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Effect of Cadmium and Zinc on Microbial Adhesion, Growth, and Metal Uptake

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Recent awareness of the biological effects of pollutants on micro-organisms has led to their use as indicators in bioassay systems and as biological monitors of environmental contamination (Anderson and Abdelghani 1980; Drobniková and Bacilek 1982).

Micro-organisms are involved in many basic ecological processes and there is a great need to screen and evaluate pollutants for adverse effects on microbial populations and on microbial-plant or microbial-animal interactions (Babich and Stotzky 1978). Heavy metals are not biodegradable and once they enter the environment, the susceptibility of micro-organisms to heavy metals and the bioaccumulation of heavy metals by micro-organisms are ecologically very relevant phenomena (Gadd and Griffiths 1978; Sterritt and Lester 1980). Another microbial parameter of fundamental importance is microbial adhesiveness as in most environments some of the organisms are attached to surfaces. The role of adhesion in the fertility of soil, corrosion, fouling of immersed surfaces and microbial virulence is well established (Rogers 1979). Previous studies have shown that adhesiveness of micro-organisms is altered by heavy metals (Bhattacherjee and Saxena 1983; Sugarman 1978). In this report, the effect of zinc and cadmium on adhesiveness based on the interaction between the micro-organism and an animal cell has been observed. At the same time, simple methods have been used to screen for changes in growth and metal uptake.

MATERIALS AND METHODS

Streptococcus faecalis obtained from the Central Drug Research Institute, Lucknow was stored on nutrient agar slants (Oxoid) at 4°C and subcultured every six weeks. For adherence studies, the organisms were grown overnight at 37°C in brain heart infusion broth (BHI Hindustan Media, Bombay) and BHI supplemented with cadmium chloride 50 µM or zinc acetate 100 µM (E. Merk). Cultures were extensively washed over 0.45 u pore size membrane filters with isotonic saline buffered with 0.05 M Tris (hydroxymethyl aminomethane from Sigma Chemicals Co., USA) at pH 7.4. Suspensions of organisms were prepared over a 100 fold range from 90×10^6 to 10×10^8 and viable counts performed. The buccal cells of three volunteers were collected by swabbing with sterile cotton swabs and adherent cells shaken off into Tris saline. The cells

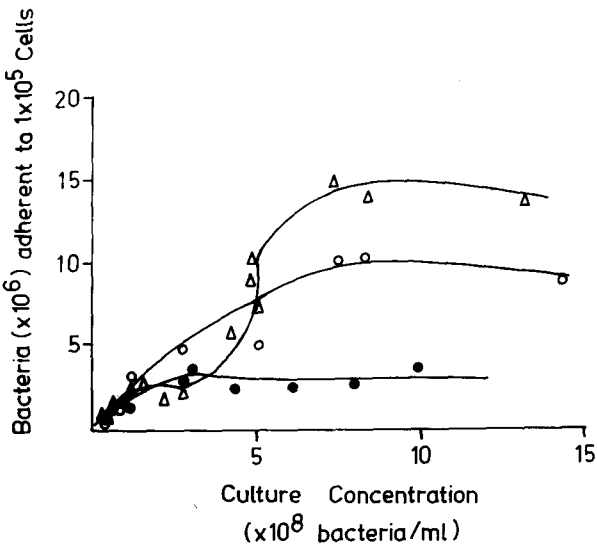

Figure 1. The effect of culture concentration on the number of bacteria which adhered to 1 x 10^5 buccal cells. (0) control (Δ) bacteria grown in presence of 100 μM zinc (•) bacteria grown in presence of 50 μM cadmium.

were washed three times and resuspended to give a concentration of 1 x 10^5 cells/ml as determined in a haemocytometer counting chamber by counting a minimum of 500 cells after thorough mixing to disperse any clumps. One ml of cells and 1 ml of bacteria of varying concentrations were then incubated in duplicate in a 37°C shaking water bath for 1 hr. The cell-bacterial mixture was drawn into a poly carbonate syringe filter holder containing a 25 mm 13 μ pore size membrane filter (F.P. 0.25/1 Schleicher and Schüll, Dassel, W. Germany). The specimen was washed with 200 ml of Tris buffered saline to remove non adherent bacteria. The cells with the adherent bacteria remaining on the surface of the membrane were resuspended in Tris saline and the number of bacteria adherent to the cells counted using viable counts.

To investigate the effect of cadmium and zinc on growth a turbimetric method was used. The organisms were growth in 5 ml of BHI amended with zinc acetate 100, 500 and 1000 μM and cadmium chloride 50, 250 and 500 μM. After 24 hr, the turbidity was measured at 480 nm in a spectrophotometer (Systronics 106 MK11). Each experiment was carried out a minimum of three times and each result represents the mean of four readings. The results were analysed by Student's 't' test as described by Fisher (1950).

Bacterial cultures derived from the growth studies were well washed and dried to constant weight (20-25 mg). These samples were digested in a 6:1 nitric acid perchloric acid mixture and analysed for zinc and cadmium

in a Perkin Elmer model 5000 atomic absorption spectrophotometer. Where sufficient numbers of specimens were available, the results were analysed by the Student 't' test, otherwise the mean of the available data is given.

RESULTS AND DISCUSSION

A range of bacterial densities was used in the adherence experiments as the number of adherent bacteria varied with the density. The control organisms at first showed a rapid increase in the number of attached cells which levelled out as the attachment surface became saturated. At low bacterial densities the micro-organisms grown in the presence of 100 μM zinc showed impaired adherence compared with the control (Fig. 1). With increasing bacterial density this inhibitory effect was overcome and at high densities zinc grown organisms exhibited enhanced adherence compared with the control.

Cultures grown in the presence of 100, 500 and 1000 μM zinc exhibited a small but statistically significant adverse effect on growth as measured by cell densities (Table 1). No detetectable increase in the zinc

Table 1. Effect of cadmium and zinc on growth of S. faecalis as determined by absorbance

μmol conc. zinc in BHI	OD	μmol conc. cadmium in BHI	OD
100	1.04±0.04**	50	1.03±0.07*
500	0.93±0.07*	250	0.85±0.01*
1000	0.95±0.01**	500	0.32±0.07**
Control	1.14±0.02	-	-

*P <0.05; **P <0.01

content of these organisms was observed (Table 2). Little information is available on the effect of microbial exposure to high levels of zinc. These findings confirm the presence of some effective regulatory mechanism for zinc uptake (Failla et al. 1976).

Table 2. Uptake of cadmium and zinc by S. faecalis

μmol conc. zinc in BHI	Zinc nmol/mg dry weight	μmol conc. cadmium in BHI	Cadmium nmol/mg dry weight
100	6.80	50	0.84±0.12**
500	7.21	250	3.35
1000	6.69	500	4.40
Control	7.39±0.81	-	0.22±0.05

**P <0.01

Although it is clear there is no acumulation of zinc by the species studied, sufficient zinc may have attached to the microbial surface to induce changes in adhesiveness. Zinc has a known affinity and ability to interact with bacterial cell walls and membranes (Beveridge & Murray 1976; Chvapil 1973). Metals are able to influence the hydrophobic and hydrophilic nature of microbial cell walls which may be related to the

decrease in adhesiveness at lower bacterial densities. Another contributory factor in this enhanced adhesiveness may be the ability of zinc to stabilise interactions between cell components and other surfaces (Ou 1973).

The highest level of cadmium in the medium which permitted the collection of adequate numbers of micro-organisms for adhesion studies was 50 μM. In this cadmium stressed culture the curvilinear adhesion pattern obtained with the control and zinc grown organisms (Figure 1) was lost. It was replaced by a linear pattern showing a steady increase in adhesion with increase in bacterial density over a narrow range of lower cell densities which quickly reached a plateau with no further increase in adhesion at higher cell densities. This suggests adhesion had become non-specific. A failure in production of adhesive polymers or proteins due to the direct action of cadmium on synthesis may be related to the changed adhesiveness (Blundell & Wild 1969). Here the adhesion studies have only examined an animal cell surface, studies using other organisms exposed to cadmium or zinc with plant cells or inert materials will indicate whether the modifications observed in adhesion represent general phenomena.

Increase in cadmium levels caused a steady decrease in growth in terms of turbidity (Table 1). This was accompanied by accumulation of cadmium by the tolerant microbial population (Table 2). Cadmium is known to adversely affect the growth kinetics of several bacteria (Babich & Stotzky 1978) and studies using E. coli suggested that the extended lag phase was related to the period of accommodation required for the micro-organism to develop some mechanism for detoxifying cadmium (Mitra et al. 1975).

Similar investigations of other pollutants utilizing the microbial parameters described here may provide useful preliminary information on their toxicity for microbial populations which form a part of all ecosystems.

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