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Full Length Research Paper

Effect of concentration and contact time on heavy metal uptake by three bacterial isolates

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The effect of heavy metal concentration and contact time (exposure period) on heavy metal up take by pure cultures of three bacteria (*Pseudomonas*, *Bacillus* and *Aeromonas*) isolated from a crude oil impacted brackish aquatic system in the Niger Delta were investigated. Heavy metals employed included metals found in this Bonny light crude oil (Fe, Zn, Cd, Cu, Ni and Pb). Accumulation of these metals was gradual and the amount increased in direct proportion to initial metal concentration up to an extent that ranged from 1 - 100 (mg/l) after which uptake remained either constant or declined. Maximum uptake of Fe, Pb, Cd, Cu, Zn and Ni were obtained at initial concentrations of 10, 10, 10, 100, 100 and 10 (mg/l), respectively and the values were 8.75, 0.01, 1.3, 0.06, 4.2 and 0.001 milligram per gram dry weight (mg/g dry wt) of *Bacillus* cells. For *Pseudomonas* sp. initial metal concentration that resulted in maximum uptake were 10 mg/l (Fe), 1 mg/l (Zn,) 10 mg/l (Pb), 0.1 mg/l (Cd), 10 mg/l (Cu) and 10 mg/l (Ni). Values accumulated at these concentrations were 14, 0.7, 1.0, 0.08, 0.3 and 0.11 (mg/g dry wt), respectively. Whereas, maximum amounts accumulated by *Aeromonas* sp. were 1.65, 0.1, 0.001, 0.9, 0.2 and 0.013 (mg/g dry wt) respectively. The respective initial concentration that yielded these uptake values was 100, 10, 1.0, 10, 10 and 10 (mg/l). Contact duration increased the amount of metal bioconcentrated by each test organism. At all tested concentrations maximum uptake of Fe, Zn, Cu, Cd, Pb and Ni by *Bacillus* were at the 8^{th}, 8^{th}, 8^{th}, 4^{th}, 4^{th} and 4^{th} hours of exposure respectively. Slight decreases in uptake were noticed on further incubation beyond these durations. Maximum accumulation of Fe, Zn, Cu, Cd, Pb and Ni by *Pseudomonas* sp. were obtained at incubation durations of 8^{th}, 12^{th}, 12^{th}, 8^{th}, 2^{nd} and 24^{th} hours. 12^{th}, 12^{th}, 12^{th}, 4^{th}, 8^{th} and 4^{th} h were the incubation periods that resulted in maximum bioconcentration of Fe, Zn, Cu, Cd, Pb and Ni by *Aeromonas* sp. The three test organisms presented distinct uptake capacities which decreased thus: *Pseudomonas* sp. > *Bacillus* sp. ≥ *Aeromonas* sp. Affinities of *Bacillus* sp., *Pseudomonas* sp. and *Aeromonas* sp. for the various heavy metals followed the pattern Fe > Zn > Cd > Cu > Ni > Pb, Fe > Pb ≥ Zn > Cu > Ni ≥ Cd and Fe > Cd > Cu ≥ Zn > Ni > Pb respectively. Results showed that heavy metal concentrations between 10 – 100 mg/l and exposure periods of between 4 - 12 h depending on the metal and the test organism rapidly promoted accumulation in heavy metal polluted sites.

Key words: Accumulation bioconcentration, heavy metal, toxicity, contact time, bacteria.

INTRODUCTION

The threat of heavy metal pollution to public health and the ecosystem (Davies et al., 2006; Mallampoti et al., 2007; Vinodhini and Narayanam, 2008) has led to an increased interest in developing systems that can remove or neutralize its toxic effects in soil, sediments and wastewater (Kotrba et al., 1999; Gonzalez et al., 2005). Unlike organic contaminants, which can be degraded to harmless chemical species, heavy metals can neither be degraded nor destroyed. Remediation of the pollution they cause can therefore only be brought about mainly by bioaccumulation, biosorption or their re-speciation into less toxic forms (Abou-Shanab et al., 2007; Adenipekun and Isikhuemhen, 2008).

Microorganisms can physically remove heavy metals

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from solution through either bioaccumulation or biosorption. Bioaccumulation is the retention and concentration of a substance by an organism (Odiete, 1999). Metals are transported from the outside of microbial cell, through the cellular membrane and into the cell cytoplasm, where the metal is sequestered and therefore immobile bioaccumulation consume cellular energy while biosorption does not (Bull et al., 1981; Mullen et al., 1989; Strandberg et al., 1995).

A number of factors may affect the rate at which heavy metals are accumulated in bacterial cells. Several authors such as Galun and Gelun (1987), Asku et al. (1992) and Fourest and Roux (1992) have shown that biosorption rates are greatly influenced by factors such as temperature, pH, metal concentration, contact time as well as concentration of biomass. Asku et al. (1992) reported that temperature ranging from 20 - 33°C seems not to influence the biosorption performance but at higher temperatures, biosorption increases. Galum and Gelun, 1987 have shown that pH affects the solution chemistry of the metals, the activity of the functional group in the biomass and the competition of metallic ion. Fourest and Roux (1992) reported that biomass concentration in solution seems to influence the specific uptake. They also attributed the interference between the binding sites resulting from an increase in biomass concentration to metal concentration shortage in solution. Bioaccumulation increased with increase in contact time for low concentration of metals (0.001 - 1.0 mg/l) of CdS, ZnO and Fe_2O_3 until equilibrium is achieved (Odokuma and Emedolu, 2005). However, at higher concentration of 10.0 - 100 mg/l of these metal salts, bioaccumulation decreased with increasing contact time (Odokuma and Emedolu, 2005). Bioaccumulation varies between individual organisms as well as between individual species. Odokuma and Emedolu (2005) have shown that bioaccumulation of CdS at 0.1, 11.0, 10.0 100 mg/l was 80, 64, 52 and 15% for *Bacillus* and 94, 57, 73 and 13% for *Aeromonas.* Some chemicals bind to specific site whereas, others move freely in and out. Those im-mediately eliminated do not bioaccumulate. Odokuma and Emedolu (2005) showed that the bioaccumulation of heavy metals by *Bacillus* and *Aeromonas* followed the trend Fe > Zn ≥ Cd > Pb

The bioconcentration potentials of heavy metals (Cd, Pb, Zn and Fe) associated with crude oil by *Bacillus*, *Chromobacteiium*, *Staphylococcus* and *Aeromonas* have been investigated by Odokuma and Emedolu (2005). The authors observed that both *Bacillus* and *Aeromonas* were tolerant to the salts of these metals at 0.001, 0.1, 1.0 10, 100 and 1000 and thus bioconcentrated these metals. The objective of this study was to enhance the bioconcentration capabilities of these three organisms by determining the optimum concentrations and contact time required for bioconcentration of these metals. Armed with this information, one could suggest the inclusion of these organisms in the protocol for bioreme diation of heavy metal contaminated environments in the Niger Delta.

MATERIALS AND METHODS

Source of samples

River water used in this study was collected from the New Calabar River located in Choba, River State of Nigeria. The New Calabar River is a short coastal river about 200 km in length. It is under the influence of tidal cycles and consists of brackish water due to marine water influx during the tidal cycles.

Water sample collection

Composite samples of water (0 - 30 cm depth) were collected from the river about 1 km southwest of the University of Port- Harcourt with 100 ml sterile plastic containers. Composite sampling was performed by collecting ten samples about 1 m apart and pooling them together.

Digestion of crude oil

Crude oil used was Bonny light. This was obtained from the Nigerian National Petroleum Corporation (NNPC), Port Harcourt. Crude oil sample was digested using the wet oxidation method employing a mixture of concentrated nitric acid, perchloric acid and sulphuric acid (APHA, 1998). The heavy metals in the crude oil were determined using model AA320 atomic absorption spectrophotometer.

Chemical reagents

All chemical reagents employed in this study were products of Aldrich chemical Co, Milwaukee, USA, BDH chemicals, Poole, England and Sigma chemical company St. Louis Missouri, USA.

Preparation of stock solution of heavy metal salts

The heavy metal salts employed in this study include: Nickel tetraoxosulphate (vi) salt ($NiSO_4$), copper (ii) tetraoxosulphate (vi) salt ($CuSO_4$), lead trioxonitrate (v) salt ($PbNO_3)_2$, iron (ii) tetraoxosulphate (vi) salt ($FeSO_4$), cadmium tetraoxosulphate (vi) salt ($CdSO_4$) and zinc tetraoxosulphate (vi) $ZnSO_4$ salt. A weight of each of these heavy metal salts that gave a 1 g of each of the respective heavy metal (metal without the salt) was weighed and dissolved in 1000 ml of deionised water. These were left to stand for 30 min to obtain complete dissolution. This was followed by sterilization by membrane filtration (0.2 µm pore size Aerodisc).

Isolation of heavy metal resistant bacteria from the river water

Heavy metal resistant bacteria were isolated from the river water. An amount (0.1 ml) of a 10^{-4} dilution of the river water sample was inoculated onto the surface of freshly prepared nutrient agar plates using the spread plate technique (APHA, 1998). The plates were incubated at 37°C for 24 h. Isolated colonies were purified by two subsequent single colony transfers. Pure colonies were specifically transferred into nutrient agar slants. The slants were incubated at 37°C for 18 - 24 h. These served as the stock cultures and were stored at 4°C in the refrigerator. Pure bacterial isolates were characterized and identified using criteria as in Holt et al. (1994).

Nine predominant bacterial genera; *Achromobacter*, *Alcaligenes*, *Aeromonas*, *Bacillus*, *Chromobacterium*, *Corynebacterium*, *Micrococcus*, *Pseudomonas* and *Serratia* were identified.

Preparation of standard inoculum of isolates

A loopful of cells from the respective stock cultures were incubated into 100 ml sterile nutrient broth contained in 250 ml Erlenmeyer flasks. The flasks were incubated at 37°C for 24 h with intermittent shaking. At the end of the incubation period, cells were harvested by centrifugation at 4000 rpm for 30 min and re-suspended in 100 ml sterile physiological saline. The total viable counts were carried out to estimate the number of viable organisms. During this process, the cultures were subjected to serial dilutions up to 10^6 dilutions. An amount (0.1 ml) from each dilution was inoculated by spread plate technique into freshly prepared nutrient agar plates, which were incubated at 37°C for 24 h. The dilutions produced between 30 - 300 colonies were chosen and served as inoculum for preliminary screening experiments.

Preliminary screening test

This was carried out to determine the isolates that possess resistance to all the heavy metals associated with the crude oil. One hundred millilitres of 1 mg/l of the respective heavy metal solutions were prepared as earlier described. Nine millilitres were dispensed into test tubes and sterilized. Controls contained 9 ml of physiological saline. One millilitre of respective standardized isolates' inoculum was then added and incubation followed immediately at a temperature of 25°C ± 2 for duration of 24 h. At the end of the incubation period, 0.1 ml were withdrawn and plated onto the surface of freshly prepared nutrient agar plates using the spread plate technique as described by APHA (1998). Incubation followed immediately at 25°C ± 2 for 18 - 24 h. Colonies formed were counted and percent log survival were calculated according to Williamson and Johnson (1981).

$$\%\ \log survival = \frac{\log of\ count\ in\ toxicant\ concentration}{Log of\ count\ in\ control} x\ 100$$

Based on the results, *Pseudomonas, Bacillus* and *Aeromonas* were chosen for further studies.

Toxicity test

The preliminary range finding test was carried out to determine the lowest observed effect concentration (that is, the lowest concentration tested that had a significant effect) and the highest observed effect concentration (highest concentration tested beyond which inhibition was 100%). Lethal response (or percentage log survival) was used as an index of toxicity.

Preparation of toxicant concentration

Based on the result of the preliminary range finding test, various toxicant (metal) concentrations (0.001, 0.01, 0.1, 1.0, 10 and100.0 mgl^{-1}) of each heavy metal salts were prepared from the stock solution (1 g equivalent of heavy metal-determined from the heavy metal salt in 1000 ml of deionised water) of the heavy metals. These concentrations were employed for the percentage log survival test.

Percentage log survival test

The test was carried out to determine the lethal effect of the heavy metal salts on *Pseudomonas*, *Bacillus* and *Aeromonas* by monitoring the total viable count of the bacterial isolates with time, when exposed to varying concentrations of the various salts.

The experimental set up for each of the six heavy metal salt is as follows:

1. Ninety millilitres of the various toxicant concentration (0.001, 0.01, 0.1, 1.0, 10 and 100 mgl^{-1}) contained in each of six 250-ml Erlenmeyer flask in duplicates were inoculated with 10 ml of standard inoculum of *Pseudomonas*, *Bacillus* and *Aeromonas,* respectively. Control consisted of a duplicate set of flask containing sterile normal saline without any toxicant added.
2. The flask was shaken and at exposure times of 0, 2, 4, 12, 24, 48 and 96 h, 1 ml was aseptically withdrawn from each flask and serially diluted (10^{-1} - 10^{-4}) using the ten-fold dilution technique. Using the spread plate method, each dilution was plated out in duplicate set of plates containing sterile nutrient agar medium. The plates were incubated at 37°C for 18 - 24 h and discrete colonies that developed were counted. Results were expressed as colony forming units per millilitre (cfu/ml). The same procedure was carried out for the controls.
3. Percentage log survival was calculated by dividing the log of counts in each toxicant concentration by the log of count in the control (zero toxicant concentration) and multiplying by 100 (Williamson and Johnson, 1981).

$$\text{Percentage log survival} = \frac{\log C}{\log c} \times 100$$

where C = count in each toxicant concentration
c = count in the control.

Metal uptake experiment

The methods of Kurek et al. (1982), Bauda and Block (1985) and Boularbah et al. (1992) were adopted with some modifications for the heavy metal uptake test.

Growth and preparation of test isolates

The biomass of the respective bacterial isolates were developed by growing each isolate in 250-ml Erlenmeyer flasks containing 100 ml of freshly prepared nutrient broth (pH 7.0) at 37°C for 18 - 24 h, under shaken conditions (120 rpm). Cells were harvested by centrifugation at 4000 rpm for 30 min. Harvested cells (biomass) were washed thrice with sterile phosphate buffered saline. The cells were resuspended in 100 ml-deionized water. This served as inoculum for the various bioaccumulation tests. To access viability of cells, 0.1 ml was plated onto surface of nutrient agar plates.

Metal solution

Stock solutions of the various heavy metal salts and their various concentrations were prepared. Solutions were adjusted to desired pH values (7) with 0.1 M sodium hydroxide and 0.1 M trioxonitrate (V) acid. The initial metal concentrations at the beginning of all experiments carried out were as calculated from the dilution.

Effect of initial heavy metal concentration on bioconcentration

The experiment was conducted to ascertain the effect of varying concentrations of heavy metal salts on bioconcentration potentials of the test organisms. The experimental set up for each of the heavy metal was as follows: From the various concentrations of the

Table 1. Response of Isolates to the toxicity of the various heavy metals.

Isolates	Fe	Zn	Cd	Cu	Ni	Pb	Control
Alcaligenes	++	++	-	-	-	-	+++
Aeromonas	+++	+++	++	+++	++	++	+++
Bacillus	+++	+++	+++	+++	+++	+++	+++
Achromobacter	+++	++	-	-	+	-	+++
Chromobacterium	++	+	-	+	-	-	+++
Corynebacterium	++	++	-	+	+	-	+++
Micrococcus	+++	++	+	++	+	-	+++
Pseudomonas	+++	+++	+++	+++	++	++	+++
Serratia	+++	+	-	+	+	-	+++

Key: +++ = > 70% log survival, ++ = 50-69% log survival, + = 30-49% log survival, - = < 29% log survival.

respective heavy metal salts, 40 ml were withdrawn using sterile pipette into duplicate set of 100-ml Erlenmeyer flask. 10 ml of each of the standard inoculum were then added. The control however contained 40 ml of sterile normal saline and 10 ml of the respective isolate. All flasks were incubated at 25°C ± 2 for 24 h. At the end of the incubation period, cells were harvested by centrifugation at 4000 rpm for 30 min (using 800 D centrifuge), washed thrice in sterile PBS, dried, weighed, digested and analysed for heavy metal uptake. Metal uptake was expressed as mg metal/gram dry weight of cells (Vol1esky and May-Philips, 1995). All glasswares were rinsed with 0.1 M hydrochloric acid before and after each experiment to prevent sorption (precipitation) of metals on glass surface.

Effect of contact time

This experiment was done to determine the shortest exposure time for the respective isolate to maximally accumulate each of the various heavy metals (that is, to determine the equilibrium point/residence time of the metals in each test isolate). The experimental set up is as described as: Ten millilitres of the standard inoculum was contacted with 40 mls aliquots of varying concentrations of heavy metal solution in 100-ml Erlenmeyer flasks. These were then incubated at 25°C ± 2 for different time intervals ranging from 1, 2, 4, 8, 12 and 24 h. At each of the exposure periods, cells were harvested, dried, weighed, digested and analysed for heavy metal uptake using AAS.

Statistical analyses

Analyses of variance (ANOVA) and Rank correlation coefficient (Finney, 1978) were employed to determine the existence of significant statistical variations in the results

RESULTS

The results of the preliminary screening for the resistance of isolates to the toxicity of the various heavy metals are presented in Table 1. The isolate obtained from the river water sample were *Achromobacter*, *Alkaligens*, *Aeromonas*, *Bacillus*, *Corynebacterium*, *Chromobacterium*, *Micrococus*, *Pseudomonas* and *Serratia*. Three of the test isolates (*Aeromonas*, *Bacillus* and *Pseudomonas*) showed resistance to the six heavy metal salts; hence these three were selected for further experiments.

The effect of heavy metal concentration on the percentage (%) survival of organisms is presented in Figures 1a - 3f. Results showed that there was a general decrease in percentage survival with increase in concentration and contact time of heavy metals to all three organisms. Results showed that Fe and Zn were the least toxic to all three organisms while Pb, Cd and Ni were the most toxic to the organisms.

Results from the two-way analyses of variance showed a significant difference between % log survival and heavy metal concentration for *Bacillus.* The calculated F value (F_{cal} was 13.8 while the tabulated F value (F_{tab}) was 2.5. For *Pseudomonas* F_{cal} was 4.6 while F_{tab} was 2.5. For *Aeromonas* F_{cal} was 3.8 while F_{tab} was 2.5. There was also a strong positive correlation between metal concentration and % log survival

Figures 4a - f shows the effect of initial metal concentration on metal uptake by *Bacillus*. The data showed that high concentrations (10 - 100 mg/l) of heavy metals favoured the increase of metal uptake as against low concentrations (0.1 - 1.0 mg/l). Only the low concentration of Pb (1.0) promoted metal up-take.

In Figures 5a - f, the effect of initial heavy metal concentration on metal up-take by *Pseudomonas* is presented. Results showed that heavy metal concentrations (10 – 100 mg/l) favoured metal up-take. However, low concentrations (1.0 mg/l) of Pb, Cd and Fe also favoured accumulation of these metals by *Pseudomonas.*

In Figure 6a - f the effect of initial metal concentration on metal uptake by *Aeromonas* is presented. High metal concentrations (10 – 100 mg/l) favoured metal up-take in *Aeromonas*.

However, low concentrations (0.1 - 1.0 mg/l) of Pb, Cd and Fe also promoted heavy metal uptake by this

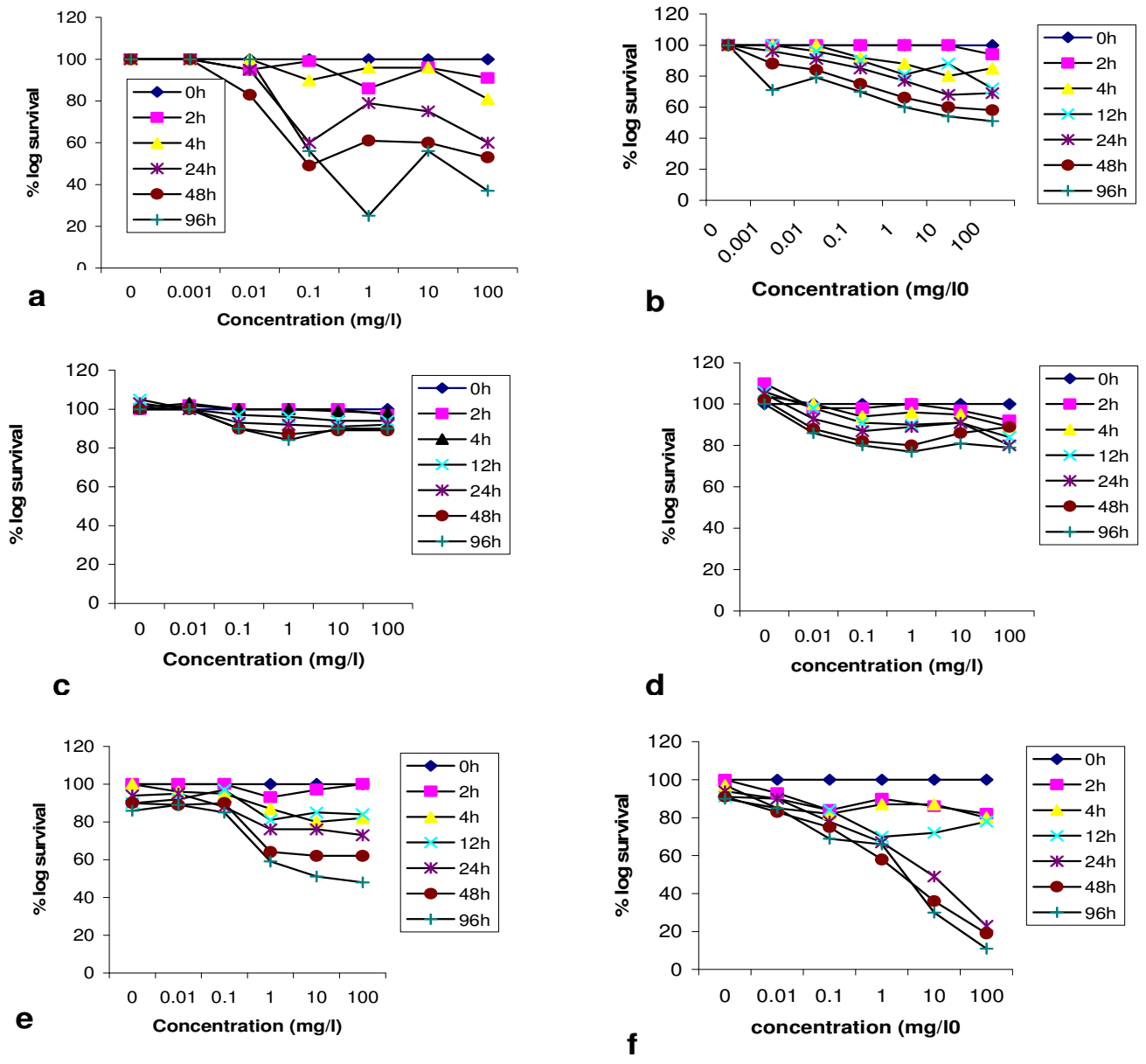

Figure 1. Effects of heavy metals; (a) $CuSO_4$, (b) Ni SO_4, (c) $FeSO_4$, (d) $ZnSO_4$, (e) $Pb(NO_3)_2$ and (f) $CdSO_4$; on the survival of *Bacillus* sp.

organism.

The effect of contact time on metal uptake by *Bacillus* is presented in Figure 7 (a – f). An increase in metal uptake with increase in contact time from 0 - 4 h followed by an equilibrium position till the 8 h then a decrease till the 24 h was observed. Similar results were observed in *Pseudomonas* (Figures 8a - f) and *Aeromonas* (Figures 9a -f) with very slight variations depending on the metal. At all tested initial concentrations, results showed that contact duration affected uptake a great deal.

There was a significant difference between metal uptake and contact time for all three organisms. For iron uptake by *Bacillus* F_{cal} was 13.8 while F_{tab} was 3.2. For *Pseudomonas* F_{cal} was 13.2 while F_{tab} was 3.2. For *Aeromonas* F_{cal} was 8.2 while F_{tab} was 3.2. For cadmium uptake by *Bacillus* F_{cal} was 5.5 while F_{tab} was 4.1. For *Pseudomonas* F_{cal} was 5.5 while F_{tab} was 3.2 while for *Aeromonas* F_{cal} was 18.4 while F_{tab} was 4.1. For lead uptake by *Bacillus* F_{cal} was 4.2 while F_{tab} was 3.2. For *Pseudomonas* F_{cal} was 28.9 while F_{tab} was 3.2. For *Aeromonas* F_{cal} was 6.7 while F_{tab} was 3 .2

A strong positive correlation between metal uptake and contact time was observed until the 12^{th} h at the various concentrations of heavy metals used for all organisms. The r value at 0.1.1.0, 10.0 and 100 mg/l of Fe for *Aeromonas* were 0.8811, 0.8712, 0.8276 and 0.8663,

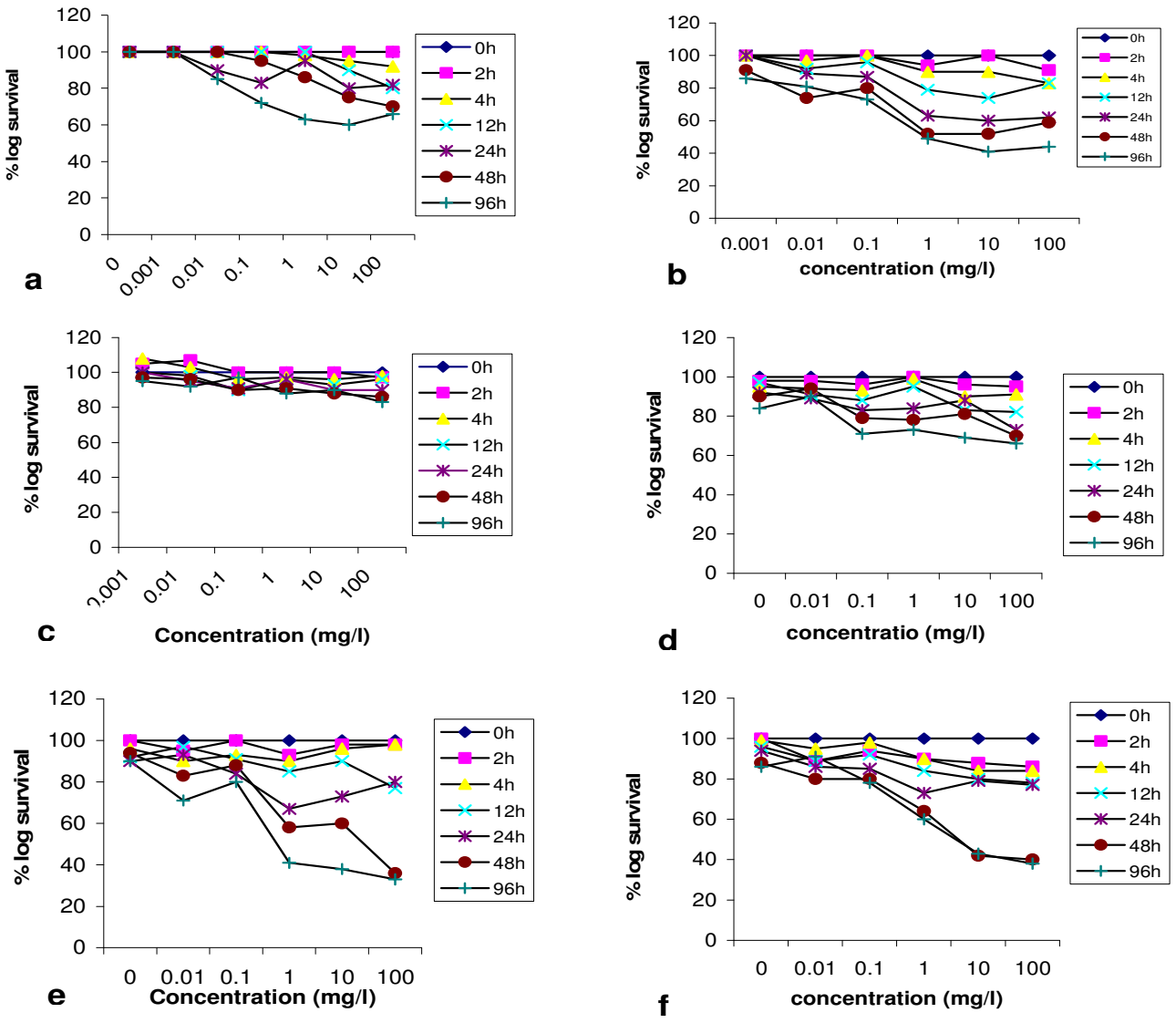

Figure 2. Effects of heavy metal salts; (a) $CuSO_4$, (b) $NiSO_4$, (C) $FeSO_4$, (D) $ZnSO_4$, (e) $Pb(NO_3)_2$ and (f) $CdSO_4$; on the survival of *Pseudomonas* sp.

respectively. The r values at similar concentrations for Zn for *Bacillus* were 0.7848, 0.7856, 0.7821 and 0.7950 respectively. Zinc uptake by *Pseudomonas* within 12 h exposure time showed positive r values of 0.8773, 0.8783, 0.8543, and 0.8594 respectively at metal concentrations of 0.1, 1.0, 10.0 and 100 mg/l. At similar concentrations metal uptake and contact time of Zn by *Aeromonas* showed strong positive concentrations of 0.8890, 0.8550, 0.7625 and 0.8127.

However for Cd, Cu and Pb correlation was less than 0.5 at these concentrations for all test organisms. For *Aeromonas* r values for Cd at these concentrations were 0.2356, 0.1296, 0.1092 and 0.2894. For *Bacillus* r values for Cd at 10.0 and 100 mg/l were 0.4887 and 0.3707 respectively. However at low concentrations of Fe, 0.0 and 1.0 mg/l r values were 0.8095 and 0.7856, respectively. For *Pseudomonas* r values for Cd at all concentrations were 0.7865, 0.7900, 0.7895 and 0.7878 respectively. Correlation value for Pb uptake by *Bacillus* were negative at all concentrations, -0.41903, -02155, -0.3300 and -0.3498, respectively.

DISCUSSION

The results of the preliminary screening for resistance to the toxicity of the various heavy metals showed that six of the nine genera were not capable of survival when exposed to the various heavy metal salts associated with the crude oil sample within 24 h exposure duration.

Aeromonas, *Bacillus* and *Pseudomonas* were however, resistant to the toxicity of all the heavy metals within

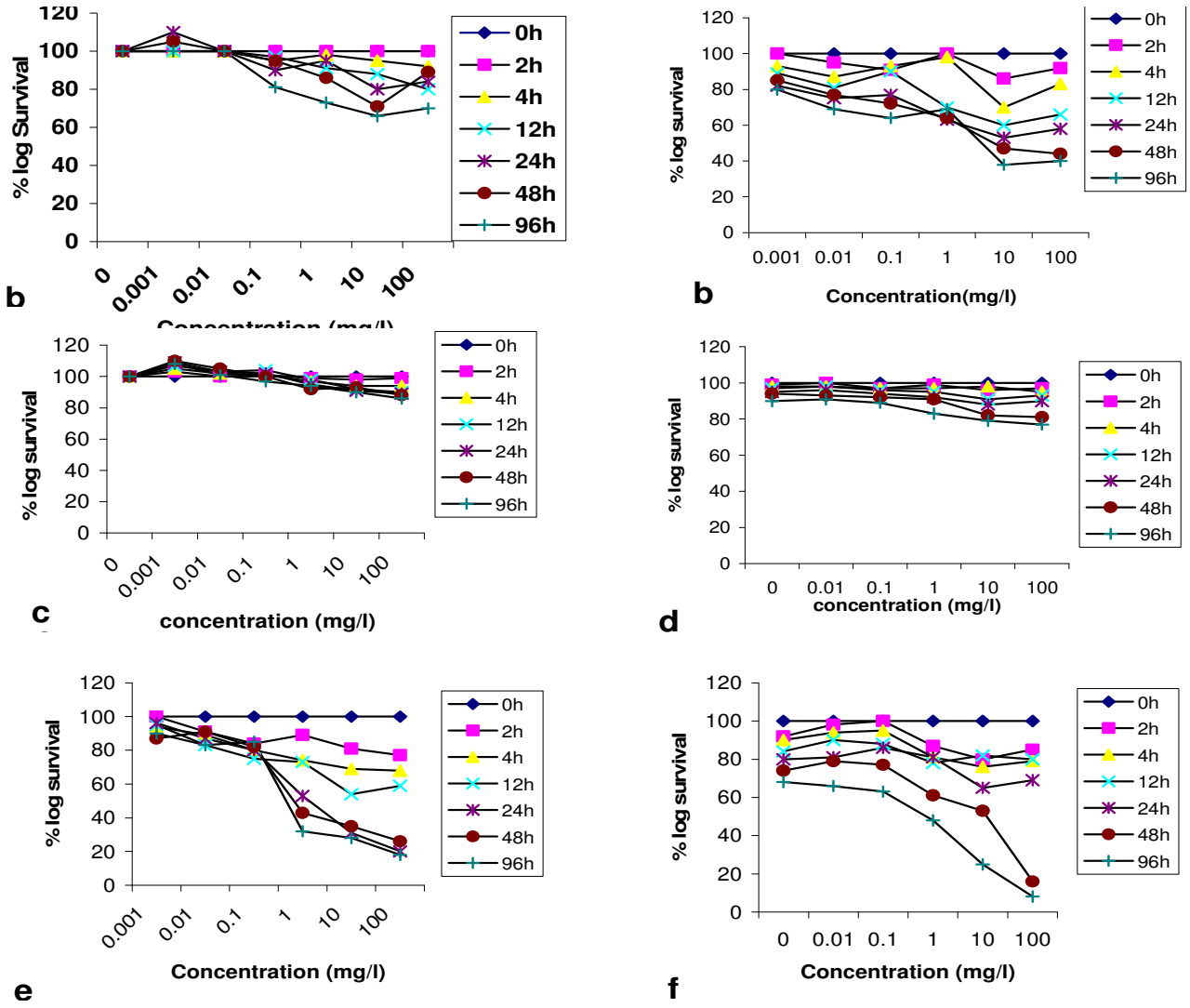

Figure 3. Effects of various heavy metals salts; (a) $CuSO_4$, (b) Ni SO_4, (c) $FeSO_4$, (d) $ZnSO_4$, (e) $Pb(NO_3)_2$, (f) $CdSO_4$; on the survival of *Aeromonas* sp.

same exposure period; hence, they were selected for the studies. Similar results had been reported by Odokuma and Ijeomah (2003), Odokuma and Emedolu (2005). In their reports *Bacillus* sp. and *Aeromonas* sp. were shown to be resistant to the toxicity of heavy metals. The persistence of these isolates in the presence of the respective heavy metals may be as a result of the possession of heavy metal resistant plasmids (Odokuma and Oliwe, 2003). The spore forming ability of *Bacillus* sp. might also, have contributed to its ability to survive when exposed to the various heavy metal salts (Stainer et al., 1982; Dutton et al., 1990). The resistance of *Pseudomonas* sp. to the toxicity of the various salts may be due to its ability to use diverse compounds (organic and inorganic) as sole carbon source (Schlegel, 1997). In addition to its genetic make-up, complexity of its cell wall being a Gram-negative organism may have contributed to its resistance. Similar reasons can also be advanced for the resistance of *Aeromonas* sp to the toxicity of the various heavy metal salts.

All the nine isolates were observed to be resistant to Fe and Zn at the tested concentrations. This might be due to the fact that these heavy metal salts are important in certain biosynthetic activities in the organisms as well as being components of enzyme (Taylor et al., 1997). Thus, at such a low concentration, they have proved to be useful to the various test isolates. However, Pb Cd, Ni and Cu were observed to be highly toxic to *Alcaligenes* sp., *Achromobacter* sp., *Chromobacterium* sp., *Corynebacterium* sp., *Micrococcus* sp., and *Serratia* sp. as the percent survival of these respective isolates when exposed to 1 mg/l of the various heavy metal salts were less than 30% as shown in Table 1. Similar results had been observed by Odokuma and Ijeomah (2003). Results

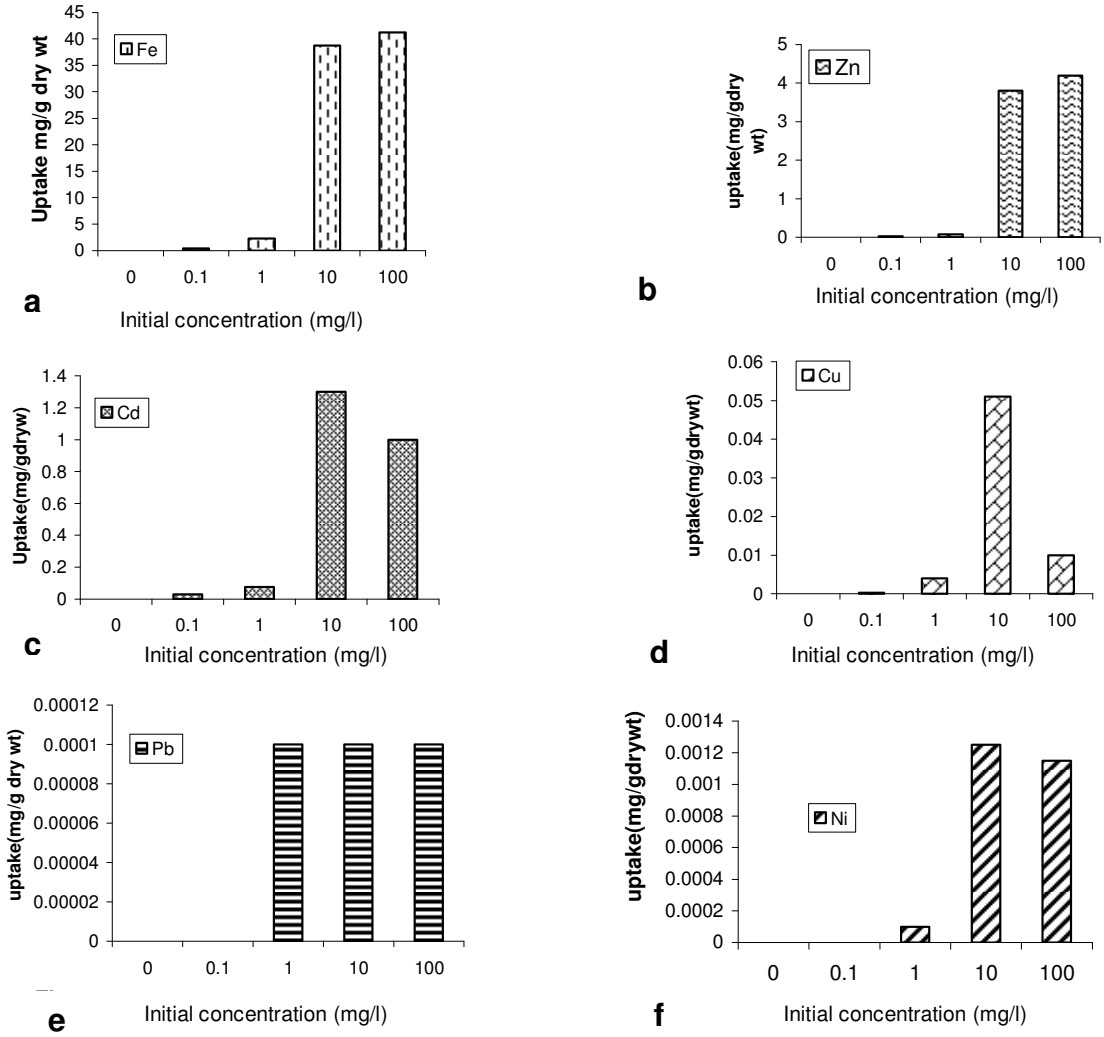

Figure 4. Uptake of various heavy metals; (a) Fe, (b) Zn, (c) Cd, (d) Cu, (e) Pb and (f) Ni; by *Bacillus* sp.

obtained therefore, suggest that the occurrence of these heavy metals in the environments will greatly reduce the population of these organisms and hence the microbial diversity of the affected ecosystem.

Results of the toxicity of the various metals to *Bacillus* sp., *Pseudomonas* sp. and *Aeromonas* sp. as presented in Figures 1a - 3f showed the mechanism of response of the isolates to the various logarithmic concentrations of the toxicants. Percentage survival decreased with increase in contact time as well as concentration (Buikema et al., 1982). The toxicity of the various heavy metals to *Bacillus* sp., *Pseudomonas* sp. and *Aeromonas* sp. respectively, followed the decreasing trend: Cd > Pb > Cu > Ni > Zn > Fe, Cd > Ni > Cu > Pb > Zn > Fe and Cd > Pb > Ni > Cu > Zn ≥ Fe respectively. Beyond the 24th h of exposure, percent log survival greatly decreased indicating that contact time is a crucial factor in establishing the resistance of organisms to the toxic pressure of the metals. Thus, further experiments were conducted within duration of 24 h.

The initial bioaccumulation experiments carried out in this study revealed that all the three isolates were not only resistant to the toxicity of the various heavy metals within the duration of exposure, but also, had the capability of accumulating these heavy metals. It is well recognized that microorganisms have affinity for metals and can accumulate heavy and toxic metals by a variety of mechanisms (Simmons et al., 1995; Malekzadeh et al., 1995; Gupta and Keegan, 1998; Odokuma and Emedolu, 2005). Several principal sites of metal-complex formation in biological systems have been proposed (Vieira and Volesky, 2000). These include accumulation in the cell wall, carbohydrate or protein polyphosphate complexes, and complexion with carboxyl groups of the peptideglycan in the cell wall or entering into cells via an present study, an increasing uptake pattern was observed in

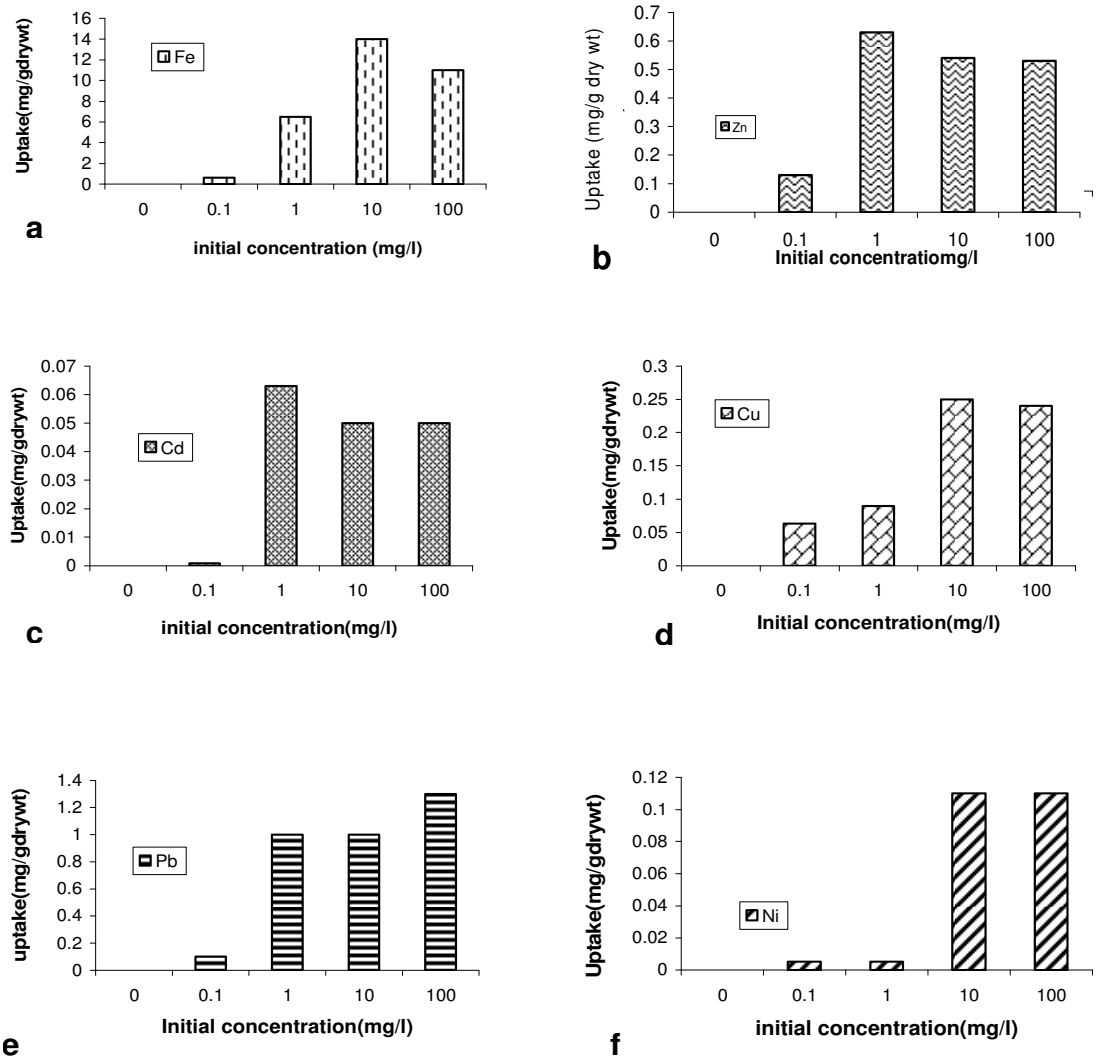

Figure 5. Uptake of various heavy metals by *Pseudomonas* sp. as a function of initial concentration. (a) Fe uptake (b) Zn uptake (c) Cd uptake (d) Cu uptake (e) Pb uptake (f) Ni uptake.

the respective test isolates as the initial concentration of the various heavy metal salts were increased. These obser-vations suggest that metal uptake may involve diffusion phenomenon whereby, metal ions move from regions of high concentrations to low concentrations and the fact that the steeper the concentration gradient, the more ra-pid is the movement of molecules or ions. (Taylor et al., 1997) Investigations indicated that under the conditions of test, maximum uptake was obtained at initial concen-trations that ranged from 1.0 - 10 mg/l after which uptake either remained constant (100.0 mg/l) or dropped to an extent. This might be suggestive of saturation of the test organism by the metal in question while the decrease in uptake at certain high initial concentration [as observed in Pb uptake by *Bacillus* sp. (Figure 4f) might be due to increasing toxic action of the metals with increase in their initial concentrations. Similar observations, had been made by Kaewehai and Prasertson (2002), Al-Garni (2005) and Odokuma and Emedolu (2005).

Bacillus sp. showed a selective uptake: Fe > Zn > Cd > Cu > Ni > Pb. Iron > Pb >Zn > Cd > Cu > Ni and Fe > Cd > Cu > Zn > Ni > Pb were the trends demonstrated by *Pseudomonas* and *Aeromonas* respectively. All the three isolates accumulated Fe the most. This may be attributable to the low toxicity of this metal to the test organisms as revealed by the results of the toxicity tests. The production of siderophores by organisms especially *Pseudomonas* had been advanced for Fe accumulation (Ford and Mitchel, 1992). These authors also reported that in the absence of Fe, accumulation of its analogs, which include Cu and Cd, could be enhanced by siderophore production. This might have contributed to the uptake of these metals. Results of toxicity test energy-dependent mechanism (Silver, 1991). In the (Figures 1e, 2e and 3e) showed that Pb and Ni were the

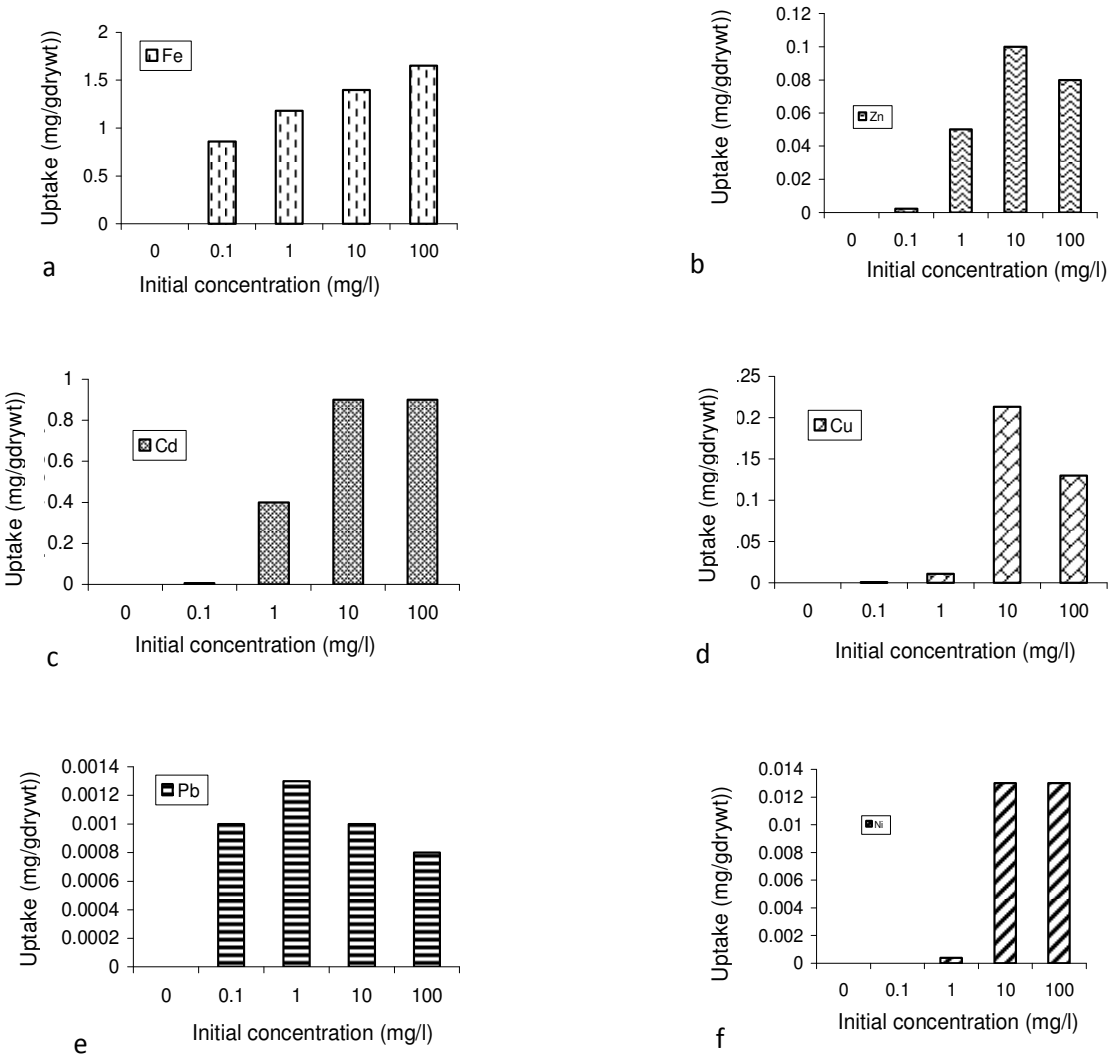

Figure 6. Uptake of various heavy metals; (a) Fe, (b) Zn, (c) Cd, (d) Cu, (e) Pb and (f) Ni; by *Aeromonas* sp. as a function of initial concentration.

most toxic to the test isolates. This might also be responsible the poor capability of *Bacillus* sp. and *Aeromonas* sp. in accumulating these metals. Again the high molecular weight of these metals might have resulted in this limitation observed in their uptake by the isolates. *Pseudomonas* however, demonstrated a good ability of accumulating this metal. A similar observation had been made by Malekzadeh et al. (1995). They reported that a strain of *Pseudomonas* was highly efficient in accumulating highly toxic uranium up to 174 mg/g dry weight of the bacterial biomass which far exceeded its ability to ac-cumulate less toxic metals (Cu and Cd). This is indicative of the fact that an organism may have specificity for a particular metal. Also, it has been reported that toxicity increases cell membrane permeability, hence toxicity can increase uptake if the toxic pressure is not sufficient to kill the organisms (Malekzadeh et al., 1995). Thus, this probably accounts for the increased influx of Pb ions into *Pseudomonas* sp.

Data obtained on the effect of contact time on the uptake of the various heavy metals by the respective test isolates, showed rapid uptake up to contact time of between 4 and 8 h depending on the heavy metal as well as the test isolates. Time for attaining equilibrium was less than 24 h. The rate of metal uptake is influenced by agitation or shaking (Gadd, 1988) diffusion of metal through a hydrodynamic boundary layer around the biosorbent surface (Weber, 1985) and adsorption of metal ions by active sites of the biomass. In this case of metal accumulation by the tested bacteria, the shaken conditions employed, probably allowed a thorough mixing of solutes and biomass in the system and hence suppressing the kinetic limitations of bulk transport of metal ions across the cell membrane. Thus, equilibrium

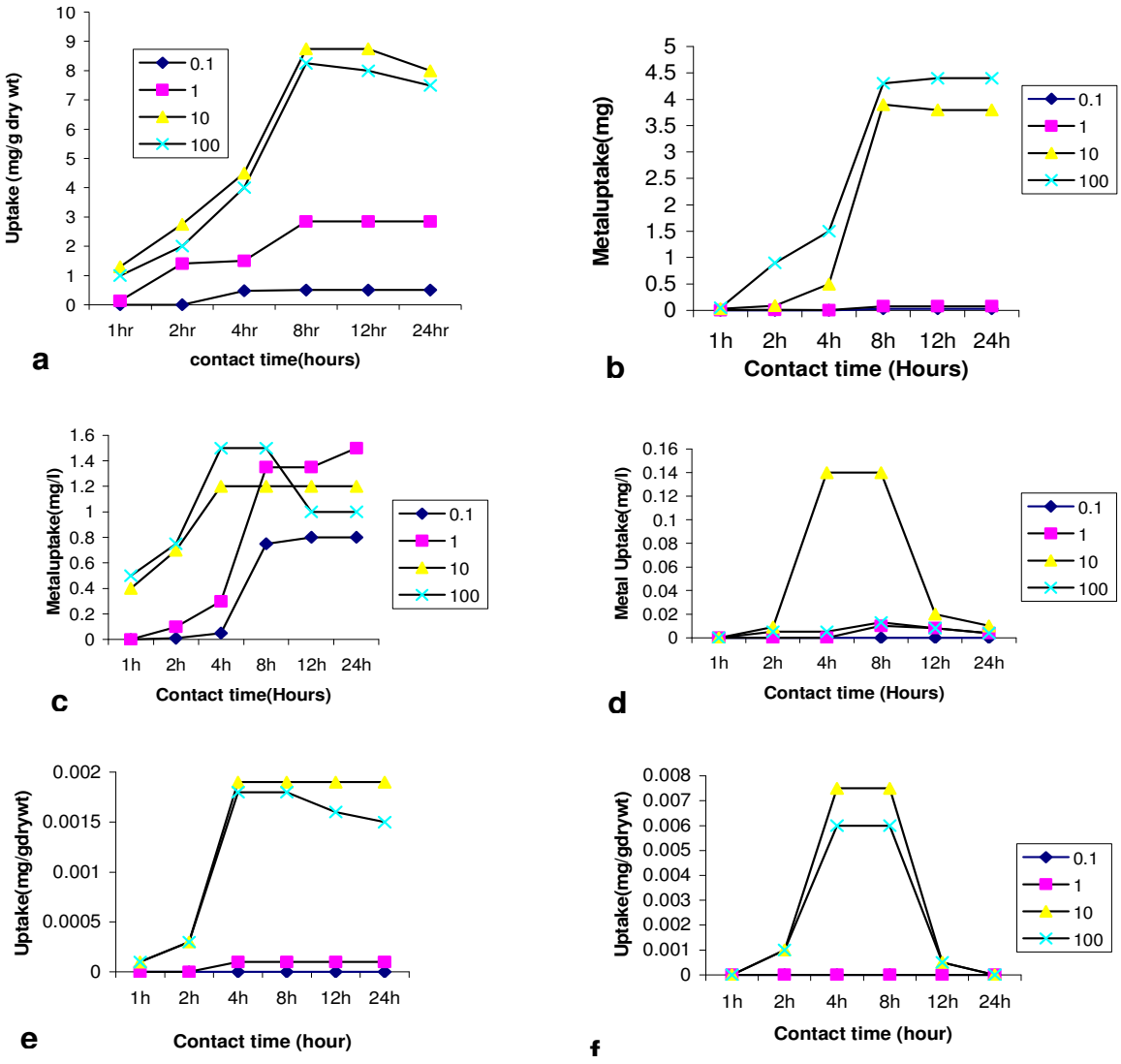

Figure 7. Effects of contact time on bioconcentration of various heavy metals by *Bacillus* sp. (a) Fe (b) Zn (c) Cd (d) Cu (e) Ni (f) Pb.

was attained in less than 24 h. The results of the effect of contact time on uptake indicates that the respective test isolates had an optimum residence time for each heavy metal and once this time elapsed, uptake remained either constant or diminished slightly. This agrees with metal uptake models, where the process can be considered as an equilibrium that involves adsorption and desorption due to saturation (Panchanadikar, 1994). Maximum nickel adsorption at 2 and 6 h by different bacteria in-cluding *Pseudomonas*, *Acinectobacter* and *Enterobacter agglomerans* have been reported (Panehanakikar, 1994; Kaewehai and Prasertson, 2002; Abu Al-Rub et al., 2002). Also, it had been reported that the biosorption of Pb by *Phanerochaete chrysporium* was rapid in the first 15 min and equilibrium was attained after 3 h (Yetis and Ceribass, 2001). The three test isolates showed a similar pattern in accumulating the heavy metals with time although the uptake of Zn by *Bacillus* and *Aeromonas* were not significantly different with respect to time.

Conclusion

The three test organisms (*Bacillus, Pseudomonas* and *Aeromonas*) had the capability of accumulation of the test metals. Results showed that metal concentrations between 10 - 100 mg/l promoted rapid metal uptake. Therefore to promote accumulation of heavy metals in polluted aquatic systems, it is necessary to ensure initial metal concentrations of between 10 - 100 mg/l. Metal up-take by the three test isolates increased between 4 - 12 h depending on the metal then reached equilibrium. The rate of attaining equilibrium varied between test organism

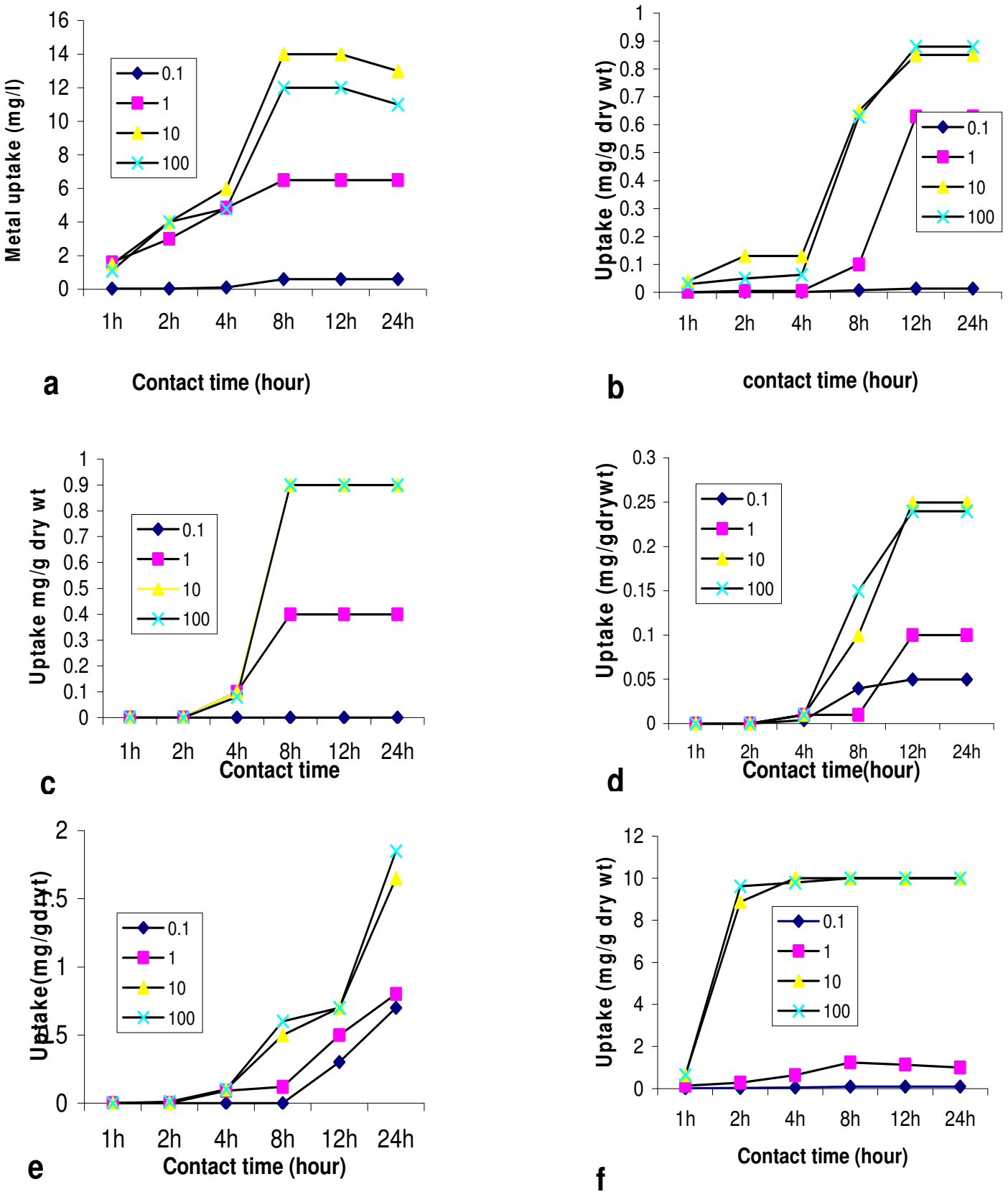

Figure 8. Effects of contact time on bioconcentration of various heavy metals; (a) Fe (b) Zn (c) Cd (d) Cu (e) Ni (f) Pb; by *Pseudomonas* sp.

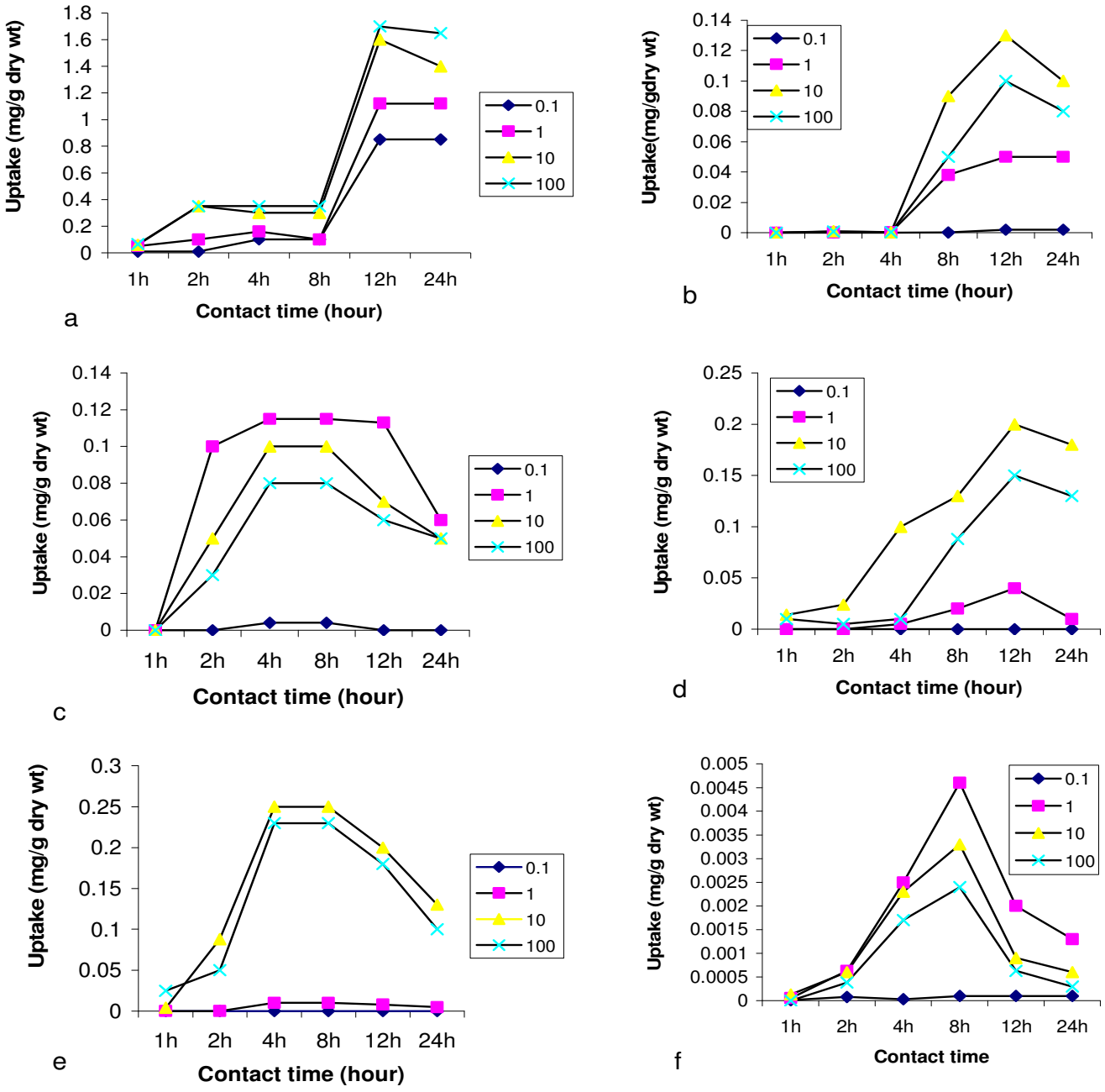

Figure 9. Effects of contact time on bioconcentration of various heavy metals; (a) Fe (b) Zn (c) Cd (d) Cu (e) Ni (f) Pb; by *Aeromonas* sp.

and metal type. Thus exposing test organisms to metal concentrations of above 24 h may not improve accumulation of these metals. Harvesting these organisms within 12 h and introducing fresh organisms will promote further accumulation of the metals. Thus, the combination of maintaining initial metal concentration of between 10 - 100 mg/l at test site and exposure for not more than 12 h followed by subsequent harvest and repeated introduction of test organism would promote rapid accumulation.

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