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# Accumulation of zinc and cadmium by *Cytophaga johnsonae*

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**Passive and active accumulation of zinc and cadmium by a common soil and freshwater bacterium, *Cytophaga johnsonae*, was studied using a radio-tracer batch distribution technique. The effects of variation of pH (3–10), as well as of ionic strength (0.007 and 0.07 M) on passive accumulation of the metals were examined. For both zinc and cadmium, accumulation was mainly due to passive processes, such as surface adsorption and/or diffusion into the periplasm. However, at low zinc concentrations, accumulation increased when glucose was added, suggesting an active uptake; at higher zinc concentrations such uptake was not detected, probably because it was masked by the stronger sorption properties of the cell wall. Adsorption of the metals was pH dependent: at higher ionic strength, accumulation was enhanced at pH values above 7; at lower ionic strength, adsorption differed and was markedly higher, with increased accumulation between pH 5 and 8.**

**Keywords:** bacteria, adsorption, uptake, metals

## Introduction

Bacteria may accumulate dissolved heavy metals from their immediate surroundings. Many of these metals are indispensable for bacterial growth although they are required at only very low concentrations. Zinc, for example, is essential for (i) the structure and activity of numerous enzymes, (ii) for the nucleic acid metabolism and cell division, and (iii) for the stabilization of macromolecules, organelles and membranes (Failla 1977). On the other hand, some heavy metals, e.g. cadmium, are non-essential and actually toxic, even at low concentrations.

Microbial heavy metal accumulation often comprises two stages (Failla 1977, Trevors *et al.* 1986, Belliveau *et al.* 1987, Gadd 1988). An initial rapid and passive process involving physical adsorption or ion exchange at cell surfaces, and occasionally a subsequent phase that is slower and involves active metabolism-dependent transport into bacterial cells. Owing to its metabolism dependence, this second phase is inhibited by low temperatures, by absence of an energy source, and by metabolic inhibitors and uncouplers. In Gram-negative bacteria, passive diffusion of metals through pores in the outer membrane is another possible accumulation mechanism. In the present text, the term *adsorption* indicates passive adsorption to the cell surface, *uptake* means metabolism-dependent intracellular transport and *accumulation* is used as a general term, i.e. when referring to one or both of the indicated mechanisms.

Metabolism-dependent, element-specific, intracellular transport would be required for the uptake of essential metals (Silver 1978) and such uptake frequently leads to higher concentration levels than those attained through accumulation by energy-independent processes. This was the case for zinc accumulation in yeasts and fungi (Paton & Budd 1972, Failla *et al.* 1976, Failla & Weinberg 1977, Lawford *et al.* 1980, Mowll & Gadd 1983), and in *Escherichia coli* (Bucheder & Broda 1974). Metals with no known biological function should not readily enter the cell unless specific transport pathways are present (Hughes & Poole 1986). However, active uptake of non-essential metals has been demonstrated for some organisms, possibly reflecting energy-driven uptake systems that are specific for certain essential metals (Perry & Silver 1982, Laddaga *et al.* 1985).

Rather more is known about zinc accumulation by fungi and yeasts (Hughes & Poole 1989) than about zinc uptake by bacteria, especially when considering nutrient conditions relevant in natural systems. Consequently, information is inadequate regarding the quantitative relationship between active and passive zinc accumulation in natural environments. Based on this lack of knowledge, the present study

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was initiated to investigate how the essential metal zinc and the non-essential metal cadmium are accumulated by *Cytophaga johnsonae*. Both active and passive accumulation were considered *per se* and in light of the effects at various pH values and ionic strengths. The studied species is a Gram-negative, gliding bacterium that is abundant in soil and freshwater, where it is involved in the decomposition of a wide range of organic compounds (Reichenbach & Dworkin 1981, Reichenbach 1989).

## Materials and methods

### *Organism and media*

*C. johnsonae* strain DSM 425 (CCUG 179355) was used throughout the study. The stock culture was stored at 4°C on Vy/2 agar slants (Reichenbach & Dworkin 1981) and was sub-cultured every 3 months.

Two experimental media, denoted CJ and CJ/10, were prepared. The CJ medium was used for culturing *C. johnsonae* and also for accumulation studies; it contained (per liter) 1 g (or 0.1 g, where stated) glucose, 2 g $(NH_4)_2SO_4$, 0.4 g magnesium glycerophosphate ($C_3H_7MgO_6 \cdot 2H_2O$), 1 g KCl and 4 g 2(-*N*-morpholino)-ethane sulphonic acid (MES buffer). Essential salts of inorganic ions ($FeSO_4 \cdot 7H_2O$ and $MnSO_4 \cdot H_2O$) were added from an acidic (pH 2) sterile stock solution to achieve final concentrations of 0.1 mg $l^{-1}$. The pH of CJ medium was adjusted to 6.5 for growth of bacteria and for accumulation experiments with non-starved bacteria (see below). In experiments with starved bacteria, the pH was adjusted to a range of values between pH 3 and 10. The ionic strength of the CJ medium was 0.07 M (contribution from MES not considered). The CJ/10 medium ($I = 0.007$) was used in the passive accumulation studies; it was obtained by diluting CJ medium 10 times with Milli-Q water. The pH value of this medium was adjusted to a range of values between pH 3 and 10.

### *Metal accumulation by starved bacteria in the absence of glucose*

*Passive* adsorption was studied as a function of two physicochemical parameters of considerable importance in soil, i.e. pH and ionic strength. *C. johnsonae* was grown in CJ medium at pH 6.5 and 23°C to late exponential phase, and then harvested by centrifugation (10 000 *g*, 10 min, 4°C), washed once with glucose-free CJ or CJ/10 medium and starved for 2 days. After starvation the bacteria were washed once more and suspended in glucose-free CJ or CJ/10 medium.

The adsorption of zinc and cadmium was studied separately at two ionic strengths, i.e. in glucose-free CJ ($I = 0.07$ M) and CJ/10 ($I = 0.007$ M) medium; experiments were performed in 50 ml centrifuge tubes using a total sample volume of 20 ml. Portions of acidic stock solutions (pH 1–2) of zinc and cadmium (as chlorides of the radioisotopes $^{65}Zn$ and $^{109}Cd$ together with non-radioactive carriers) were added to the tubes to give a total concentration of $10^{-7}$ M zinc and $10^{-9}$ M cadmium. This difference in concentration was chosen since zinc is more common than cadmium in natural environments. Portions of 1 M HCl or KOH solutions (filter sterilized) were added to each tube to obtain pH values ranging from 3 to 10. A suspension of bacteria was added to yield a density of 0.065 mg (DW) $ml^{-1}$ ($2 \times 10^8$ c.f.u. $ml^{-1}$, and the tubes were placed on a rotary shaker (120 r.p.m.) at 23°C for 24 h. The bacteria were then separated by centrifugation (4400 *g*, 10 min) and the pH was measured in the supernatants. Zinc and cadmium concentrations were determined by gamma counting (Compu Gamma, LKB Products, Bromma, Sweden) on 1 ml aliquots taken from the suspensions after 24 h of shaking and from the supernatants after centrifugation.

After the adsorption studies, the effects of metals, pH and ionic strength on the survival of starved bacteria were investigated. This was done by viable counting of bacterial suspensions grown on nutrient agar plates after dilution with 0.01 or 0.1 M KCl solutions.

### *Metal accumulation by non-starved bacteria in the presence of glucose*

Accumulation was investigated in the presence of glucose (0.1 g $l^{-1}$) to determine *active* metal uptake. *C. johnsonae* was grown in CJ medium (pH 6.5) at 23°C on a rotary shaker (120 r.p.m.) and then harvested by centrifugation (10 000 *g*, 10 min, 4°C) during the mid-exponential phase. The cells were washed twice by centrifugation in CJ medium without glucose, resuspended in CJ medium without glucose to yield a suspension of cells and immediately used in the accumulation experiments. Metabolism-dependent metal uptake was measured according to the method described by Brynhildsen & Rosswall (1989). The accumulation experiments were conducted in 125 ml Erlenmeyer flasks containing 50 ml portions of CJ medium at pH 6.5 with 0.1 g $l^{-1}$ (0.55 mM) glucose; glucose-free controls were run in parallel. To the medium in a flask, zinc or cadmium solutions were added in the form of the isotopes $^{65}Zn$ or $^{109}Cd$ together with non-radioactive carrier of the same metal (in both cases as chlorides) to give the desired metal concentration (i.e. in the range $5 \times 10^{-9}$ to $10^{-4}$ M for zinc and $10^{-9}$ to $10^{-3}$ M for cadmium). The bacterial cell suspension (1 ml) was added to yield an initial density of 0.026 mg (DW) $ml^{-1}$ ($1 \times 10^8$ c.f.u. $ml^{-1}$). Samples (1 ml) were removed after 2, 4, 6, 8, 10, 20, 30 and 40 min of incubation, and the bacteria were filtered using polycarbonate membrane filters (pore size 0.22 μm; Nucleopore, Pleasanton, CA). To remove loosely bound metal from the cell surface of the bacteria, cells were washed twice with 5 ml of non-radioactive metal solution (pH 6.5) of the same concentration as that used in the corresponding experimental flasks. The choice of this type of washing solution was based on results of preliminary investigations using various rinsing liquids, i.e. Milli-Q water, CJ medium with non-radioactive metal (with and without glucose), and solutions of non-radioactive metal at pH 3.5 and 6.5. The non-radioactive metal solutions with pH values at 3.5 and 6.5 were most efficient at removing exchangeable metals from the cell surface. The metal solution with a pH of 6.5 was used because it represented a pH value similar to that in the interior of the bacterial cell and that of the growth medium. After washing with the non-radioactive metal solution (pH 6.5), the filters were assayed for radioactivity by gamma counting (see above) and the values were corrected for any zinc or cadmium adsorption by the filters by comparing with the assay values for the bacteria-free controls. The number of viable cells in a flask was then determined as described above, at the beginning and the end of the metal-accumulation experiments.

### *Chemicals and equipment*

All chemicals used were of analytical reagent grade. Water of Milli-Q quality, obtained from a Millipore water system, was used in all solutions. The radionuclides (Amersham International, Buckinghamshire, UK) were stored in HCl solutions. Polypropylene or polyallomer equipment (Nalgene Labware, Nalge/Sybron, Rochester, NY) was used throughout the study. All equipment was cleaned by soaking overnight in a mixture of $HNO_3$ (3 M) and HCl (0.1 M), and then rinsing six times with Milli-Q water.

## Results

### *Metal accumulation by starved bacteria in the absence of glucose*

Figure 1 illustrates metal accumulation by starved *C. johnsonae* in the absence of glucose, expressed as percent adsorption and as the distribution coefficient $K_d$ [the concentration in the solid phase (mol $g^{-1}$) divided by the concentration in solution (mol $cm^{-3}$)] as a function of pH. The adsorption was clearly pH dependent at both ionic strengths. A similar pH dependence was obtained for both metals at the higher ionic strength (i.e. in CJ medium), i.e. metal accumulation was low at pH values below 7–8 and increased at pH values above 8. At pH below 8, the adsorption of zinc was greater than that of cadmium ($\log K_d = 3.0$–3.4, corresponding to 10–15%, and $\log K_d$ about 3.0, corresponding to 5–6%, for zinc and cadmium, respectively; those values also respectively correspond to 100–230 and 0.7–0.9 nmol $g^{-1}$ [DW]). For zinc and cadmium, respectively, adsorption was approximately 430 and 6 nmol $g^{-1}$ (DW) ($\log K_d = 3.6$ and almost 4.0) at pH around 9. In contrast, a different adsorption behaviour was observed at the lower ionic strength (in CJ/10 medium): accumulation was markedly enhanced at the neutral pH range, with a maximum at pH 7 ($\log K_d = 4.2$ and 50% adsorption for zinc and $\log K_d$ around 4.0 and 40% for cadmium, corresponding to 800 and 7 nmol $g^{-1}$ [DW], respectively).

Bacterial survival was pH dependent, and maximum between pH 5 and 8.5, and from there gradually decreasing at both higher and lower pH values (data not shown). Neither the ionic strength of the medium, nor the presence of zinc or cadmium (at concentrations of $10^{-7}$ and $10^{-9}$ M, respectively) had any noticeable effect on the survival of the bacteria.

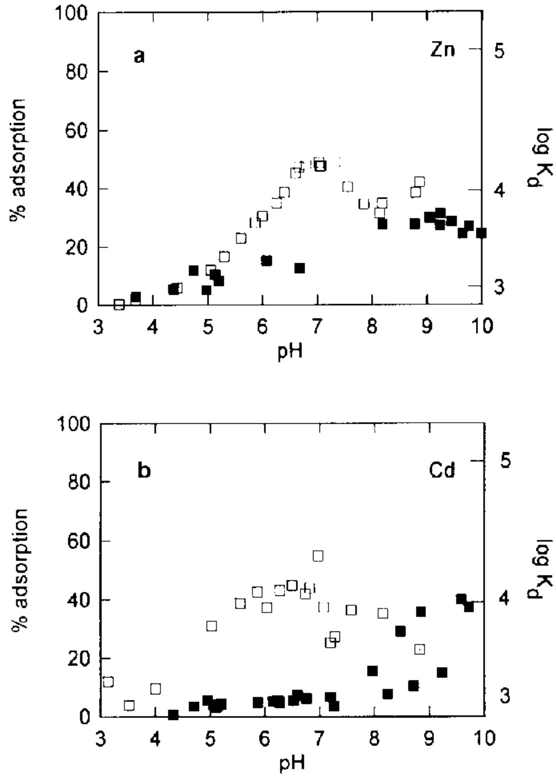

**Figure 1.** Accumulation of zinc (**a**) and cadmium (**b**) [percent and $K_d$ ($cm^3 g^{-1}$)] by starved *C. johnsonae* in the absence of glucose as a function of pH and ionic strength (metal concentrations $10^{-7}$ and $10^{-9}$ M, respectively). Each symbol corresponds to one experiment. ■, CJ medium, $I = 0.07$ M; □ CJ/10 medium, $I = 0.007$ M.

### *Metal accumulation by non-starved bacteria in the presence of glucose*

Zinc and cadmium accumulation by *C. johnsonae* was compared regarding accumulation kinetics exhibited during 40 min of incubation in the presence and absence of glucose; this was done to determine differences related to a metabolism-dependent uptake (CJ medium, pH 6.5). Generally, the addition of glucose (0.1 g $l^{-1}$) slightly enhanced accumulation of zinc at low concentrations ($5 \times 10^{-9}$ to $10^{-7}$ M): after an initial, rapid increase during the first 5–10 min, accumulation increased slowly with time (data for $10^{-7}$ M given in Figure 2a; similar graphs were obtained for $10^{-8}$ and $5 \times 10^{-9}$ M, not illustrated). This slow increase in accumulation was also observed in media without glucose at zinc concentrations below $10^{-7}$ M. Accumulation of zinc at higher concentrations ($10^{-6}$ to $10^{-4}$ M) was not affected by the addition of glucose nor was the accumulation of cadmium at any of the tested concentrations ($10^{-9}$ to $10^{-3}$ M; data for $10^{-7}$ M given in Figure 2b). In these cases, accumulation leveled off after 10 min. Cell survival did not decrease during 40 min of incubation, not even at the highest metal concentrations studied (i.e. $10^{-4}$ M for zinc and $10^{-3}$ M for cadmium; data not shown).

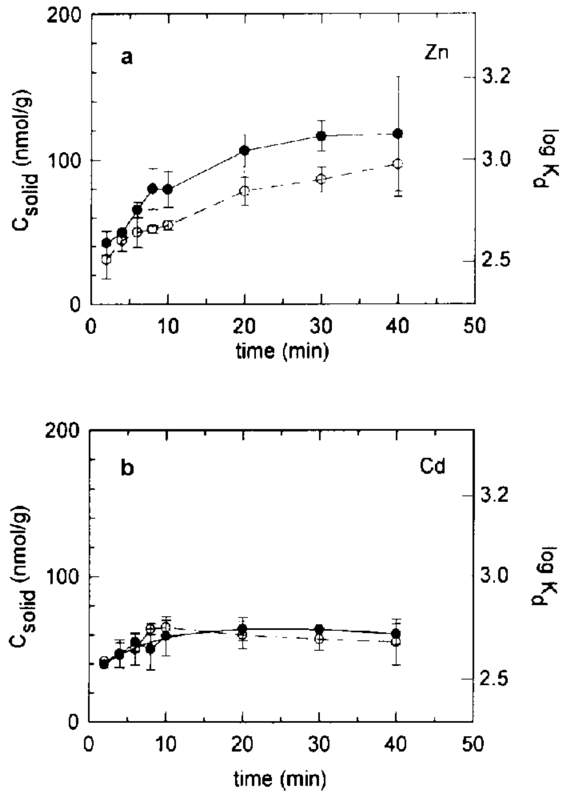

**Figure 2.** Kinetics of accumulation of zinc (**a**) and cadmium (**b**) [$C_{solid}$ (nmol $g^{-1}$ DW) and $K_d$ ($cm^3\ g^{-1}$)] by non-starved *C. johnsonae* in CJ medium (metal concentration $10^{-7}$ M, pH 6.5). The results represent the means (with standard deviations) of three (Zn) and two (Cd) experiments: ●, with glucose; ○, without glucose.

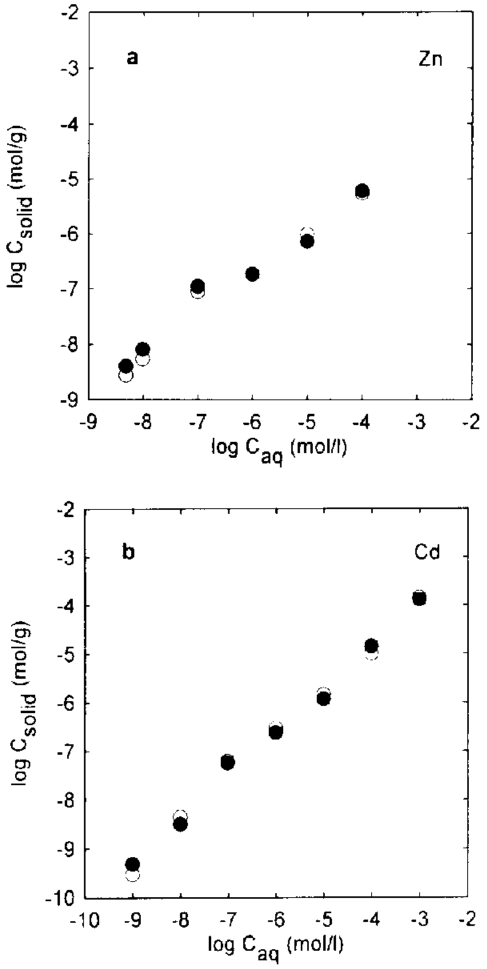

**Figure 3.** Accumulation isotherms for zinc (**a**) and cadmium (**b**) obtained using non-starved bacteria in CJ medium (pH 6.5). The concentration of metal on solid ($C_{solid}$) (mol $g^{-1}$) is plotted as a function of the concentration of metal in solution ($C_{aq}$) (mol $l^{-1}$). The results represent the means of three to six measurements; standard deviation is within the dimensions of the symbols: ● with glucose; ○, without glucose.

Metal distribution between solution and bacteria at equilibrium was determined for zinc and cadmium in the concentration ranges $10^{-4}$ to $5 \times 10^{-9}$ and $10^{-3}$ to $10^{-9}$ M, respectively, based on values from the accumulation of non-starved bacteria (pH 6.5, CJ medium). The concentration of metal on or in the solid ($C_{solid}$) was plotted as a function of the concentration of metal in the solution ($C_{aq}$) (Figure 3a and b). For zinc, this accumulation isotherm showed a clear inflection point at a concentration in solution of $10^{-7}$ M. The values for $10^{-7}$ M differed significantly (*t*-test, $P < 0.05$) from a regression line obtained through the other values. No saturation effects were observed at the highest metal concentrations, indicating a maximum capacity of more than 10 mmol $kg^{-1}$ (corresponding to the highest zinc concentration in solution, i.e. $10^{-4}$ M). The isotherm for cadmium displayed no definite inflection point in the concentration range studied. Nor did the cadmium isotherm level off at higher concentrations, suggesting that the system was far from saturated; a feasible maximum capacity would be at least 100 mmol $kg^{-1}$ (DW) (corresponding to the highest studied concentration in solution, $10^{-3}$ M). The accumulation of both metals appeared to follow almost linear Freundlich isotherms, using data for the whole concentration range ($\log C_{solid} = N \log C_{aq} + \log K$, with $N$ close to 1.0).

## Discussion

Bacterial cell walls, which usually carry a negative net charge at neutral pH, would attract cationic metal species in solution. The prime sites for metal binding by *C. johnsonae* may be similar to those found for *E. coli* (Beveridge & Koval 1981, Hoyle & Beveridge 1984), i.e. phosphate groups of lipopolysaccharides (LPS) and phospholipids, as well as carboxylate groups of both peptidoglycan and LPS. Godchaux *et al.* (1990, 1991) found a high molecular weight polysaccharide (HMPS), rich in amino sugars, that was firmly associated with the outer membrane of *C. johnsonae*.

It is likely that some functional groups of this polysaccharide may also contribute to the overall binding capacity. However, the total negative charge of the related bacterial strain *Cytophaga* U 67 was found to be relatively low (Bayer & Sloyer 1990), which could suggest a low metal-adsorption capacity. In the present system, total accumulation of zinc by the *starved* bacteria in the absence of glucose (pH 6.5; $I = 0.07$ M) was around 150–200 nmol g$^{-1}$ (DW) at a solution zinc concentration of $10^{-7}$ M. Although passive adsorption should be the dominant mechanism of metal accumulation in these energy-deprived bacteria, active uptake of the metals into the cytoplasm due to endogeneous metabolism cannot be excluded. However, if any active uptake did occur in the starved bacteria in the absence of glucose (as in the present systems), it must be much lower than uptake exhibited by the *non-starved* bacteria (also in the absence of glucose) at the same metal concentration ($10^{-7}$ M) and pH (i.e. approximately around 100 nmol g$^{-1}$ [DW]; Figure 2a). The possibility of active uptake in the starved system could have been avoided by using dead cells or purified envelopes, but then the surface composition, and therefore the metal-sorbing ability, of the bacteria probably would have been altered (Bollag & Duszota 1984). The largest amounts of zinc and cadmium adsorbed by starved *C. johnsonae* in the absence of glucose were comparable with amounts found to be adsorbed by another Gram-negative bacterium, *Klebsiella oxytoca*, at similar metal concentrations (Wallberg *et al.* 1991).

The present results illustrate that pH is an important factor in microbial metal adsorption. As expected, the adsorption of both zinc and cadmium by *C. johnsonae* was reduced at low external pH and enhanced at higher pH values, with an optimum between 8 and 9 (at an ionic strength of 0.07 M; Figure 1). In general, a similar trend in metal absorption over a wide range of pH values was obtained in previously reported experiments on other bacteria (Doyle *et al.* 1980), fungi (Gadd & Griffiths 1980) and algae (Geisweid & Urbach 1983, Les & Walker 1984). Wnorowski (1991) found that the highest adsorption of cadmium by two heat-inactivated strains of a *Cytophaga* sp. was achieved at pH 11. This observed pH dependence can reflect changes in both metal speciation in solution and the ligands available at the metal binding sites (Campbell & Stokes 1985). At low pH values, affinity of the cell wall for metal ions decreases, presumably due to competition with $H^+$ for the binding sites. An enhanced adsorption at higher pH could be due to an increase in the negative charge of surface functional groups and also partly to the formation of hydroxide and carbonate complexes with the metals, complexes that could precipitate within (Geisweid & Urbach 1983, Norberg & Persson 1984) or become associated with the cell walls. However, the accumulation of zinc by *K. oxytoca* in 0.1 M KCl was maximum at around pH 6 (Wallberg *et al.* 1991), which is similar to the pH dependence of *C. johnsonae* in CJ/10 medium (see below).

The *ionic strength* of the experimental medium is often neglected when investigating the adsorption of metals on biological surfaces. In the present study, the adsorption of zinc and cadmium was markedly enhanced at lower ionic strength (Figure 1), especially around neutral pH, where a maximum was observed. In contrast, Norberg & Persson (1984) reported that the accumulation of metals by *Zoogloea ramigera* was insensitive to changes in ionic strength. The observed increase in metal accumulation at lower ionic strength in *C. johnsonae* (Figure 1) may be ascribed to an increase in the negative charge of the cell surface as ionic groups are no longer saturated with ions from exogeneous salt (James 1979).

Accumulation isotherms were used to evaluate the metal-uptake capacity of *non-starved C. johnsonae* (at pH 6.5 and in CJ medium). The isotherm for cadmium could be described by an almost linear (i.e. $N = 1$) Freundlich isotherm at low concentrations. The maximum metal capacity was at least 100 mmol kg$^{-1}$ (Figure 3b). The isotherm for zinc had a steep slope at low metal concentrations, with a significant bend around $10^{-7}$ M and a slope similar to that exhibited by the cadmium system at higher metal concentrations. This indicates the existence of two different accumulation mechanisms for zinc (see Figure 4).

The slow increase in accumulation of zinc after the initial rapid accumulation at the lowest concentrations (i.e. below $10^{-7}$ M; Figure 2a) suggests that the metal was actively taken up, possibly by a zinc-specific energy-dependent transport system. Such metal-specific transport systems have been reported for other bacteria (Silver 1978). This corresponds to the steep isotherm at low concentrations (the dashed line in Figure 4), which suggests a maximum capacity of 0.1 mmol kg$^{-1}$ (DW) (*cf.* Figure 2a). The zinc requirement and the corresponding capacity of the zinc uptake system in *C. johnsonae* are obviously small.

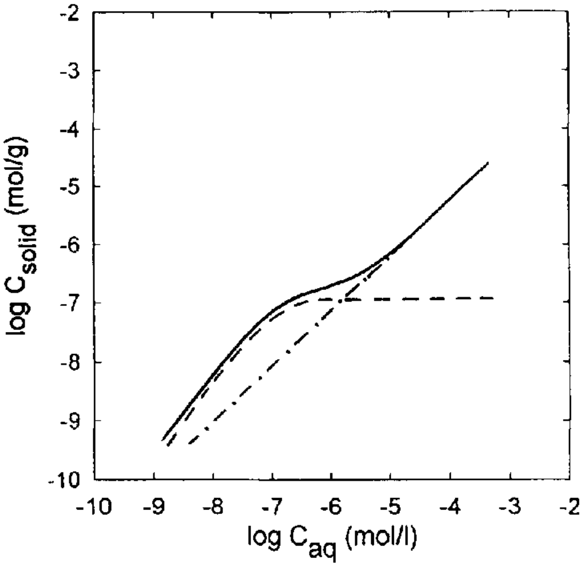

**Figure 4.** Resolution of the accumulation isotherm for zinc (pH 6.5) (the solid line) into two superimposed Freundlich isotherms ($\log C_{solid} = N \log C_{aq} + \log K$): – – –, capacity of 0.1 mmol kg$^{-1}$, $N = 1.2$ at low zinc concentrations; –·–·–, capacity of at least 100 mmol kg$^{-1}$; $N = 0.9$.

At the lowest zinc concentrations, the second, slower, phase of accumulation occurred also in the absence of an exogeneous energy source (Figure 2a), which suggests that the bacterial intracellular energy supply was not completely depleted. In contrast, at higher zinc concentrations (above $10^{-6}$ M), no such difference was observed between samples in the presence and absence of glucose, and no second accumulation phase was detected. Any active uptake system was probably saturated and masked by the presence of a passive accumulation mechanism. This passive mechanism would correspond to the lower slope of the isotherm at high metal concentrations (Figure 4).

It appears that cadmium did not enter the cells of *C. johnsonae* via an energy-dependent transport system, since the addition of glucose did not stimulate cadmium accumulation during 40 min of incubation (Figure 2b). The kinetics of the cadmium accumulation shows that almost all metal added was accumulated within the first 5–10 min, regardless of glucose supplementation. This reveals that cadmium was accumulated mainly through passive processes, such as physico-chemical interaction with negatively charged groups on the cell surface and/or diffusion through pores in the outer membrane. The same mechanisms would probably also be responsible for the passive accumulation of zinc. Most of the $^{65}$Zn or $^{109}$Cd that was loosely attached to the surface of the cells was replaced by non-radioactive zinc or cadmium during the washing procedure (see Materials and methods). Thus, it may be concluded that non-desorbed metals were bound to less accessible sites in the cell wall or in the periplasmic space, and/or that covalent bond formation took place, since the metals were difficult to remove by washing with non-radioactive metal solutions.

The absence of an intracellular transport system for cadmium was not surprising, since bacteria have no need for specific, active transport systems for metals of no known biological function (Hughes & Poole 1986, Gadd 1988). On the other hand, these elements are sometimes actively taken up by less specific systems: energy-dependent uptake of cadmium has been found to occur via a manganese uptake system in *Bacillus subtilis* (Laddaga *et al.* 1985) and *Staphylococcus aureus* (Tynecka *et al.* 1981a,b, Perry & Silver 1982), and by the zinc transport system in *E. coli* (Laddaga & Silver 1985).

The presence of zinc did not reduce cell viability, regardless of glucose supplementation, which is in agreement with the hypothesis that only small amounts of zinc were taken up by the active zinc transport system. In *E. coli*, where an energy-dependent zinc transport was described (Bucheder & Broda 1974), glucose strongly stimulated zinc uptake, which, in turn, depressed cell viability. However, the glucose concentration used by the cited authors (i.e. 8 g $l^{-1}$) was high compared to that used in the present study (0.1 g $l^{-1}$). Brynhildsen *et al.* (1988) showed that bioavailability of nutrients in media may affect the sensitivity of bacteria to metals. These researchers found that zinc, cadmium and copper were generally less toxic to a *Klebsiella* sp. at lower glucose concentrations than at higher. This was proposed to be due to deficiency of energy for the energy-driven metal uptake systems. However, other investigators (Failla & Weinberg 1977, Lawford *et al.* 1980) found that the yeast *Candida utilis* accumulated high intracellular levels of zinc without any reduction of viability, which may have been due to the presence of detoxification mechanisms such as internal compartmentalization or binding to metal-sequestering macromolecules.

## Conclusions

(i) Zinc and cadmium were accumulated mainly through *passive* processes, such as adsorption on the cell surface and/or diffusion into the periplasm through pores in the outer membrane. Accumulation of zinc by *C. johnsonae* was stimulated by the presence of glucose, suggesting also an *active* transport for this metal. However, the zinc-accumulating capacity of this system was low (0.1 mmol $kg^{-1}$) and saturation occurred even when the zinc concentration in solution was low ($10^{-7}$ M). At higher zinc concentrations, the active uptake appeared to be masked, which could reflect diffusion of zinc into the periplasmic space or binding of the metal to other sites, from which it was not easily exchangeable with the present method. For cadmium, only passive accumulation processes could be detected.

(ii) Generally, the passive adsorption of zinc and cadmium increased with increasing pH and decreasing ionic strength. In natural environments, both these parameters can vary within broad ranges (on a microscale), and may therefore profoundly influence adsorption processes and thereby also the mobility of the metals.

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## References

Bayer ME, Sloyer Jr JL. 1990 The electrophoretic mobility of gram-negative and gram-positive bacteria: an electrokinetic analysis. *J Gen Microbiol* **136**, 867–874.

Belliveau BH, Starodub ME, Cotter C, Trevors JT. 1987 Metal resistance and accumulation in bacteria. *Biotech Adv* **5**, 101–127.

Beveridge TJ, Koval SF. 1981 Binding of metals to cell envelopes of *Escherichia coli* K-12. *Appl Environ Microbiol* **42**, 325–335.

Bollag J-M, Duszota M. 1984 Effect of the physiological state of microbial cells on cadmium sorption. *Arch Environ Contam Toxicol* **13**, 267–270.

Brynhildsen L, Lundgren B, Allard B, Rosswall T. 1988 Effects of glucose concentrations on cadmium, copper, mercury, and zinc toxicity to a *Klebsiella* sp. *Appl Environ Microbiol* **54**, 1689–1693.

Brynhildsen L, Rosswall T. 1989 Effects of cadmium, copper, magnesium, and zinc on the decomposition of citrate by a *Klebsiella* sp. *Appl Environ Microbiol* **55**, 1375–1379.

Bucheder F, Broda E. 1974 Energy-dependent zinc transport by *Escherichia coli. Eur J Biochem* **45,** 555–559.

Campbell PGS, Stokes PM. 1985 Acidification and toxicity of metals to aquatic biota. *Can J Fish Aquat Sci* **42,** 2034–2049.

Doyle RJ, Matthews TH, Streips UN. 1980 Chemical basis for selectivity of metal ions by the *Bacillus subtilis* cell wall. *J Bacteriol* **143,** 471–480.

Failla ML. 1977 Zinc: functions and transport in microorganisms. In: Weinberg ED, ed. *Microorganisms and Minerals.* New York: Marcel Dekker; 151 214.

Failla ML, Benedict CD, Weinberg ED. 1976 Accumulation and storage of $Zn^{2+}$ by *Candida utilis. J Gen Microbiol* **94,** 23–36.

Failla ML, Weinberg ED. 1977 Cyclic accumulation of zinc by *Candida utilis* during growth in batch culture. *J Gen Microbiol* **99,** 85 97.

Gadd GM. 1988 Accumulations of metals by microorganisms and algae. In: Rehm H-J, Reed G, eds. *Biotechnology—A Comprehensive Treatise,* Vol. 6b. Weinheim: VCH Verlagsgesellschaft; 401–433.

Gadd GM, Griffiths AJ. 1980 Influence of pH on toxicity and uptake of copper in *Aureobasidium pullulans. Trans Br Mycol Soc* **75,** 91–96.

Geisweid HJ, Urbach W. 1983 Sorption of cadmium by the green microalgae *Chlorella vulgaris, Ankistrodesmus braunii* and *Eremosphaera viridis. Z Pflanzenphysiol Bd* **109,** 127–141.

Godchaux III W, Gorski L, Leadbetter ER. 1990 Outer membrane polysaccharide deficiency in two nongliding mutants of *Cytophaga johnsonae. J Bacteriol* **172,** 1250–1255.

Godchaux III W, Lynes MA, Leadbetter ER. 1991 Defects in gliding motility in mutants of *Cytophaga johnsonae* lacking a high-molecular-weight cell surface polysaccharide. *J Bacteriol* **173,** 7607 7614.

Hoyle BD, Beveridge TJ. 1984 Metal binding by the peptidoglycan sacculus of *Escherichia coli* K-12. *Can J Microbiol* **30,** 204–211.

Hughes MN, Poole RK. 1986 Metal mimicry and metal limitation in studies of metal–microbe interactions. In: Poole RK, Gadd GM, eds. *Metal Microbe Interactions,* Vol. 26. Oxford: IRL Press; 1–17.

Hughes MN, Poole RK. 1989 *Metals and Micro-organisms.* London: Chapman & Hall.

James AM. 1979 Molecular aspects of biological surfaces. *Chem Soc Rev* **8,** 389–418.

Laddaga RA, Silver S. 1985 Cadmium uptake in *Escherichia coli K-12. J Bacteriol* **162,** 1100 1105.

Laddaga RA, Bessen R, Silver S. 1985 Cadmium-resistant mutant of *Bacillus subtilis* 168 with reduced cadmium transport. *J Bacteriol* **162,** 1106–1110.

Lawford HG, Pik JR, Lawford GR, Williams T, Kligerman A. 1980 Hyperaccumulation of zinc by zinc-depleted *Candida utilis* grown in chemostat culture. *Can J Microbiol* **26,** 71–76.

Les A, Walker RW. 1984 Toxicity and binding of copper, zinc, and cadmium by the blue-green alga, *Chroococcus paris. Water Air Soil Pollut* **23,** 129–139.

Mowll JL, Gadd GM. 1983 Zinc uptake and toxicity in the yeast *Sporobolomyces roseus* and *Saccharomyces cerevisiae. J Gen Microbiol* **129,** 3421–3425.

Norberg AB, Persson H. 1984 Accumulation of heavy metal ions by *Zoogloea ramigera. Biotechnol Bioeng* **26,** 239–246.

Paton WHN, Budd K. 1972 Zinc uptake in *Neocosmospora vasinfecta. J Gen Microbiol* **72,** 173–184.

Perry RD, Silver S. 1982 Cadmium and manganese transport in *Staphylococcus aureus* membrane vesicles. *J Bacteriol* **150,** 973–976.

Reichenbach H. 1989 The order Cytophagales. In: Staley JT, Bryant MP, Pfennig N, Holt JG, eds. *Bergey's Manual of Systematic Bacteriology,* Vol. 3. Baltimore, MD: Williams & Wilkins; 2010–2074.

Reichenbach H, Dworkin M. 1981 The order Cytophagales (with addenda on the genera *Herpetosiphon, Saprospira* and *Flexithrix*). In: Starr MP, Stolp H, Trüper HG, Balows A, Schegel GH, eds. *The Procaryotes,* Vol. 1. Berlin: Springer-Verlag; 356–379.

Silver S. 1978. Transport of cations and anions. In: Rosen BP, ed. *Bacterial Transport.* New York: Marcel Dekker; 221–324.

Trevors JT, Stratton GW, Gadd GM. 1986 Cadmium transport, resistance, and toxicity in bacteria, algae, and fungi. *Can J Microbiol* **32,** 447–464.

Tynecka Z, Gos Z, Zajac J. 1981a Energy-dependent efflux of cadmium coded by a plasmid resistance determinant in *Staphylococcus aureus. J Bacteriol* **147,** 313–319.

Tynecka Z, Gos Z, Zajac J. 1981b Reduced cadmium transport determined by a resistance plasmid in *Staphylococcus aureus. J Bacteriol* **147,** 305–312.

Wallberg M, Brynhildsen L, Allard B. 1991 Metal binding properties of *Klebsiella oxytoca. Water Air Soil Pollut* **57/58,** 579–587.

Wnorowski AU. 1991 Selection of bacterial and fungal strains for bioaccumulation of heavy metals from aqueous solutions. *Water Sci Technol* **23,** 309–318.