intake for Chironomous midges (Baker and Bradham, 1976).

Clay Minerals and Aquatic Bacteria

Clay particles significantly influence aquatic bacteria. Clays protect bacteria against phage attack, immobilize antibiotics and toxins, and reduce the bactericidal effects of light (Reinheimer, 1980).

Clay surfaces concentrate inorganic and organic substrates. These substrates are readily accessible to attached bacteria (Fletcher and Marshall, 1982). Bacterial

attachment increases with oligotrophic conditions (Paerl, 1975; Paerl and Merkel, 1982; Bell and Albright, 1982). In nutrient-poor waters, over 90% of the bacterial population may be on particles (Campbell, 1983).

Attachment to clays enhances bacterial enzyme activity. Exoenzyme diffusion is slowed, making substrate utilization efficient (ZoBell, 1943). Additionally, clays alter enzyme orientation and increase reaction rates. Attachment also alters the structure of membrane-associated enzymes and facilitates substrate transport across the membrane (Fletcher and Marshall, 1982).

Clays increase bacterial respiration by maintaining the environmental pH (Stotzky 1966a, 1966b). Clay surface cations are exchanged with the hydrogen ions produced during metabolism. This buffering prevents enzyme denaturation by metabolic acids. The activity of aldehyde degrading enzymes is increased through clay buffering in certain soils (Kunc and Stotzky, 1970).

In general, attached bacteria have higher specific uptake rates of radiolabelled glucose and amino acids than free bacteria. Attached assimilation values in marine waters are typically less than 10% of the total uptake, while estuarine values range from 10% to 99% (Ferguson and Rublee, 1976). Epibacterial glucose uptake in freshwater is highly

variable, with values ranging from less than 10% to 72% of the total assimilation. Harvey and Young (1980) assayed heterotrophic activity in a salt marsh environment with the 2-(p-iodophenyl)-3-(p-nitrophenyl)-5 phenyl tetrazolium chloride (INT) method. They observed a strong correlation between the degree of particle colonization and the abundance of actively respiring bacteria. These data support that of Jannasch and Pritchard, (as referenced by Ferguson and Rublee, 1976) who found that the addition of inert particulates elevated bacterial respiration.

Paerl and Merkel (1982) examined differential phosphorus uptake in free and attached bacteria. They observed a six-fold increase in phosphorus assimilation when particulate material was present. This was presumably due to concentration of the phosphorus at the particle surfaces. In accordance with autoradiographic results, particle-bound bacteria had a greater specific uptake than did free bacteria.

Validity of data concerning heterotrophic activity has been questioned. Harvey and Young (1980), in justifying the INT method, questioned the use of unnaturally high concentrations of radiolabelled substrates. In addition, heterotrophic activity by attached bacteria may be underestimated due to the preferential utilization of substrates already present on the particle (Palumbo et al.,

1984). Uptake rates also have been found to be substrate specific, which can lead to inaccurate estimations of true heterotrophic activity (Bell and Albright, 1982).

There is abundant literature on the total microbial secondary production in aquatic ecosystems. However, surprisingly few studies on production by particle-bound bacteria have been conducted. Attached bacterial carbon flux and production in the Hudson river was between 3 and 10 mg C m^{-2} day^{-1} (Ducklow et al., 1982). These values were equal to 18% and 38% of total bacterial production.

Seasonal trends have been observed in attached bacterial production (Lovell and Konopka, 1985). During late summer and fall, 20% to 35% of total bacterial production occurred in the 1µm and greater size fraction. The same fraction contributed only 2% to 19% of the total during the remainder of the year.

Attached bacterial production was less than 10% of the total secondary production in a small eutrophic pond (Kirchman, 1983). He observed an order of magnitude increase in attached production from winter to summer, with values increasing from 0.08 to 1 µg C l^{-1} hr^{-1}.

In Lake Biwa, less than 10% of the bacterial production was associated with particulates in the epilimnion, while 2% to 15% was particle-bound in the meta- and hypolimnion

(Nagata, 1987). These estimates equal 1 to 9 μg C l^{-1}. Increased production by attached bacteria coincided with chlorophyll a maxima. Similar results were obtained by Bell et al. (1983), who found maximum attached bacterial production during cyanobacterial blooms. However, attached production accounted for 59% of the total bacterial production in these waters.

Attached bacteria are important to aquatic food webs. Attachment makes bacteria easier prey for zooplankton and improves the nutritional quality of particles (Ferguson and Rublee, 1976). Extensive extracellular polymers are used for particle attachment and increase the particle's carbon content (Paerl, 1975).

Bacterial-clay aggregates are an important food source for benthic communities. Ducklow et al. (1982) found that 67% of daily bacterial production was lost from the epilimnion via aggregate sinking. This sedimentation effectively links the trophogenic and tropholytic zones.

The Effects of Cadmium on Bacteria

Cadmium exerts significant physiological and ecological influences on bacteria. Excessive cadmium inhibits enzyme activity through competitive displacement of metallic cofactors (Forstner and Wittmann, 1981). Inactivation of polymerase enzymes halts RNA and protein synthesis (Blundell

and Wild, 1969). Juma and Tabatabai (1977) reported cadmium inactivation of acid and alkaline phosphatase enzymes. Cadmium also expands cell membranes making substrate transport impossible (Mitra et al., 1975). The results of enzyme inactivation are manifested in reduced growth, metabolism, and production rates.

Cadmium concentrations inhibiting growth are highly variable among species. Bacillus megaterium growth was inhibited by 1 mg Cd l^{-1} (Babich and Stotzky, 1977) while Bacillus cereus and Esherichia coli grew at 80 mg l^{-1} (Doyle et al., 1975). Gram positive bacteria are generally more sensitive to cadmium than gram negative species (Wong, 1987). Sub-lethal cadmium levels prolonged the lag phase of E. coli (Mitra et al., 1975) and prolonged the log phase of Agrobacterium tumefaciens (Babich and Stotzky, 1977).

Glucose metabolism by E. coli was inhibited by 12 mg Cd l^{-1} in Chesapeake Bay water (Zwarum, 1973). Mills and Colwell (1977) found that 10 mg Cd l^{-1} reduced glucose metabolism by 84% in Chesapeake Bay and Colgate Creek water and sediment. Remacle et al. (1982) investigated the effects of cadmium on bacterial biomass production. They found that 0.5 mg Cd l^{-1} lowered productivity by 10%. This effect was intensified at low temperatures, with productivity dropping by 50% at 10°C.

Bacteria bind and accumulate cadmium. (Kurek et al., 1982). Pfister (1982) found that bacteria accumulated 1 to 3 mg cadmium gm^{-1} biomass in model aquatic systems. These levels were an order of magnitude greater than those in the water and sediments. Bacillus subtilis accumulated .15%-1.22% of its dry weight as cadmium (Macaskie and Dean, 1982).

Cadmium is taken up intracellularly or bound on the cell wall (Gadd and Griffith, 1978). In experiments with E. coli, Khazaeli and Mitra (1981) found that 80% of the accumulated cadmium was cytoplasmic. Sixty percent was associated with a high molecular weight protein that sequestered the metal.

Cadmium associated with the cell wall is bound by negatively charged exopolymers. Attached bacteria accumulate more cadmium than free-living bacteria because of these exuded polymers (Remacle et al., 1982). Remacle (1981) found that bacteria attached to microcosm walls accumulated five times more cadmium than those in the water. This enhanced cadmium accumulation leads to biomagnification through aquatic food webs (Duxbury, 1985; Doyle et al., 1975).

The Effects of Clays on Cadmium Toxicity

Clay minerals have negatively charged surfaces that adsorb cations. Cadmium is exchanged with cations such as potassium and magnesium present on the exchange complex of

clays. These exchange reactions are reversible, and the cadmium is only temporarily bound (Babich and Stotzky, 1980).

Cadmium adsorption depends upon pH. Adsorption is enhanced as pH increases from 3 to 6, due to formation of hydroxyl bridges between surface cations and the divalent metal ions. At pH's greater than 6, the metal begins precipitation as monovalent cadmium hyroxide (Babich and Stotzky, 1980).

Adsorption also depends upon the quantity and type of ligands present. Ammonia, sulfate, nitrate, and free hydroxyl ions all complex cadmium. Additionally, organic compounds such as humic and fulvic acids, amino acids, sugars, and phenolics chelate cadmium (Manahan, 1984).

Babich and Stotzky (1977) found that 10 mg Cd l^{-1} reduced Bacillus megaterium growth by 83% in the absence of clays. However, in media containing 2% montmorillonite growth was only reduced by 37%. A similar response was observed for Agrobacterium tumefaciens. At 100 mg Cd l^{-1}, growth was reduced by 67% in the absence of clays. Growth was reduced by 8% in media containing 2% montmorillonite. They hypothesized that the clays complexed and immobilized the cadmium.

Bacterial Productivity Measurements

Bacterial growth and biomass measurements are necessary to assess the effect of environmental variables on bacterial activity (Davalos, 1986). Satisfactory techniques for measuring hetrotrophic bacterial growth in aquatic systems have been developed only recently (Moriarty, 1986).

Fuhrman and Azam (1980) and Moriarty and Pollard (1981) developed the use of the tritiated thymidine incorporation method for estimating bacterial growth rates. This method relates the incorporation of [methyl-^{3}H]-thymidine into DNA synthesis rates. DNA synthesis is coupled to cell division (Lark, 1969; VanEs and Meyer-Reil, 1982).

The tritiated thymidine method meets the criteria for measuring bacterial growth rates as described by Azam and Fuhrman (1984). First, the method is specific for heterotrophic bacteria (Moriarty and Pollard, 1982). Second, the method does not rely on balanced growth. Third, growth rates are not altered by experimental manipulations of the sample. Fourth, conversion factors between incorporation and growth rates are easily calculated.

The tritiated thymidine technique is based upon several assumptions such as rapid and efficient bacterial uptake of thymidine, stability during uptake, rapid conversion into nucleotides, and absence of dilution by intracellular

thymidine pools (Kornberg, 1980; Moriarty, 1986; Davalos, 1986). Other assumptions are that bacteria preferentially utilize exogenous thymidine over de novo synthesis (Fuhrman and Azam 1980, 1982; Reimann, 1984), and that naturally occuring thymidine does not reduce the specific activity of the radiolabeled thymidine (Moriarty, 1986).

The major violations of these assumptions are non-specific labelling of macromolecules and isotope dilution by naturally occuring thymidine. Catabolism of thymidine to thymine releases the tritium label. The tritiated methyl group is then distributed to cellular proteins, lipids, and RNA. Retention of these molecules on filters leads to overestimation of DNA synthesis rates. The proportion of ^{3}H in DNA is variable and dependent upon the organisms' physiological state and the environment's species composition (Staley and Konopka, 1985). Non-specific labelling can be reduced by using short incubation times of 10-30 minutes (Pollard and Moriarty, 1984; Reimann,1984). A more favorable solution is to measure the radioactivity in purified DNA fractions (Moriarty and Pollard, 1981; Reimann et al., 1982). The phenol-chloroform extraction method of Wicks and Robarts (1987) can be used to remove labelled proteins and lipids.

De novo nucleotide synthesis dilutes the specific activity of tritiated thymidine. This dilution results in underestimation of cell synthesis rates (Davalos, 1986).

Isotope dilution can be prevented by adding sufficient radiolabeled thymidine to inhibit de novo synthesis (Reimann et al., 1982; Moriarty, 1986). A 20 nM concentration completely inhibited de novo synthesis in seawater (Pollard and Moriarty, 1984). Davalos (1986) used a 18 nM concentration in Lake Brazos.

Thymidine Incorporation into DNA

There are two pathways for incorporation of thymidine into DNA. The first is de novo synthesis, in which thymidine is syntehsised from precursor molecules (Pollard and Moriarty, 1984). The precursor molecules are converted to uracil monophosphate (dUMP). dUMP is then converted into thymidine monophosphate (dTMP) by thymidilate synthetase enzymes. dTMP is sequentially phosphorylated to thymidine triphosphate (dTTP). DNA polymerase then incorporates dTTP into DNA as thymidine nucleotides. The second pathway is the salvage pathway, in which excess nucleotides and nucleic acids are converted to dTTP (Moriarty and Pollard, 1984).

Bacterial Biomass Determinations

Cell synthesis measurements are coupled to biomass determinations in order to assess bacterial carbon production. Few methods have been available in the past to accurately measure the number and volume of aquatic bacteria (Zimmerman, 1977). The two most widely used methods are scanning electron microscopy (SEM) and epifluorescence

microscopy (Lee and Fuhrman, 1981). SEM has been shown to underestimate cell volume due to cell shrinkage during preparation (Fuhrman, 1981; Montesinos et al., 1983).

Hobbie et al. (1977) developed the acridine orange direct count (AODC) method. Preserved samples are stained with acridine orange and filtered onto stained polycarbonate filters. Acridine orange binds with nucleic acids, forming fluorescent complexes. These complexes fluoresce orange (RNA) or bright green (DNA) when viewed with an epifluorescent microscope with the appropriate filters. Orange or green fluorescent particles having a distinct bacterial shape are counted as bacteria. Biomass estimates are made by measuring the bacterial volume and converting to carbon units (Lee and Fuhrman, 1987; Bratbak, 1985).

METHODS and MATERIALS

The variables measured were fixed and volatile suspended solids concentrations, pH, and bacterial cell synthesis rates, production, and specific production at various concentrations of clay and cadmium.

Sampling

Water samples were collected from Lake Brazos, Waco, Texas. Lake Brazos is 15.12 km long with an average width of 94.8 m. The maximum depth is 8 m (Davalos, 1986). Water chemical characteristics are listed in Table 1. Five samples were collected from December 1, 1989 to July 10, 1990. Integrated 0-2 m samples were collected with an acid washed PVC pipe and transported to the laboratory in sterile polyethylene containers. Well mixed sub-samples were removed aseptically for bacterial biomass determinations and pH measurements. Biomass sub-samples were preserved in 2% formalin (final concentration) and refrigerated (4°C) until processed.

Fixed and Volatile Suspended Solids

Fixed and volatile solids analyses were conducted according to Standard Method #224C

Table 1. Water chemical characteristics of Lake Brazos, McLennan County, Texas. Data are from the Texas Water Commission.

PARAMETER	1984	1985	1986
Dissolved Oxygen (mg l^{-1})	7.9	11.5	11.8
pH	7.7	8.2	7.4
Alkalinity (mg l^{-1} as $CaCO_3$)	103	114	124
Total Residue (mg l^{-1})	14	42	20
Volatile Residue (mg l^{-1})	4	5	1
Ammonia (mg l^{-1})	0.14	0.02	0.43
Total Nitrate (mg l^{-1})	0.02	0.02	1.2
Total Phosphate (mg l^{-1})	0.21	0.24	0.04
Orthophosphate (mg l^{-1})	0.03	0.06	0.02
Chloride (mg l^{-1})	231	370	145

(American Public Health Association, 1971). Samples (500-800 ml) were filtered through acid washed, pre-weighed Whatman 934-AH glass fiber filters using gentle vacuum (<5 in. Hg). The filters were dried at 103°C for 1 hour, cooled, and weighed on an analytical balance, and results were recorded as mg l^{-1} total suspended matter. The filters were ignited in a 600°C muffle furnace for 15 min. The weight loss on ignition was recorded as milligrams per liter volatile suspended matter and the weight of the remaining ash as milligrams per liter fixed suspended matter (i.e. clays).

Clay Preparation

Clay samples were collected with an Ekman dredge from Lake Brazos on December 1, 1989. The clays were dried overnight at 60°C. Organic matter was removed via hydrogen peroxide oxidation (Black, 1965). Ten to twenty grams of dried clay were transferred to a 1-liter beaker with sufficient distilled water to make a slurry. The slurry was acidified with 10 drops of 1N HCl. Hydrogen peroxide (30%, Fisher Scientific) was added in 5-10 ml increments as the suspension was stirred. Additional peroxide was added as the frothing subsided. When the reactions were complete, the suspension was heated to 70°C on a hot plate for 30 min. or until further reactions ceased.

The peroxide/clay slurries were transferred to separatory funnels and allowed to sit overnight. The coarse sediment layer was drawn off and discarded. Fine particles and suspended clays were washed by centrifuging at 2000 rpm for 10 min. The clay pellets were re-suspended in organic-free, deionized water. This washing procedure was repeated three times. Clay pellets were dried at 60°C and stored in a dessicator until needed.

Bacterial Productivity Determinations

Lake water samples (300) ml were transferred to acid washed, sterile flasks and amended with 0, 20, 40, 80, or 160 mg l^{-1} organic free clays. The suspensions were vigorously mixed with a magnetic stirrer for 30 min. to allow coating of the clays with dissolved organic compounds.

The suspensions were dispensed into sterile screw-top test tubes (10 ml) which contained 0, 1, 5, 10, or 20 mg l^{-1} cadmium as cadmium chloride. Five replicates at each cadmium concentration and each clay concentration were prepared. To determine cadmium exposure time effects on productivity, these tubes were incubated at room temperature for 30, 60, 90, or 120 min. Killed controls for each cadmium concentration at each clay concentration were prepared by adding 0.5 ml 5N NaOH.

Bacterial productivity was measured by the thymidine incorporation method (Fuhrman and Azam, 1980). This method measures cell synthesis rates. Production is the product of cell synthesis rate and a constant equating mean cell volume to organic carbon. Samples were incubated 30 min. at room temperature with 18 nmol [methyl-^{3}H]-thymidine (ICN Biomedical, 55 Ci/mmol). Incubations were terminated by adding 0.5 ml 5N NaOH. The tubes were incubated at room temperature for 15 min. and then refrigerated. The samples were processed within 24 hr to assure complete recovery of the labelled DNA (Wicks and Robarts, 1987).

The samples were placed in crushed ice for 15 min. prior to filtration and extraction. DNA purification was performed with the phenol-chloroform method of Wicks and Robarts (1987). The tubes were acidified by adding 2.8 ml ice-cold trichloroacetic acid (100%). After 15 min., the samples were filtered (8 in. Hg) through chilled nitro-cellulose filters (Whatman, 0.2 µm pore size, 25 mm diameter). The filters were washed with 5 ml ice-cold phenol-chloroform (50% w/v) to remove labelled proteins. Lipids were extracted by washing the filters with 5 ml cold 80% ethyl alcohol. After filtration, the margin of the filter plus 5.2% of the effective filtration area were removed with a cork borer. Cut filters were placed in glass scintillation vials with 1

ml ethyl acetate. After the filters dissolved, 10 ml scintillation cocktail (Beckman, Ready-Protein) were added to the vials. The radioactivity was determined with a Beckman LS1800 scintillation counter. Activities were counted to a 2% confidence limit.

Radioactivity counts (CPM) were converted to disintegrations per minute (DPM) by applying external quench correction. Quench, or loss of counting efficiency, was recorded as an H#. The H# was converted to efficiency measurements with a quench curve equation. The curve was prepared by adding a constant radioactivity to vials containing 10 ml scintillation cocktail and various amounts of carbon tetrachloride.

DPM measurements were converted to cell synthesis rates with the equation of Moriarty and Pollard (1984):

$$\text{Cells Synthesized } l^{-1}\ hr^{-1} =$$

$$(DPM/2.2\times10^{6}/sa)(1/time)(vol/1000)(1\times10^{-9})(2.1\times10^{18})$$

DPM= radioactivity of filtered sample.

2.2×10^{6}= the number of DPM per µCi.

sa= specific activity of the added isotope (Ci $mmol^{-1}$).

time= the incubation time in hours.

vol= the sample volume.

$1x10^{-9}$= conversion factor for nmoles to moles.

$2.1x10^{18}$= conversion factor for cells produced per mole of thymidine.

Bacterial Biomass Measurements.

Bacterial abundance and cell volume were determined with the acridine orange technique of Hobbie et al. (1977). Nuclepore filters (0.2 µm pore size, 25 mm diameter) were soaked in Irgalan Black (2g l^{-1} 2% acetic acid) for 1 hr and rinsed in sterile deionized water. The filters were then placed on a fritted glass filter support and a sterile filter tower attached. One milliliter of a well-mixed preserved sample was added to the filtration apparatus with 5 ml of sterile water. The bacteria were stained by adding 2 drops Acridine Orange (0.1%) and incubating 1-2 min. prior to filtration (8 in. Hg).

When all the sample had passed through the filter, the filter was placed on a microscope slide. One drop of non-fluorescing immersion oil was placed on the filter followed by a coverslip and another drop of oil. Attached and free cells were counted on a Nikon Optiphot epifluorescent microscope (1000x). A minimum of 10 randomly chosen fields

containing a minimum of 30 cells were counted. Duplicate slides were counted for each sample.

Cell volumes were measured by photographing 3-4 fields on each slide. Micrographs were made on Kodak Ektachrome film (400 ASA). The cell lengths (L) and widths (W) were measured by projecting the images on white poster paper. The scale was determined by measuring the projected image of a stage micrometer. The volume of each cell was determined with the following formula:

$$\text{Cell Volume}(\mu m^3) = (.7854)(W^2)(L-W/3)$$

This formula applies to both rods and cocci (Bratbak, 1985).

Biomass was calculated as:

$g\ C\ \mu m^{-3} =$ Mean Volume x Mean Abundance x 1.21×10^{-13}

(Fuhrman and Azam, 1980; Reimann et al.,1982).

Data Analysis

Bacterial production and specific production rates at each clay concentration, cadmium concentration, and time interval were compared with a three-way factorial analysis of variance (ANOVA). The data were normalized with a natural logarithm transformation. The analyses were performed with the General Linear Models (GLM) procedure of SAS. The GLM procedure uses the least squares method to fit linear models to the data. The resulting ANOVA model tests for the main

and interactive effects of independent variables (clay, cadmium, time) on dependent variables (cell synthesis, bacterial production, specific production). Each component of the model is an effect at some specified combination of the independent variables. The GLM procedure also performs pairwise comparisons of all main effects specified in the ANOVA model (SAS Institute, 1985). The probability value chosen as the rejection limit for the model was 0.05.

RESULTS

Bacterial production was greatly reduced even at the lowest cadmium concentration examined. Further, clay and cadmium had a significant interactive effect on the bacteria. Cadmium toxicity increased with the addition of small quantities of clay, but generally decreased with the addition of higher concentrations.

Environmental Variables

The fixed solids (clay) concentrations in the original samples ranged from 10.22 to 43.18 mg l^{-1} (Table 2). Volatile particulates varied from 2.51 to 19.46 mg l^{-1}. The wide fluctuation among samples in the particulate concentrations was probably due to frequent lake drainage and refilling. The lake water pH measurements remained fairly constant throughout the study, ranging from 7.6 to 8.3 (Table 2).

The Lake Brazos Bacterial Community

Bacterial Abundance and Biomass.

Bacterial numbers ranged from 4.19×10^9 to 8.4×10^9 cells l^{-1} (Table 3). Attached cells were less abundant than free-

Table 2. Total, volatile, and fixed solids concentrations (mg l^{-1}) and pH of Lake Brazos samples.

Date	Total Solids	Volatile Solids	Fixed Solids	pH
1 Dec 89	21.5	3.9	17.5	8.2
29 Jan 90	62.6	19.4	43.1	7.6
28 May 90	12.7	2.5	10.2	8.1
3 July 90	84.1	68.5	15.5	8.3
10 July 90	20.7	3.7	17.0	8.2

Table 3. Bacterial abundance (10^9 cells l^{-1}) and mean cell volume (μm^3) measurements for Lake Brazos samples.

	Abundance			
Date	Free	Attached	Total	Volume
1 Dec 89	4.02	0.17	4.19	0.127
29 Jan 90	7.71	0.43	8.14	0.074
28 May 90	4.74	0.17	4.91	0.089
3 July 90	5.00	0.17	5.18	0.129
10 July 90	4.74	0.04	4.79	0.100

living bacteria, comprising 3.5% of the total.

Rod shaped cells were most common and had an average volume of 0.125 μm^3 per cell. Coccoid bacteria had an average volume of 0.082 μm^3. Vibrio shaped cells also were occassionally observed. The average volume of free cells was 0.105 μm^3. Attached cells were slightly smaller with an average volume of 0.102 μm^3. The overall mean cell volume was 0.104 μm^3 (Table 3).

Free biomass ranged from 50.57 to 96.96 $\mu g\ C\ l^{-1}$ and the attached biomass from .057 to 5.47 $\mu g\ C\ l^{-1}$ (Table 4). The total bacterial biomass ranged from 52.73 to 102.43 $\mu g\ C\ l^{-1}$ (Table 4).

Bacterial Productivity Measurements

Bacterial cell synthesis rates were variable among sample dates and ranged between 4.23×10^4 and 1.69×10^6 cells $l^{-1}\ hr^{-1}$ with an average rate of 6.38×10^5 (Table 5). Consequently, production values ranged from 5.3×10^{-4} to 2.13×10^{-2} $\mu g\ C\ l^{-1}\ hr^{-1}$ with an average of 9.3×10^{-3} $\mu g\ C\ l^{-1}\ hr^{-1}$ (Table 6). The specific production was also variable, ranging from 2.08×10^{-4} to 8.1×10^{-6} $\mu g\ C\ \mu g\ C^{-1}\ hr^{-1}$ with an average of 1.02×10^{-4} $\mu g\ C\ \mu g\ C^{-1}\ hr^{-1}$ (Table 6).

Table 4. Bacterial biomass estimates ($\mu g\ C\ l^{-1}$) for Lake Brazos samples.

	Biomass		
Date	Free	Attached	Total
1 Dec 89	50.57	2.16	52.73
29 Jan 90	96.96	5.47	102.43
28 May 90	59.66	2.13	61.79
3 July 90	62.95	2.24	65.19
10 July 90	59.71	0.567	60.28

Table 5. Bacterial cell synthesis rates (10^5 cells l^{-1} hr^{-1}) in Lake Brazos samples.

Date	Cell Synthesis
1 Dec 89	1.79
29 Jan 90	16.9
28 May 90	16.3
3 July 90	0.42
10 July 90	1.52

Table 6. Calculated bacterial production ($\mu g\ C\ l^{-1}\ hr^{-1}$) and specific production ($10^{-4}\ \mu g\ C\ \mu g\ C^{-1}\ hr^{-1}$) in Lake Brazos samples.

Date	Production	Specific Production
1 Dec 89	0.0023	0.436
29 Jan 90	0.0213	2.08
28 May 90	0.0205	3.32
3 July 90	0.00053	0.081
10 July 90	0.0019	0.315

The Effect of Cadmium on Bacteria

Cadmium significantly reduced production and specific production ($p<0.0001$). Reduction of production ranged from 79% at 1 mg Cd l^{-1} to 98.4% at 20 mg Cd l^{-1} (Table 7). However, cadmium was less toxic at 5 and 10 mg l^{-1}. Specific production dropped by 70.5% at 1 mg Cd l^{-1} and by 82.2% at 20 mg l^{-1}, with lower toxicity at the 5 and 10 mg l^{-1} concentrations (Table 7).

Apparently, the short exposure time used was sufficient to produce the maximum cadmium effect. The cadmium toxicities at the various exposure times were not significantly different ($p=0.0567$). Thus, the exposure time data were pooled and included in productivity calculations at each cadmium concentration.

The Effects of Added Clays on Bacteria

The added clay in the samples did not significantly affect bacterial production ($p=0.1829$), or specific production ($p=0.6262$). With the addition of 20 mg l^{-1}, production was 0.0191 µg C l^{-1} hr^{-1} and specific production was 2.36×10^{-4} µg C µg C^{-1} hr^{-1} (Table 8).

Table 7. Reduction of bacterial production ($\mu g\ C\ l^{-1}\ hr^{-1}$) and specific production ($10^{-4}\ \mu g\ C\ \mu g\ C^{-1}\ hr^{-1}$) by cadmium in Lake Brazos samples.

	Cadmium Concentration ($mg\ l^{-1}$)				
	0	1	5	10	20
Production	0.0093	0.0019	0.0035	0.0032	0.0015
% Reduction	-	79	62.4	65.1	98.4
Specific Production	1.29	0.38	0.58	0.64	0.23
% Reduction	-	70.5	55.0	50.4	82.2

Table 8. Bacterial production (μg C l^{-1} hr^{-1}) and specific production (10^{-4} μg C μg C^{-1} hr^{-1}) with added clays (mg l^{-1}) in Lake Brazos samples.

Added Clay Concentration	Production	Specific Production
0	0.0097	0.89
20	0.0191	2.36
40	0.0163	2.01
80	0.0141	1.75
160	0.0156	1.99

The Effect of Added Clays on Cadmium Toxicity to Bacteria

Clay and cadmium had a significant interactive effect on bacterial production ($p<0.0001$). Except at the highest cadmium concentration, the greatest toxicity was observed in samples with 20 mg added clay l^{-1} (Table 9). Cadmium toxicity either decreased or was unchanged with the addition of 40, 80, or 160 mg clay l^{-1} , approaching that observed in the unamended samples (Tables 10-12).

Table 9. Reduction of bacterial production ($\mu g\ C\ l^{-1}\ hr^{-1}$) and specific production ($10^{-4}\ \mu g\ C\ \mu g\ C^{-1}\ hr^{-1}$) by cadmium in Lake Brazos samples amended with 20 mg l^{-1} clay.

	Cadmium Concentration (mg l^{-1})				
	0	1	5	10	20
Production	0.0191	0.0018	0.0057	0.003	0.0015
% Reduction	-	90.6	70.2	85.9	92.4
Specific Production	2.36	0.332	1.0	1.3	0.37
% Reduction	-	85.9	57.6	44.9	84.3

Table 10. Reduction of bacterial production ($\mu g\ C\ l^{-1}\ hr^{-1}$) and specific production ($10^{-4}\ \mu g\ C\ \mu g\ C^{-1}\ hr^{-1}$) by cadmium in Lake Brazos samples amended with 40 mg l^{-1} clay.

	Cadmium Concentration (mg l^{-1})				
	0	1	5	10	20
Production	0.0163	0.0041	0.0055	0.0046	0.0019
% Reduction	-	75	66.3	71.8	88.3
Specific Production	2.01	0.69	0.93	1.2	0.44
% Reduction	-	65.7	53.7	40.3	78.1

Table 11. Reduction of bacterial production ($\mu g\ C\ l^{-1}\ hr^{-1}$) and specific production ($10^{-4}\ \mu g\ C\ \mu g\ C^{-1}\ hr^{-1}$) by cadmium in Lake Brazos samples amended with 80 mg l^{-1} clay.

	Cadmium Concentration (mg l^{-1})				
	0	1	5	10	20
Production	0.0141	0.0039	0.0056	0.0044	0.0018
% Reduction	-	72.3	60.3	68.8	87.2
Specific Production	1.75	0.55	0.83	0.38	0.46
% Reduction	-	68.5	52.6	78.3	73.7

Table 12. Reduction of bacterial production ($\mu g\ C\ l^{-1}\ hr^{-1}$) and specific production ($10^{-4}\ \mu g\ C\ \mu g\ C^{-1}\ hr^{-1}$) by cadmium in Lake Brazos samples amended with 160 mg l^{-1} clay.

	Cadmium Concentration (mg l^{-1})				
	0	1	5	10	20
Production	0.0156	0.0054	0.0104	0.0044	0.0032
% Reduction	-	65.4	33.3	71.8	79.5
Specific Production	1.99	4.6	1.5	0.86	0.52
% Reduction	-	-	24.62	56.8	73.9

DISCUSSION

Bacteria produce significant amounts of particulate carbon in aquatic systems (Rheinheimer, 1980). The effects of pollutants on bacterial production are unknown. The purposes of this investigation were to assess the toxicity of cadmium to bacterial production and to determine how this toxicity is modified by clay turbidity.

The cell synthesis rate observed in the samples was 7.39×10^5 cells l^{-1} hr^{-1}. Davalos (1986) reported synthesis rates between 1.97×10^7 and 4.79×10^7 cells l^{-1} hr^{-1} in Lake Brazos. However, she measured thymidine incorporation into cold TCA insoluble fractions. This method has been shown to overestimate DNA synthesis (Wicks and Robarts, 1987). Consequently, bacterial production and specific production also were much lower in this study.

Bacterial biomass values in this study were slightly higher than those previously reported for Lake Brazos (Davalos, 1986). This can be attributed to the higher bacterial abundance I observed because the cell volumes in the two investigations were similar.

The small percentage of attached bacteria I observed is common. Attached bacteria comprise less than 10% of the total bacterial population in many waters (Hobbie and Wright, 1979; Simon and Tilzer, 1982; Kirchman and Mitchell, 1982; Kirchman, 1983; Nagata, 1987). I did not observe the seasonal pattern of attachment reported by other investigators (Fallon and Brock, 1979; Lovell and Konopka, 1985; Laanbroek and Verplanke, 1986). In general, attachment increases during the summer due to the presence of algal exudates. Goulder (1986) reported increased attachment during winter due to elevated particulate loads and flow rates. I observed a single peak in attachment in January, which coincided with the highest clay concentration.

No production stimulation was observed with the addition of clay (Fig. 1). This was unexpected because clays typically concentrate organic substrates and increase bacterial growth rates. Apparently, the majority of the organic compounds were bound at the lower clay levels, and increasing the clay concentration resulted in little further substrate concentration.

Cadmium reduced bacterial cell production in this study (Fig. 2). Bacterial cell synthesis and specific production were greatly reduced by 1 and 20 mg Cd l^{-1} and less reduced at 5 and 10 mg Cd l^{-1}. These responses are atypical and

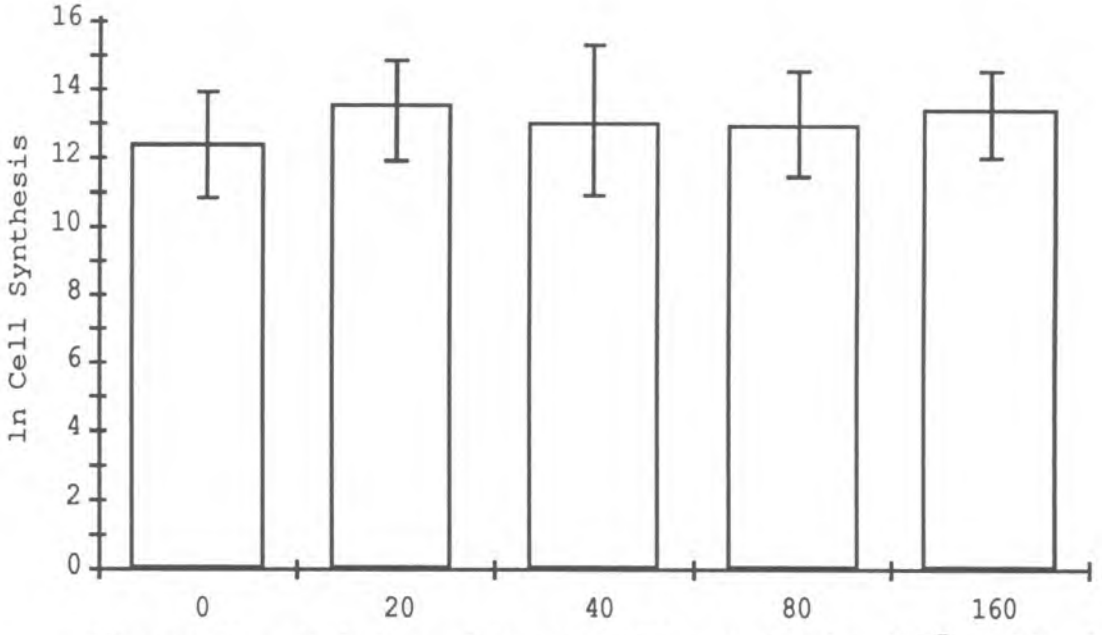

Figure 1. Relationship of cell synthesis (10^5 cells l^{-1} hr^{-1}) to added clay concentration (mg l^{-1}) in Lake Brazos.

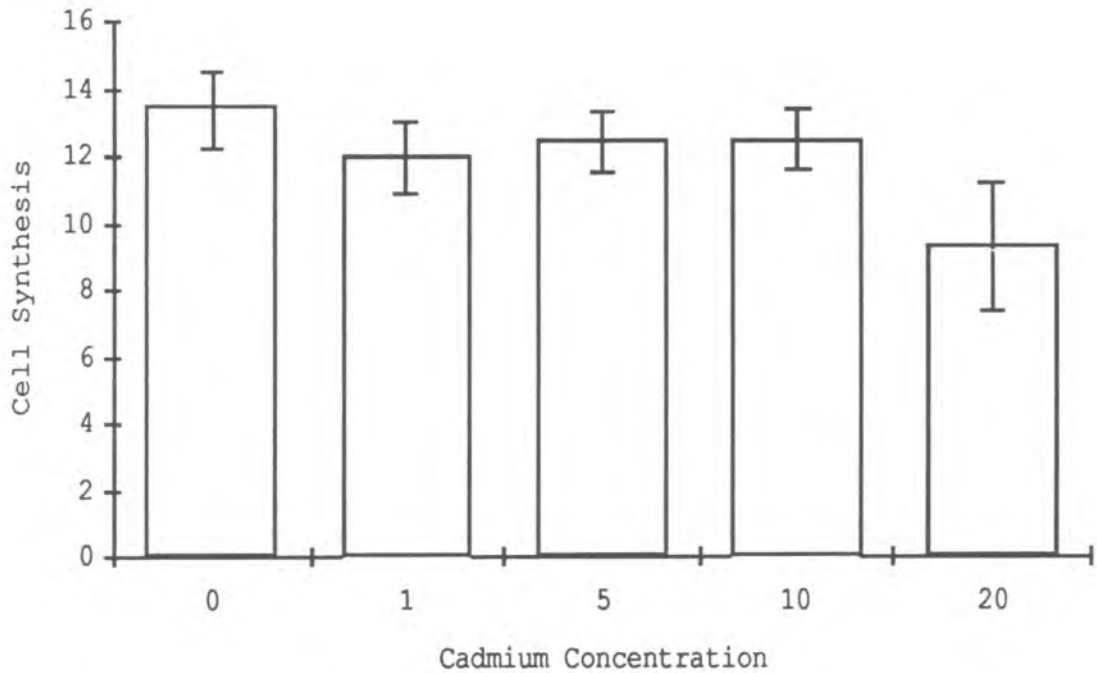

Figure 2. Reduction of bacterial cell synthesis (ln 10^5 cells l^{-1} hr^{-1}) by cadmium (mg l^{-1}) in Lake Brazos samples..

difficult to explain. Babich and Stotzky (1977) found that cadmium toxicity to bacteria and fungi increased with increasing cadmium concentration. My data suggest that the responses of bacteria to various levels of cadmium are more complex than originally believed. Others have shown that bacteria are relatively insensitive to cadmium at low concentrations (i.e. 1 mg Cd l^{-1}) (Wong,1987). However, these studies were conducted on laboratory cultures grown in synthetic media, and cannot accurately predict the response of naturally occuring bacterial communities (Babich and Stotzky, 1978). My data indicate that bacterial productivity is a sensitive indicator of cadmium toxicity at low cadmium concentrations, and support the data of Remacle et al. (1982), who observed reduction of bacterial biomass production at 0.5 mg l^{-1} cadmium. The effects of cadmium at lower concentrations (i.e. µg l^{-1}) need to be evaluated.

An important consideration in any toxicological study is the role of ambient environmental variables in the modification of the toxic response. Clays are an important component in many aquatic ecosystems. The addition of clays to the samples significantly affected cadmium toxicity to bacterial cell synthesis. Toxicity increased in the samples amended with 20 mg clay l^{-1}. Cadmium toxicity generally decreased with higher clay additions, approaching that in the

unamended samples (Fig.3). This relationship was not as prominant with the specific productivity data (Fig.4). This response may have been due to binding of the cadmium on the particles. The cadmium concentration on the particle surfaces was higher at the lower clay concentrations, resulting in the higher toxicity in these samples. At elevated clay concentrations, the amount of cadmium per particle was much lower, and fewer of the bacteria were exposed to the metal. However, since few of the bacteria were associated with particles in the original samples and the extent of bacterial colonization of the added clays was not investigated, this explanation is tentative. Other possible explanations are that the added clays released some inorganic component such as metal ions into solution that increased cadmium toxicity to the bacteria (Button, 1970). Metal analyses on the clays used in this study are forthcoming. Conversely, the clays may have adsorbed a production-limiting compound from solution or adsorbed some inorganic ligand such as chloride that protected bacteria from the toxic effects of cadmium (Babich and Stotzky, 1980). However, these explanations do not explain the lower toxicity at the higher clay concentrations. Addition of cadmium and clay to the samples probably induced changes in the chemical equilibria on the clay surfaces and in solution. Because the chemical characteristics of the clays and the water samples

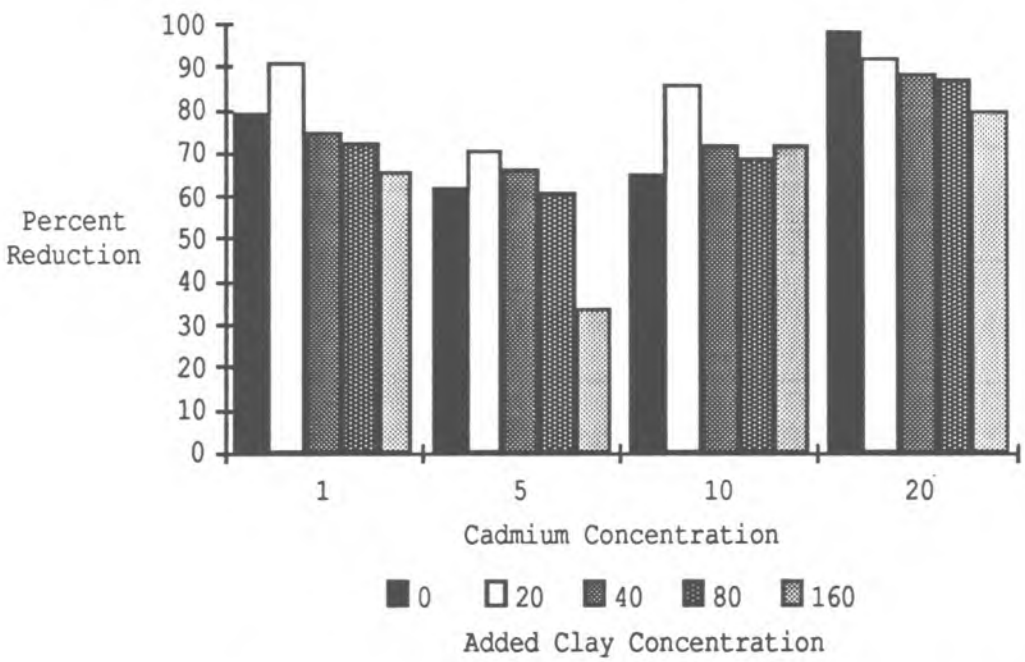

Figure 3. Toxicity of cadmium (mg l^{-1}) to cell synthesis at various added clay concentrations (mg l^{-1}) in Lake Brazos.

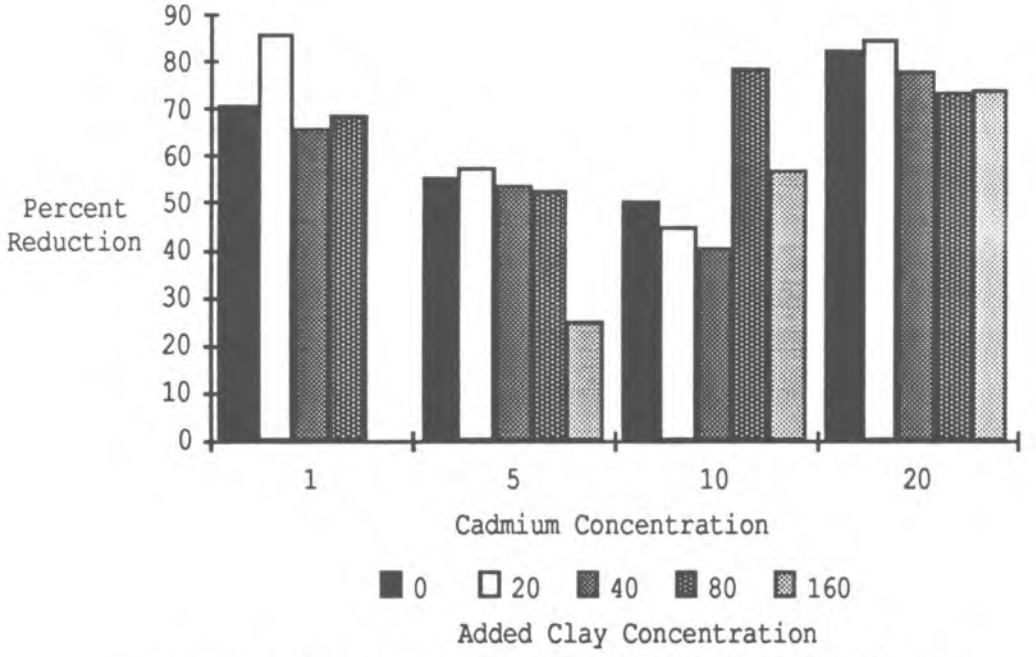

Figure 4 . Toxicity of cadmium (mg l^{-1}) to specific production at various added clay concentrations (mg l^{-1}) in Lake Brazos.

were not investigated, an explanation of the data is problematic. A previous study indicated that clay minerals protect microorganisms from the adverse effects of cadmium (Babich and Stotzky, 1977). The clay concentrations used in this investigation were 2-3 orders of magnitude higher than those I used, resulting in complete immobilization of the cadmium. My data show that at more realistic clay concentrations, clays may potentiate cadmium toxicity to bacteria.

This study indicates that bacterial productivity measurements are useful indicators of toxicity, and that conclusions drawn from conventional methodologies may not be accurate. Because bacterial production is important to aquatic carbon dynamics, further investigation of pollutant effects and the impact of environmental variables on this process is essential.

LITERATURE CITED

American Public Health Association. 1971. Standard methods for the examination of water and wastewater. Thirteenth edition. Washington, D. C.

Azam, F. and J. Fuhrman. 1984. Measurement of bacterioplankton growth in the sea and its regulation by environmental conditions. Pages 179-196. In: Hobbie, J. and P. J. leB Williams, (eds). Heterotrophic activity in the sea. Plenum Press, New York. 638 pp.

Babich, H. and G. Stotzky. 1977. Reductions in the toxicity of cadmium to microorganisms by clay minerals. Appl. Environ. Micro. 3:696-705.

Babich, H. and G. Stotzky. 1978. Effect of cadmium on microbes in vitro and in vivo: Influence of clay minerals. Pages 412-415. In: Loutit, M. and J. A. R. Miles, (eds). Microbial ecology. Springer- Verlag, Berlin. 452 pp.

Babich, H. and G. Stotzky. 1980. Environmental factors that influence the toxicity of heavy metals and gaseous pollutants to microorganisms. CRC Crit. Rev. Micro. 8:99-145.

Babich, H. and G. Stotzky. 1983. Developing standards for environmental toxicants: the need to consider abiotic environmental factors and microbe-mediated ecologic processes. Environ. Health Persp. 49:247-260.

Baker, J. H. and L. A. Bradham. 1976. The role of bacteria in the nutrition of aquatic detritivores. Oecologia. 24:95-104.

Barsdate, R. S., T. Fenchel, and R. T. Prentki. 1974. Phosphorus cycle of model ecosystems: Significance for decomposer food chains and effect of bacterial grazers. Oikos. 25:239-251.

Bell, R. T., and J. Kuparinen. 1984. Assessing phytoplankton and bacterioplankton production during early spring in Lake Erken, Sweden. Appl. Environ. Microbiol. 47:49-55.

Bell, R. T., G. M. Ahlgren, and I. Ahlgren. 1983. Estimating bacterioplankton production by measuring [^{3}H]- thymidine incorporation in a eutrophic Swedish lake. Appl. Environ. Microbiol. 45:1709-1721.

Black, C. A., (ed). 1965. Methods of soil analysis. Pages 572-574. In: Agronomy. No. 9. Amer. Soc. Agron. Madison, Wisconsin.

Blundel, M. R. and D. G. Wild. 1969. Inhibition of bacterial growth by metal salts: A survey of effects on the synthesis of ribonucleic acid and protein. Biochem. J. 115:207-212.

Bratbak, G. 1985. Bacterial biovolume and biomass estimation. Appl. Environ. Microbiol. 49:1488-1493.

Button, D. K. 1970. Effect of clay on on the availability of dilute organic nutrients to steady-state heterotrophic populations. Limnol. Oceanogr. 14:95-100.

Campbell,R. 1983. Microbial ecology. Blackwell Scientific Publications, Oxford. 191 pp.

Davalos, L. 1986. The relationship of turbidity and organic matter to bacterial productivity and biomass in a small Texas lake. Master's Thesis, Baylor University. Waco, Texas.

Doyle, J. J., R. T. Marshall, and W. H. Pfander. 1975. Effects of cadmium on the growth and uptake of cadmium by microorganisms. Appl. Microbiol. 29:562-564.

Ducklow, H. W., D. L. Kirchman, and G. T. Rowe. 1982. production and vertical flux of attached bacteria in the Hudson River plume of the New York Bight as studied with floating sediment traps. Appl. Environ. Microbiol. 43:769-776.

Duxbury, Trevor. 1985. Ecological aspects of heavy metal responses in microorganisms. Pages 185-228. In: Marshall, K. C., (ed). Advances in microbial ecology. Volume 8. Plenum Press, New York. 307 pp.

Fallon, R. D. and T. D. Brock. 1979. Lytic organisms and photooxidative effects: Influence on blue-green algae (Cyanobacteria) in Lake Mendota, Wisconsin. Appl. Environ. Microbiol. 38:499-505.

Fenchel, T. 1975. The quantitative importance of the benthic microflora of an arctic tundra pond. Hydrobiologia. 46:445-464.

Fenchel, T. M. and B. Barker Jorgensen. 1977. Detritus food chains of aquatic ecosystems: The role of bacteria. Pages 1-58. In:Alexander, M., (ed). Advances in microbial ecology. Volume 8. Plenum Press, New York. 268 pp.

Ferguson R. L. and P. Rublee. 1976. Contribution of bacteria to standing crop of coastal plankton. Limnol. Oceanogr. 21:141-145.

Fleischer, M., A. Sarofim, D. Fasset, P. Hammond, H. Shacklette, I. Nisbett, and S. Epstein. 1974. Environmental impact of cadmium: a review by the panel on hazardous trace substances. Environ. Health Perspect. 7:253-323.

Fletcher, M. and K. C. Marshall. 1982. Are solid surfaces of ecological significance to aquatic bacteria? Pages 199-236. In: Marshall, K. C., (ed). Advances in microbial ecology. Volume 6. Plenum Press, New York. 240 pp.

Forstner, U. and G. T. W. Wittmann. 1981. Metal pollution in the aquatic environment. Springer-Verlag. Berlin. 486 pp.

Friberg, L., M. Piscator, G. F. Nordberg, and T. Kjellstrom. 1974. Cadmium in the environment. CRC Press, Cleveland. 432 pp.

Fuhrman, J. A. 1981. Influence of method on the apparent size distribution of bacterioplankton cells: epifluorescence microscopy compared to scanning electron microscopy. Mar. Ecol. Prog. Ser. 5:103-106.

Fuhrman, J. A. and F. Azam. 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl. Environ. Microbiol. 39:1085-1095.

Fuhrman, J. A. and F. Azam. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol. 66:109-120.

Gadd, G. M. and Griffiths, A. J. 1978. Microorganisms and heavy metal toxicity. Microb. Ecol. 4:303-317.

Goulder, R. 1986. Seasonal variation in the abundance and heterotrophic activity of suspended bacteria in two lowland rivers. Freshwater Biology 16:21-37.

Harvey, R. W. and L. Y. Young. 1989. Enumeration of particle-bound and unattached respiring bacteria in the salt marsh environment. Appl. Environ. Microbiol. 40:156-160.

Hobbie, J. E. and R. T. Wright. 1979. An assessment of quantitative measurements of aquatic microbes. Arch. Hydrbiol. Beih. Ergebn. Limnol. 13:85-95.

Hobbie, J. E., R. J. Daley, and S. Jasper. 1977. Use of Nuclepore filters for counting bacteria by epifluorescence microscopy. Appl. Environ. Micro. 33:1225-1228.

Jannasch, H. W. and P. H. Pritchard. 1972. The role of inert particulate matter in the activity of aquatic microorganisms. Mem. Ist. Ital. Idrobiol. 29(suppl.): 289-308.

Jefferey, W. H. and J. H. Paul. 1986. Activity of an attached and free-Living Vibrio sp. as measured by thymidine incorporation, p-iodonitrotetrazolium reduction, and ATP/DNA ratios. Appl. Environ. Microbiol. 51:150-156.

Juma, N. G., and M. A. Tabatabai. 1977. Effects of trace elements on phosphatase activity in soils. Soil Sci. Soc. Am. J. 41:343-346.

Khazaeli, M. B. and R. S. Mitra. 1981. Cadmium binding component in Escherichia coli during accomodation to this ion. Appl. Environ. Microbiol. 41:46-50.

Kirchman, D. 1983. The production of bacteria attached to particles suspended in a freshwater pond. Limnol. Oceanogr. 28:858-872.

Kirchman, D. and R. Mitchell. 1982. Contribution of particle-bound bacteria to total microheterotrophic activity in five ponds and two marshes. Appl. Environ. Microbiol. 43:200-209.

Kornberg, A. 1980. DNA replication. W. H. Freeman and Company, San Francisco. 724 pp.

Kunc, F. and G. Stotzky. 1970. Breakdown of somealdehydes in soils with different amounts of montmorillonite and kaolinite. Folia Microbiol. 15:216-222.

Kurek, E., J. Czaban, and J. M. Bollag. 1982. Sorption of cadmium by microorganisms in competition with other soil constituents. Appl. Environ. Microbiol. 43:1166-1172.

Laanbroek, H. J. and J. C. Verplanke. 1986. Seasonal changes in percentages of attached bacteria enumerated in a tidal and a stagnant coastal basin: relation to bacterioplankton productivity. FEMS Microbiology Ecology. 38:87-98.

Lark, K. G. 1969. Initiation and control of DNA synthesis. Annu. Rev. Biochem. 38:569-604.

Larsson, U. and A. Hagstrom. 1982. Fractionated phytoplankton primary production, exudate release, and bacterial production in a Baltic eutrophication gradient. Mar. Biol. 67:57-70.

Lee, S. and J. A. Fuhrman. 1987. Relationships between biovolume and biomass of naturally derived marine phytoplankton. Appl. Environ. Microbiol. 53:1298-1303.

Lovell, C. R. and A. Konopka. 1985. Thymidine incorporation by free-living and particle-bound bacteria in a eutrophic dimictic lake. Appl. Environ. Microbiol. 49: 501-509.

Macaskie, L. E. and A. C. R. Dean. 1982. Cadmium accumulation by microorganisms. Environ. Technol. Lett. 3:49-56.

Manahan, S. E. 1984. Environmental chemistry. Willard Grant Press, Boston. 612 pp.

Mills, A. L. and R. R. Colwell. 1977. Microbiological effects of metal ions in Chesapeake Bay water and sediment. Bull. Env. Contam. Toxicol. 18:99-105.

Mitra, R. S., R. H. Gray, B. Chin, and A. I. Bernstein. 1975. Molecular mechanisms of accomadation in Escherichia coli to toxic levels of cadmium. J. Bacteriol. 121:1180-1188.

Montesinos, E., I.Esteve, and R. Guerrero. 1983. Comparison between direct method of determination of microbial cell volume: electron microscopy and electron particle sizing. Appl. Environ. Microbiol. 45:1651-1658.

Moriarty, D. J. W. 1986. Measurement of bacterial growth rates in aquatic systems from rates of nucleic acid synthesis. Pages 245-292. In: Marshall, K. C., (ed). Advances in Microbial Ecology. Vol. 9. Plenum Press. New York. 402 pp.

Moriarty, D. J. W. and P. C. Pollard. 1981. DNA synthesis as a measure of bacterial productivity in seagrass sediments. Mar. Ecol. Prog. Ser. 5:151-156.

Moriarty, D. J. W. and P.C. Pollard. 1982. Diel variation of bacterial productivity in seagrass (Zostera capricorni) beds measured by rate of thymidine incorporation into DNA. Mar. Biol. 72:165-173.

Nagata, Toshi. 1987. Production rate of planktonic bacteria in the north basin of Lake Biwa, Japan. Appl. Environ. Microbiol. 53:2872-2882.

Paerl, H. 1975. Microbial attachment to particles in marine and freshwater systems. Microb. Ecol. 2:73-83.

Paerl, H. and S. Merkel. 1982. Differential phosphorous uptake in attached vs. unattached microorganisms. Arch. Hydrbiol. 93:125-134.

Palumbo, A., R. L. Ferguson, and P. A. Rublee. 1984. Size of suspended bacterial cells and association of heterotrophic activity with size fractions of particles in estuarine and coastal waters. Appl. Environ. Microbiol. 48:157-164.

Pfister, R.M. 1982. Evaluation of bacterial binding and release of cadmium from aquatic sediments. U. S. Department of the Interior Report 712437.

Pollard, P. C. and D. J. W. Moriarty. 1984. Validity of the tritiated thymidine method for estimating bacterial growth rates: Measurement of isotope dilution during DNA synthesis. Appl. Environ. Microbiol. 48:1076-1083.

Pomeroy, L. R. 1974. The ocean's food web, a changing paradigm. BioScience. 24:499-504.

Pomeroy, L. R. 1984. Significance of microorganisms in carbon and energy flow in marine ecosystems. Pages 405-411. In: Klug, M. J. and C. A. Reddy, (eds). Current Perspectives in Microbial Ecology. American Society for Microbiology, Washington, D. C. 710 pp.

Reimann, B. 1984. Determining growth rates of natural assemblages of freshwater bacteria by means of ^{3}H-thymidine incorporation into DNA: comments on methodology. Arch. Hydrobiol. Beih. Ergebn. Limnol. 19:67-80.

Reimann, B. and M. Sondergard. 1984. Maesurements of diel rates of bacterial secondary production in aquatic environments. Appl. Environ. Microbiol. 47:632-638.

Reimann, B., J. Fuhrman, and F. Azam. 1982. Bacterial secondary production in freshwater measured by the ^{3}H-thymidine incorporation method. Microb. Ecol. 8:101-114.

Remacle, J. 1981. Cadmium uptake by freshwater bacterial communities. Wat. Res. 15:67-71.

Remacle, J., C. Houba, and J. Ninane. 1982. Cadmium fate in bacterial microcosms. Water, Air, and Soil Pollution. 18:455-465.

Rheinheimer, G. 1980. Aquatic microbiology. John Wiley and Sons, New York. 257 pp.

Riley, G. A. 1970. Particulate organic matter in sea water. Adv. Mar. Biol. 8:1.

SAS Institute. 1985. SAS user's guide: Statistics. Version 5 edition. 956 pp.

Simon, M. and M. M. Tizler. 1982. Bacterial decay of the autumnal phytoplankton in Lake Constance. Schweiz. Z. Hydrobiol. 44:263-275.

Sorokin, J. I. 1973. On the feeding of some scleractinian coralls with bacteria and dissolved organic matter. Limnol. Oceanogr. 18:380-385.

Staley,F. T. and A. Konopka. 1985. Measurement of in situ activities of non-photosynthetic microorganisms in aquatic and terrestrial habitats. Annu. Rev. Microbiol. 39:321-346.

Stotzky, G. 1966a. Influence of clay minerals on microorganisms. I. Montmorillonite and kaolinite on bacteria. Can. J. Microbiol. 12:547-563.

Stotzky, G. 1966b. Influence of clay minerals on microorganisms. II. Effect of various clay species, homoionic clays, and other particles on bacteria. Can. J. Microbiol. 12:831-848.

Stotzky, G. 1966c. Influence of clay minerals on microorganisms. III. Effect of particle size, cation exchange capacity, and surface area on bacteria. Can. J. Microbiol. 12:1235-1246.

Van Es, F. B. and A. Meyer-Reil. 1982. Biomass and metabolic activity of heterotrophic marine bacteria. Pages 111-170. In: Marshall, K. C., (ed). Advances in Microbial Ecology. Vol 6. Plenum Press, New York. 240 pp.

Wetzel, R. G., P.H. Rich, M. C. Miller, and H. L. Allen. 1972. Metabolism of dissolved and particulate detrital carbon in a temperate hard water lake. Mem. Ist. Ital. Idrobiol. Suppl. 29(suppl.):185.

Wicks, R. J. and R. D. Robarts. 1987. The extraction and purification of DNA labelled with [methyl-^{3}H] thymidine in aquatic bacterial production studies. J. Plank. Res. 9:1159-1166.

Williams, P. J. LeB. 1981. Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food web. Kieler. Meresforch. Sonderh. 5:1-28.

Wolter, K. 1980. Bacterial incorporation of organic substances released by natural phtoplankton populations. Mar. Ecol. 7:287-295.

Wong, P.T.S. 1987. Toxicity of cadmium to freshwater microorganisms, phytoplankton, and invertebrates. Pages 117-138. In: Nriagu, J. O. and J. B. Sprague, (eds). Cadmium in the Aquatic Environment. Wiley Interscience Series, New York. 512 pp.

Zhucova, A. I. 1963. Onthe quantitative significance of microorganisms in the nutrition of aquatic invertebrates. Pages 699-710. In: Oppenheimer, C. H. (ed). Symposium on marine ecology. Springfield. 752 pp.

Zimmerman, R. 1977. Estimation of bacterial number and biomass by epifluorescence microscopy and scanning electron microscopy. In: Rheinheimer, G.,(ed). Microbial ecology of a brackish water environment. Springer-Verlag, Berlin. 291 pp.

ZoBell, C. E. 1943. The effect of solid surfaces on bacterial activity. J. Bacteriol. 46:39-56.

Zwarum, A. A. 1973. Tolerance of Escherichia coli to cadmium. J. Environ. Qual. 2:353-355.