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Bioavailability of Zinc and Cadmium and Their Effect on Microbial Growth and Metal Uptake

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Much of the information concerning the influences of heavy metals on microorganisms and the processes they mediate, is fragmentary and scattered over a wide range of scientific literature (Duxbury 1985). Heavy metals are not biodegradable and once they enter the environment, the susceptibility of microorganisms to heavy metals and the bioaccumulation of heavy metals by microorganisms are ecologically very significant phenomenon (Gadd and Griffiths 1978; Sterritt and Lester 1980; Bhattacherjee 1986). The ability of heavy metals to bind organic molecules plays an important, though still poorly understood, part in their action (Ramamoorthy and Kushner 1975). Microbiological studies on heavy metals are usually carried out in growth media. It is well understood that several of the constituents of common growth media can bind heavy metals, and this can influence the interaction of these metals with the microorganisms. Den Dooren de Jong (1965) suggested that using algae (which can grow in a completely inorganic medium) as test organisms would permit workers to estimate the true inhibitory concentrations of heavy metals.

Whether this is true or not, such a suggestion does reflect the difficulties experienced by microbiologists working with heavy metals. It is therefore desirable to know how much of the added metal to the bacterial growth medium remains mobile and how this proportion fluctuates with the different strengths of the medium studied with respect to metal complexation and the ways in which such complexes can affect bioaccumulation of the metal. Accordingly, in this report, the effect of Zn and Cd on bacterial growth, metal uptake and the influence of different strengths of the growth media in altering the availability of these metals to the organism has been observed.

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MATERIALS AND METHODS

Alcaligenes faecalis NCL 2105 obtained from National Chemical Laboratory, Poona, was stored on nutrient agar slants (Oxoid) at 4°C and sub-cultured every six weeks. At the time of each experiment with the heavy metals Zn and Cd, bacteria were inoculated into test tubes containing 10 mL of nutrient broth and grown overnight on a rotary shaker housed in a 37°C incubator. The bacterial suspension was then diluted with the nutrient broth to yield appoximately 75% transmission at 420 nm (Systronic Spectrophotometer, having an IP 39 phototube), and 0.1 mL of the aliquots of this diluted bacterial suspension in full strength nutrient broth containing 22 x 10^9 bacteria/mL was used for subsequent experiments on metal interactions with the bacteria in different strengths of the nutrient broth (HI-MEDIA, BOMBAY). Full strength nutrient broth consists of 0.5% Peptone, 0.5% Sodium Chloride, 0.15% Beef Extract and 0.15% Yeast Extract. Half strength and quarter strength broth were prepared by diluting full strength nutrient broth proportionately to its half and quarter strengths. The number of cells as determined by plate count method in half and quarter strength nutrient broth were 16 x 10^9 and 9 x 10^7 bacteria/mL respectively.

To investigate the effect of Zinc and Cadmium on the growth of the bacteria in different strengths of the nutrients broth, a turbidimetric method was used. The organism was grown in the test tubes containing 10 mL of nutrient broth amended with 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg of pure Zn. Similarly for Cd, 10 mL of nutrient broth was amended with 5 to 50 µg pure Cd and adjusted with NaOH to pH 7.4 ± 0.2. The tubes were placed in the rotary shaker, and after 24 hours the growth was measured at 420 nm.

Bacterial cultures derived from the growth studies with the metal Zinc, were well washed and dried. The dry weight culture samples were digested in a (6:1) nitric:perchloric acid mixture and analyzed for Zinc employing atomic absorption spectrophotometer (AAS) Perkin Elmer 5000 model (1982).

RESULTS AND DISCUSSION

The growth of bacteria under different culture conditions, indicate that the bacterial density in full strength nutrient broth amended with different levels of Zinc showed good growth upto concentration of 10 µg/mL Zn, and in half strength nutrient broth, the growth of bacteria was observed upto 7 µg/mL Zn (Table 1). In contrast to full strength and half strength growth media, the quarter strength nutrient broth had adverse effect on the growth of culture at 0.8 µg/mL Zn (Table 2). So also in the case of A. faecalis

Table 1. Effect of Zinc on the growth of A. faecalis as determined by absorbance in full and half strength nutrient broth

µg/mL conc. Zinc in N.B.*	Optical density (Full strength N.B.)	Optical density (Half strength N.B)
Control (without Zn)	0.28 ± 0.03**	0.25 ± 0.01**
1	0.23 ± 0.01	0.21 ± 0.01
2	0.23 ± 0.01	0.19 ± 0.03
3	0.23 ± 0.01	0.18 ± 0.03
4	0.22 ± 0.01	0.19 ± 0.03
5	0.21 ± 0.01	0.20 ± 0.03
6	0.19 ± 0.01	0.12 ± 0.02
7	0.16 ± 0.01	0.05 ± 0.03
8	0.10 ± 0.03	No growth
9	0.08 ± 0.01	"
10	0.06 ± 0.03	"

* Nutrient broth
** Mean ± the S.D. of the mean of three samples.

Table 2. Effect of Zinc on the growth A. faecalis as determined by absorbance in quarter strength nutrient broth

µg/mL conc. Zinc in N.B.*	Optical density
Control (without Zn)	0.11 ± 0.01**
0.2	0.06 ± 0.003
0.4	0.04 ± 0.003
0.6	0.01 ± 0.002
0.8	No growth

* Nutrient broth.
** Mean ± the S.D. of the mean of 3 samples.

Table 3. Effect of Cadmium on the growth of *A. faecalis* as determined by absorbance in different strengths of nutrient broth**

µg/mL conc. Cd in N.B.*	Optical density Full strength	Optical density Half strength	Optical density Quarter strength
Control (No Cd)	0.28 ± 0.03	0.22 ± 0.03	0.09 ± 0.01
0.5	0.25 ± 0.01	0.17 ± 0.03	No growth
1.0	0.20 ± 0.03	0.11 ± 0.03	"
1.5	0.18 ± 0.03	0.02 ± 0.01	"
2.0	0.10 ± 0.03	No growth	"
2.5	0.05 ± 0.03	"	"
3.0	0.02 ± 0.01	"	"
3.5	No growth	"	"

* Nutrient broth.
** Data presented as the mean ± the S.D. of the mean of three samples.

grown in presence of Cadmium, showed good growth in full strength nutrient broth upto 3.0 µg/mL Cd and in half strength broth upto 1.5 µg/mL Cd and total inhibition of growth was observed in quarter strength nutrient broth amended with least concentration of Cadmium of the order of 0.5 µg/mL (Table 3). Ramamoorthy and Kusher (1975) have observed binding of mercuric and other heavy metals in the commonly used microbial growth media and media components. They have evaluated the metal binding capacity in growth media by estimating free metal species by Orion ion specific metal electrodes. In the present study, although metal ion quantitation in the medium employed was not done, the effect of metal availability to the bacteria in terms of growth was evaluated by altering the strengths of the nutrient broth. Interestingly, the results of the study indicate that, metal availability (Zn and Cd) to the culture *A. faecalis* was strongly influenced by the strengths of the nutrient broth as indicated by its luxuriant growth in the concentrated broth than in the diluted broth. In so much so, bacteria grown in quarter strength nutrient broth amended with cadmium concentration experienced least or nil growth. Cadmium is known to adversely affect the growth kinetics of several bacteria (Babich and Stotzky 1978; Bhattacherjee 1986). The lower toxicity of Zn and Cd in full strength nutrient broth, may

have been the result of the complexation of the added metals to the components of the growth medium, which protected the organism from uptake of these metals.

The ability of heavy metals to bind organic substances, play an important ecological role that affects uptake and toxicity of the metals to microbes. The binding of metals to different organic ligands may indeed seem quite capricious, because of the manifold possibilities that can occur in complex and chelate formation (Jernelov and Martin 1975). These formations depend on such factor as pH, concentration of the metal ion in the actual solution and the presence of suitable organic ligands for complex formation. Complex and chelate formation of the metals may occur with the organic ligands and the binding of metal to S, N and O. Chelated heavy metals are, in general, less toxic when complexed with organics (Babich and Stotzky 1983). Consequently, studies to establish the concentration of a heavy metal that is inhibitory to microbial growth or activity must recognize the mediating influence of the organic and inorganic components present in the medium used.

It is evident from Table 3 that Cadium was more toxic to bacteria at lower concentrations. Bacterial growth was completely inhibited in the quarter strength nutrient broth. Inhibition was also evident in half strength and full strength nutrient broth, probably due to least complex nature of this medium as it contains peptone as the dominant nitrogen containing organic substrate, which was relatively ineffective in protecting against Cadmium. Ramamoorthy and Kushner (1975) have observed the following sequence of binding Cadmium by organics - Casamino acids > proteose peptone > tryptone > yeast extract and with peptone not binding Cadmium. It seems likely that the availability of heavy metals and their complexes for microbial flora in natural waters depends very much on the form in which these metals exist; the latter will in its turn depend on the properties of water, that is on its pH, Eh and on the dissolved substances present. Eutrophic waters, rich in phosphates, sulphates, amino acids and other organic compounds, could have considerable binding capacity towards metal pollutants (Siegel 1971). In growth media (which are of course much richer than most natural waters) somewhat similar conditions exist. Hence, an assay of total metals might be misleading in not showing the biological availability of these metals in most of the studies.
The metal uptake study in A. faecalis was performed only on Zinc and not Cadmium, since bacterial culture derived from the growth studies in half strength nutrient broth amended with pure Cadmium were not sufficient enough to get dry weight concentration of the

Table 4. Uptake of Zinc by A. faecalis grown in Half strength Nutrient broth

µg/mL conc. Zinc in 20 mL Nutrient broth	O.D. Mean ± S.D.	Dry weight (mg) Mean ± S.D.	Zinc µg/mg dry weight Mean ± S.D.
2	0.41 ± 0.01	20.5 ± 0.5	0.070 ± 0.01
6	0.34 ± 0.01	15.5 ± 0.5	1.279 ± 0.25
10	0.37 ± 0.02	15.5 ± 0.5	1.262 ± 0.26
Control	0.61 ± 0.01	26.5 ± 4.5	0.038 ± 0.03

culture for acid digestion and subsequent analysis of the metal on AAS. Cadmium showed adverse effect on the growth of A. faecalis at lower concentration in half strength nutrient broth (Table 3). Metal uptake studies in A. faecalis with Zinc showed appreciable increase of the metal at lower concentration and as the concentration of the metal in the growth medium increased, no detectable increase in the Zinc content of these organisms was observed (Table 4). 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). The Alcaligenes isolate grown in basal medium supplemented with Zinc exhibited no evidence of metal accumulation and toxicity to cells thereby showing resistance to the metal (McEntee et al 1986). Resistance may depend upon membrane alterations which either prevent Zinc influx or produce Zinc efflux once the metal has entered the cells. Zinc has a known affinity and ability to interact with bacterial cell walls and membranes (Beveridge and Murray 1976).

Similar investigations of other metal 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. With such studies, it may also be possible to give an acceptable concentration of the metal pollutant, before the organism is deemed resistant at particular concentration; since when determining, if an organism is metal resistant, there is no standard acceptable concentration which designates the boundary between metal resistance and susceptibility in bacterial isolates (Trevors 1986).

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REFERENCES

Babich H, Stotzky G (1978) Effect of Cadmium on the biota. Influence of environmental factors. Adv Appl Microbiol 23 : 55-116

Babich H, Stotzky G (1983) Physicochemical factors of natural reservoirs affect the transformation and exchange of heavy metals toxic to microbes. Environmental Biogeochemistry, Hallberg R (ed) Ecol Bull (Stockholm) 35 : 315-323

Beveridge TJ, Murray GF (1976) Uptake and retention of metals by cell walls Bacillus subtilis. J Bact 127 : 1502-1518

Bhattacherjee JW (1986) Effect of Cadmium and Zinc on microbial adhesion, growth and metal uptake. Bull Environ Contam Toxicol 36 : 396-400

Chvapil M (1973) New aspects in biological role of Zinc. A stabiliser of macromolecules and biological membranes. Life Sci 13 : 1041-2049

Den Dooren de Jong LE (1965) Tolerance of Chlorella vulgaris for metallic and non-metallic ions. Antonie Van Leeuwenhoek 31 : 301-313

Duxbury T (1985) Ecological aspects of heavy metal responses in microorganisms. In: Marshall KC (ed) Advances in Microbial Ecology, Plenum Press, New York 8 : 185-235

Failla ML, Benedict CD, Weinberg ED (1976) Accumulation and storage of Zinc by Candida utilis. J Gen Microbiol 94 : 23-36

Gadd GM, Griffiths AJ (1978) Microorganisms and heavy metal toxicity. Microbiol Ecol 4 : 300-317

Jernelov A, Martin AN (1975) Ecological implications of metal metabolism by microorganisms. Ann Rev Microbiol 29 : 61-77

McEntee JD, Woodrow JR, Quirk AV (1986) Investigation of Cadmium resistance in an Alcaligenes sp. Appl Environ Microbiol 51 : 515-520

Ramamoorthy S, Kushner DJ (1975) Binding of mercuric and other heavy metal ions by microbial growth media. Microbial Ecol 2 : 162-176

Siegell M (1971) Metal-organic interactions in the marine environment. In: Faust SJ, Hunter JV (eds) Organic Compounds in Aquatic Environments, Marcel Dekker, New York pp 265-295

Sterritt RM, Lester JN (1980) Interactions of metals with bacteria. Sci Total Environ 14 : 5-17

Trevors JT (1986) Mercury resistant bacteria isolated from sediment. Bull Environ Contam Toxicol 36 : 405-411

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