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Multiple Heavy Metal Tolerant Ciliates, *Oxytricha fallax* and *Paramecium caudatum*, Isolated From Industrial Effluents and Their Potential Use in Wastewater Treatment

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Abstract.- The multiple heavy metal resistant ciliates, *Oxytricha fallax* and *Paramecium caudatum*, isolated from industrial wastewater have been shown to be potential bioremediator of contaminated wastewater. The *O. fallax* showed tolerance against Zn^{2+} (17 µg/ml), Hg^{2+} and Ni^{2+} (18 µg/ml), and Cu^{2+} and Cd^{2+} (16 µg/ml). *P. caudatum* was found to tolerate Ni^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, and Cd^{2+} at a concentration of 18, 15, 17, 15 and 14 µg/ml, respectively. The metal ions slowed down the growth of the ciliates as compared to the culture grown without metal stress. The reduction in cell population of *O. fallax* was 70% for Cd^{2+}, 59% for Hg^{2+}, 28% for Zn^{2+}, 71% for Cu^{2+} and 70% for Ni^{2+} after 8 days of metal stress. The decrease in cell population of *P. caudatum* was 56% for Cd^{2+}, 44% for Hg^{2+}, 34% for Zn^{2+}, 80% for Cu^{2+} and 70% for Ni^{2+} after 8 days of metal stress. *O. fallax* decreased 91% of Zn^{2+}, and 90% of Hg^{2+} from the medium after 96 hours of incubation in a culture medium containing10 µg/ml of the respective metal ions. Besides this, the ciliate could also remove 94% of Cu^{2+} and Cd^{2+} and 88% Ni^{2+} from the medium containing 5µg/ml of each metal after 96 hours, respectively. *P. caudatum* removed 95% of Zn^{2+} and 78% of Hg^{2+} from the medium containing10 µg/ml of the respective metal ions after 96 hours of incubation. The protozoan could also remove 94% of Cu^{2+}, 82% of Cd^{2+} and 76% Ni^{2+} from the medium containing 5µg/ml of each metal after 96 hours, respectively. The ability of ciliates to take up multiple heavy metals from the medium could be exploited for metal detoxification and environmental clean-up operations.

Key words: Heavy metals, *Oxytricha fallax, Paramecium caudatum,* bioremediation.

INTRODUCTION

Industrial and mining wastewaters are the major source of heavy metal pollution. Metals can pose health hazards (Kang *et al.,* 2007) if their concentrations exceed allowable limits. Even when the concentration of metals does not exceed these limits, there is still a potential for long-term contamination, since heavy metals are known to be accumulative within biological systems (El-Sherif *et al.,* 2008). Common heavy metals which are known to cause health problems to higher organisms include arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc (Zhang *et al*., 2009).

Cadmium (Cd) is a heavy metal pollutant, widely distributed in the environment (Kalantari, 2008). Cd is one of the most toxic and carcinogenic heavy metals to organisms. Exposure to the metal occurs mainly through environmental pollution and its wide range of uses in industrial fields (Semra and Sefik, 2009). Cd is an element with no known biological function and is one of the most serious environmental pollutants. It is known to be carcinogenic and mutagenic in humans and plants (Song *et al.,* 2004; Akiko *et al.,* 2007)**.**

Copper is rarely found in natural water but is found in man-polluted environments (Udom *et al.,* 2004). Copper ions inhibit macromolecules synthesis and other enzymatic reactions (Company *et al.,* 2004). Mercury is a unique element that has no essential biological function. Compounds of mercury such as mercuric chloride and organomercurials are toxic to both eukaryotic and prokaryotic cells. Mercury is also genotoxic; inorganic Hg (II) is capable of strong reversible interactions with the nitrogens in purines and pyrimidines, and organic mercury compounds, *e.g.,* methylmercury, also produce irreversible damage to nucleic acids (Sletten and Nerdal, 1997).

Zinc is a major inorganic pollutant, which has shown inhibitory and promotory effects on the

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growth along with accumulation in plants (Kumar, 1989). Some reports indicate that Zn may inhibit apoptosis (Zalewski *et al.,* 1991), others suggest that Zn actually induces apoptotic cell death (Haase *et al.,* 2001; Iitaka *et al*., 2001) and propose Zn as a potential cytotoxic agent in treatment of thyroid cancer (Iitaka *et al*., 2001). Nickel is natural component of the soil. Human activities such as metal processing, land-application of sludges, and the use of certain fertilizers can lead to an accumulation of Ni at potentially toxic levels (Kabata-Pendias and Pendias, 2001). Nickel compounds are found to be nephrotoxic, hepatotoxic, immunotoxic and teratogenic (Ross, 1995). Nickel sub-sulfide, a component of nickel refinery dust, can cause lung, throat and nasal cancer (Liesegang *et al.,* 1993).

Conventional wastewater treatments such as chemical precipitation, lime coagulation, solvent extraction, ion exchange and adsorption have several disadvantages including high energy requirements, incomplete metal removal, high capital investment and running costs, and generation of toxic sludges (Ciba *et al.,* 1999). Recently, microbial bioremediation has emerged as an alternative technique to such traditional chemical treatments (Brierley, 1990). Microbial metal bioremediation is an efficient strategy due to its low cost, high efficiency and eco-friendly nature (Rehman *et al.,* 2007, 2009).

The objective of the present study was to evaluate the survival of *Oxytricha fallax* and *Paramecium caudatum* in media containing heavy metals such as Cd^{2+}, Cu^{2+} Hg^{2+}, Zn^{2+} and Ni^{2+} and to determine the efficiency of uptake of these metals by the ciliates. This information could be later used for remediation of heavy metal contaminated wastewater.

MATERIALS AND METHODS

Sample collection

Wastewater samples from a tannery effluent were collected in screw capped sterile bottles from Kasur (Pakistan). Some physicochemical parameters of wastewater *viz.* temperature (ºC), pH, dissolved oxygen (mg/l), and chromium (µg/ml) were measured. The samples were inoculated in Bold-basal salt medium in 100 ml conical flasks (Haq *et al*., 2000). A large number of bacteria, yeast, algae, rotifers, and various protozoa were present in the original wastewater sample.

Isolation and culturing of protozoa

For isolation of protozoa, antibiotics, *i.e*., ampicillin (25 µg/ml), chloramphenicol (35 µg/ml) and gentamicin (25 µg/ml), were used to prevent growth of bacteria. Algae were excluded by keeping the culture in semidarkness. Yeast was excluded by absence of any organic substance in the medium. Axenic culture of protozoa was made according to Shakoori *et al*. (2004). One hundred milliliter of Bold-basal salt medium with 8 boiled wheat grains in 250 ml conical flask was inoculated under aseptic conditions with 10µl of inoculum containing 40-50 ciliates. Glucose as carbon source was only added as 1g/l in Bold-basal salt medium (Shakoori *et al.,* 2004; Rehman *et al*., 2006). The culture was maintained in the laboratory for one week at room temperature (25-27°C) and at pH 7.5. The growth of ciliates was observed in the cultures by counting the number of ciliates in a haemocytometer at regular intervals.

Identification of Protozoa

Identification of the protozoans was done by observing their body shape, other morphological features, movements and behaviour (Edmondson, 1966; Curds, 1982; Curds *et al*., 1983; APHA, 1992).

Determination of growth curves

The effect of different metal ions on growth of the culture was checked by counting the number of protozoan cells in the medium. The cells were grown in the salt medium, to which Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, and Ni^{2+} ions were added at a concentration of 1µg/ml per day for eight days. At least three counts were taken every day to get a mean of every reading. The growth was compared with that of the control culture, which contained no added metal ions. The activity, shape and size of the protozoans were also noted. The size was measured with an ocular micrometer after restricting the movement of the ciliates by putting the culture in methylcellulose and staining with 1% neutral red.

Determination of metal resistance

Resistance of *O. fallax* and *P. caudatum* to five metal ions *i.e*., Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, and Ni^{2+} was checked by addition of the respective metal salts *viz*., $CdCl_2.H_2O$, $CuSO_4$, $HgCl_2$, $HgCl_2$, $ZnSO_4.7H_2O$ and $NiCl_2$ to Bold-basal salt medium. Metals ions were sterilized separately and added to the medium when the temperature of the salt medium was slightly less than 50°C. For Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, and Ni^{2+} the concentration in the medium on the first day was 1µg/ml with an increase of 1µg/ml every day for 17 days for Zn^{2+}, 16 days each for Cu^{2+} and Cd^{2+}, and for 18 days each for Hg^{2+} and Ni^{2+}. For *Paramecium* it was 1µg/ml every day for 18 days for Ni^{2+}, 15 days each for Cu^{2+} and Zn^{2+}, 17 days for Hg^{2+} and 14 days for Cd^{2+}. Although the death of protozoa is confirmed by the lysis of the cell, movement is considered to be a vital sign of life. When the protozoa became inactive, no more metal was added.

Heavy metal uptake

For determining uptake of heavy metals by *O. fallax* and *P. caudatum*, the ciliates were grown by inoculating 100 ml of Bold-basal medium in five 250 ml conical flasks, with 10 µl of original laboratory culture (40±2 cells) at 25°C. Mercury, and zinc were added at a concentration of 10 µg/ml of each in the medium containing ciliate cells but cadmium, copper, and nickel each was added at a concentration of 5 µg/ml. The control culture medium was also run for each metal containing the same concentration as in the treated one but without the ciliates. The cultures were incubated for 6 days and from each medium (control and treated) 5 ml culture was taken out under sterile conditions after 0, 48, 72, 96 hours, respectively. The cultures were spun down at 350g for 15 min and the supernatants were used for the estimation of Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, and Ni^{2+} by atomic absorption spectrophotometer (Varian, U.S.A) at wavelengths 228.8, 324.7, 253.7, 213.9, and 232.0 nm, respectively. The amount of metals in the supernatants was determined using standard curves. The percentage reduction in the amount of Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, and Ni^{2+} in the medium was calculated.

Statistical analysis

Observations were made and all the experiments were repeated two or more times and the results reported are average values. Standard deviation and standard error of the mean were also calculated. For determining significance of differences between the control and the experimental, Student's "t" test was applied.

RESULTS

Some physicochemical characteristics of industrial wastewater of five different ponds, from where the ciliates were isolated, were recorded. The temperature of ponds harboring the ciliates ranged between 19.66ºC and 34ºC, pH between 7.46 and 8.93, dissolved oxygen between 0.36±0.01 and 1.77±0.03 mg/ml and Cr^{6+} ranging between 0.30±0.04 and 1.60±0.08 µg/ml (Rehman *et al.*, 2007).

Ciliates identification

A wide variety of micro-organisms was observed in wastewater samples obtained from tannery effluents. The protozoans observed in the samples were *Paramecium, Stylonychia, Euplotes, Chilodonella, Colpoda, Cyclidium, Metopus, Plagiopyla, Tachysoma, Tetrahymena, Vorticella, Oxytricha, Spirostomum, Amoeba* and *Spathidium.* On the basis of metal tolerance and frequent occurrence in the wastewater samples *Oxytricha fallax* and *Paramecium caudatum* were selected for further study.

Effect of metals on the growth of ciliates

Figures 1 and 2 show growth curves of *O. fallax* and *P. caudatum* in a medium with and without metal ions. The growth of ciliates, which is indicated by cell population, has been affected by the presence of metal ions in culture media. The control culture of *O. fallax* contained $0.058x10^3$ cells/ml on day 1, which increased to $2.100x10^3$ cells/ml after 8 days. However, when Cu^{2+} (8 µg/ml) was added the number increased from $0.100x10^3$ to $0.615x10^3$ cells/ml in 8 days, whereas the number of cells increased from $0.100x10^3$ to $0.630x10^3$ cells/ml in the presence of Cd^{2+} (8 µg /ml) in 8 days. In the presence of Zn^{2+} (8 µg/ml) the number of

cells increased from $0.058x10^3$ to $1.508x10^3$ cells/ml, $0.027x10^3$ to $0.860x10^3$ cells/ml in Hg^{2+} (8 µg/ml) after 8 days, whereas the number of cells increased from $0.039x10^3$ to $0.617x10^3$ cells/ml in the presence of Ni^{2+} (8 µg /ml) in 8 days. The addition of metal ions into the medium resulted in slower growth and delayed cell division (Fig.1). The reduction in cell population of *O. fallax* was 70% ($P< 0.05$) for Cd^{2+}, 71% ($P< 0.05$) for Cu^{2+}, 59% for Hg^{2+}, 28% for Zn^{2+}, and 70% ($P< 0.05$) for Ni^{2+} after 8 days of metal stress.

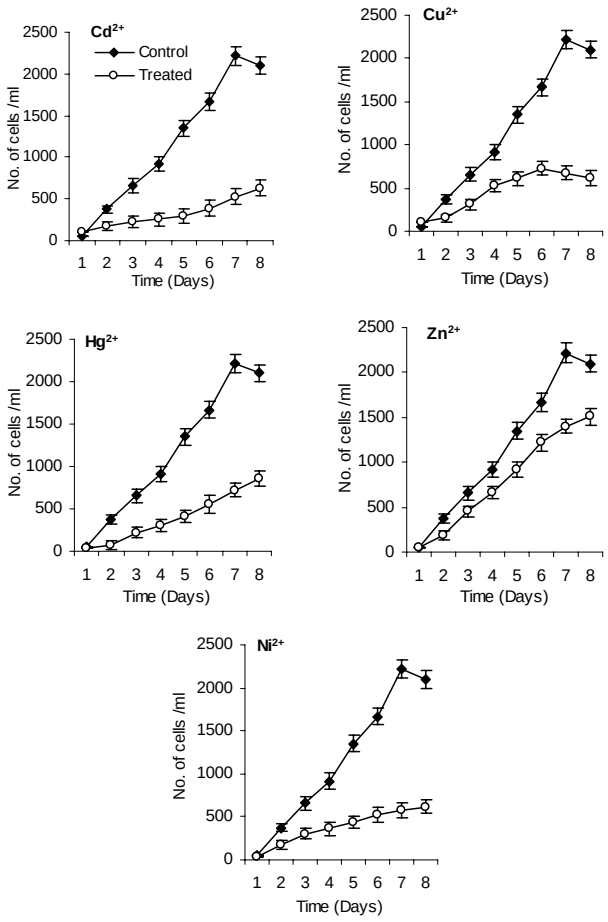

Fig. 1. Growth curves of *Oxytricha fallax* in Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+} and Ni^{2+} containing medium. Control culture did not contain any metal ions.

Maximum number of *Oxytricha* cells for control (without metal) and in each metal containing medium was achieved on day 8 except for copper containing medium, where it was achieved on day 6. The maximum number of cells for *Oxytricha* in control culture (2217.33±00.5) were obtained in 7 days. The number of protozoan cells in Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, and Ni^{2+} containing media was 629.00±01.00, 724.66±01.53, 860.33±01.53, 1508.00±01.57, and 616.66±01.53, respectively during this period. The growth rate of *Oxytricha* was slower in the presence of all metal ions as compared with the control.

For *Paramecium,* the control culture contained 100 cells/ml on day 1, which increased to 1553 cells/ml after 8 days. However, when Cu^{2+} (8 µg/ml) was added the number increased from 144 to 293 cells/ml in 8 days, whereas the number of cells increased from 183 to 683 cells/ml in the presence of Cd^{2+} (8 µg /ml) in 8 days (Fig.2). In the presence of Zn^{2+} (8 µg/ml) the number of cells increased from 67 to 1027 cells/ml, 120 to 860 cells/ml in Hg^{2+} (8 µg/ml) after 8 days, whereas the number of cells increased from 80 to 460 cells/ml in the presence of Ni^{2+} (8 µg /ml) in 8 days. The decrease in cell population of *P. caudatum* was 56% for Cd^{2+}, 44% for Hg^{2+}, 34% for Zn^{2+}, 80% ($P< 0.05$) for Cu^{2+}, and 70% ($P< 0.05$) for Ni^{2+} after 8 days of metal stress.

Maximum number of *Paramecium* cells for control and in each metal containing medium was achieved on day 8 except for copper and nickel containing medium, where it was achieved on day 6. The control contained 1553.33±01.53cells /ml and the maximum number of ciliate cells in Cd^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, and Ni^{2+} containing medium was 684.66±01.53, 393.00±02.00 1026.66±00.57, 860.66±01.15, and 592.00±01.00, respectively. It is clear from the growth curves that cell division was slowed down in the presence of metal ions as compared to control. Growth rate of *Paramecium* was highly affected by Cu^{2+} and least by Zn^{2+}.

Heavy metal tolerance

O. fallax was found to resist Zn^{2+} up to a concentration of 17 µg/ml. The metal-resistant ciliate could also tolerate $Hg^{2+}$and Ni^{2+} at a concentration of 18 µg/ml each, respectively while the ciliate resisted $Cd^{2+}$and Cu^{2+} up to a concentration of 16 µg/ml each, respectively. *Paramecium* showed maximum resistance against Ni^{2+} at a concentration of 18 µg/ml. The ciliate was

also found to tolerate Cu^{2+}, Hg^{2+}, Zn^{2+}, and Cd^{2+} at a concentration of 15 µg/ml, 17 µg/ml 15 µg/ml and 14 µg/ml, respectively. The order of resistance regarding the metal concentration in both ciliates was, therefore, $Ni^{2+}>Hg^{2+}>Zn^{2+}>Cu^{2+}>Cd^{2+}$. There was apparently no reduction in the size of *O. fallax* and *P. caudatum* cells. Movement, which is a vital sign of life, was taken as a parameter of effect on growth rate. The movements of the ciliates slowed down in the presence of $ZnSO_4$ but almost stopped in the $CdCl_2.H_2O$, $CuSO_4$, $HgCl_2$, and $NiCl_2$.

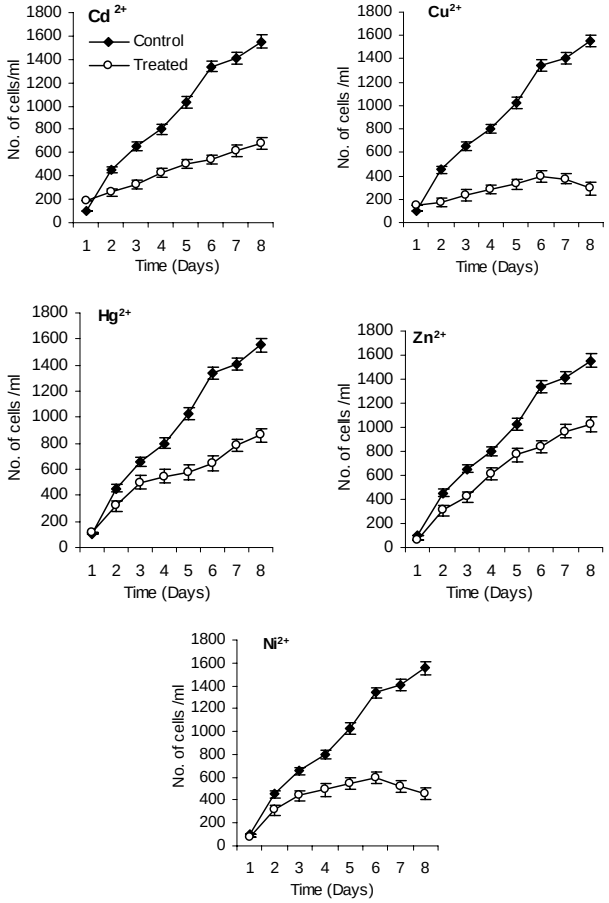

Fig. 2. Growth curves of *Paramecium caudatum* in Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, and Ni^{2+} containing medium. Control culture did not contain any metal ions.

Metal uptake by O. fallax *and* P. caudatum

The *O. fallax* growing in medium containing mercury (10 µg/ml) could decrease 75% of mercury from the medium after 48 hours, 82% after 72 hours and 90% after 96 hours. Likewise ciliate reduced 84% zinc (10 µg/ml) from the medium after 48 hours, 88% after 72 hours and 91% after 96 hours. It could also decrease 80% of cadmium (5 µg/ml) from the medium after 48 hours, 88% after 72 hours and 94% after 96 hours. *O. fallax* also removed 82% of copper (5 µg/ml) from the medium after 48 hours, 88% after 72 hours and 94% after 96 hours. Likewise ciliate decreased 78% nickel after 48 hours, 82% after 72 hours and 88% after 96 hours from the medium containing Ni^{2+} at a concentration of 5 µg/ml (Fig.3).

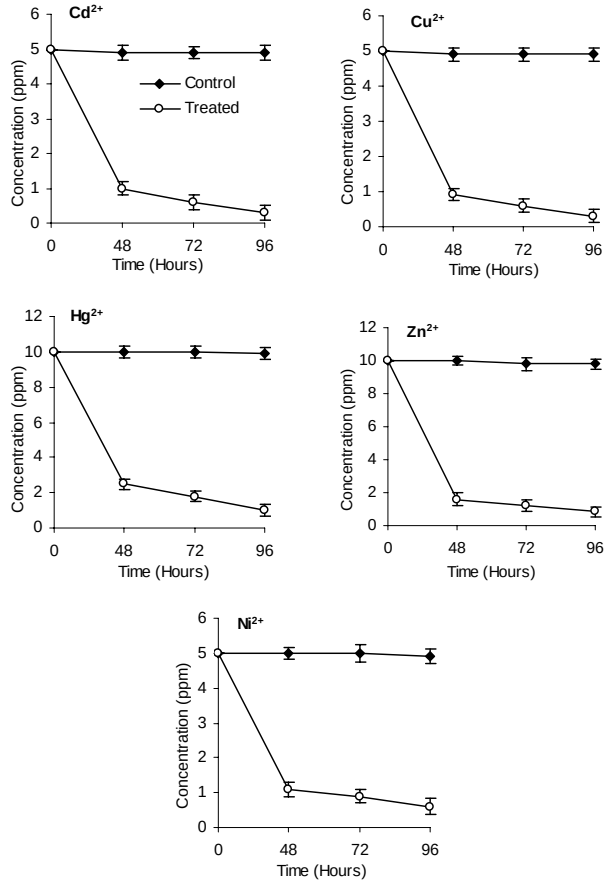

Fig. 3. Uptake of Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+} and Ni^{2+} by *Oxytricha fallax* growing in Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, and Ni^{2+} containing medium. The control did not contain cells of the ciliate.

P. caudatum showed remarkable ability to pick up heavy metal ions from the culture medium. The concentration of Zn^{2+} and Hg^{2+} was reduced 95%, and 78% after 96 hours in a culture medium

each containing10 µg/ml of the respective metal ions. The ciliate was able to remove 83% and 88% zinc after 48 and 72 hours, respectively. Likewise ciliate removed 65% and 74% mercury from the medium after 48 and 72 hours, respectively. On the other hand, 94% of Cu^{2+} (5 µg/ml), 82% of Cd^{2+} (5µg/) and 76% of Ni^{2+} (5 µg/ml) were removed from the medium after 96 hours, respectively (Fig.4). It could also decrease 70% and 76% cadmium from the medium after 48 and 72 hours. *P. caudatum* was also capable to decrease 76% and 88% copper, whereas the decrease in nickel concentration was 58% and 66% after 48 and 72 hours from the medium, respectively (Fig. 4).

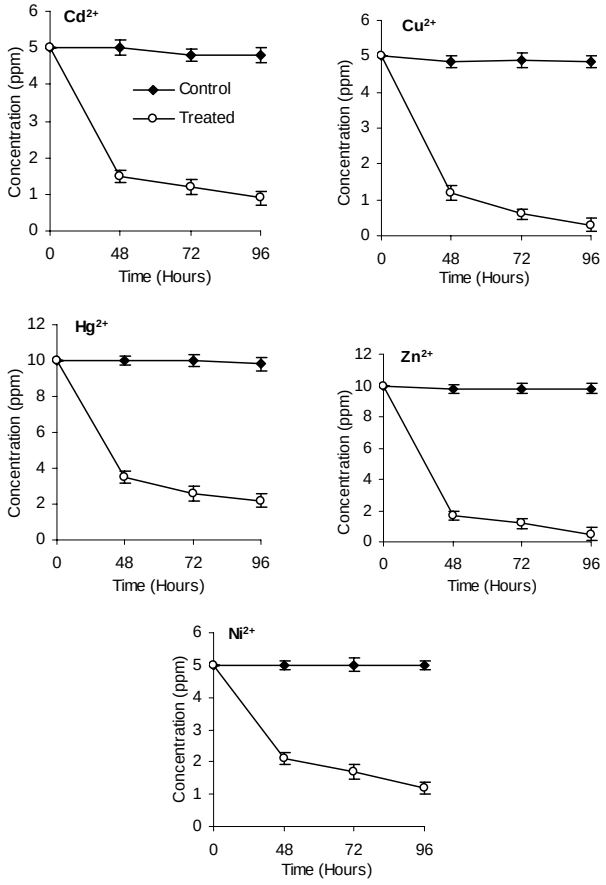

Fig. 4. Uptake of Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+} and Ni^{2+} by *Paramecium caudatum* growing in Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, and Ni^{2+} containing medium. The control did not contain cells of the ciliate.

DISCUSSION

Industrialization lead to environmental pollution which has become a global problem with heavy metals being prominent pollutants (Shokrzadeh and Saravi, 2009). In recent years, increasing awareness of the environmental impact of heavy metals has prompted a demand for the purification of industrial wastewaters prior to discharge into natural waters (El-Sherif *et al.,* 2008; Rehman *et al.,* 2008).

The heavy metals, in the present study, have significantly hampered the growth of the ciliates cells. When the cell populations of metal-treated cultures were compared with those of the corresponding control culture, it was observed that the cadmium-treated *Oxytricha* culture had 70% lesser cell population when compared with 8 days of control culture. In the presence of Cu^{2+} ions, this decrease was 71%, whereas for Hg^{2+} it was 59%, for Zn^{2+} it was 28%, and for Ni^{2+} it was 70% as compared with control after 8 days of metal exposure. The order of resistance, in terms of reduction in the cellular population of *Oxytricha*, was $Zn^{2+}>Hg^{2+}>Cu^{2+}>Cd^{2+}=Ni^{2+}$. For *Paramecium* culture it had 56% lesser cell population when compared with 8 days of control culture. In the presence of Cu^{2+} ions, this decrease was 80%, whereas for Hg^{2+} it was 44%, for Zn^{2+} it was 34%, and for Ni^{2+} it was 70% as compared with control after 8 days of metal exposure. The order of resistance, in terms of reduction in the cellular population of *Paramecium*, was $Cu^{2+}>Ni^{2+}>Cd^{2+}>Hg^{2+}>Zn^{2+}$. Metal resistant protozoa have been reported in wastewaters and metal-polluted environments (Shakoori *et al.,* 2004; Madoni and Romeo, 2006; Rehman *et al.,* 2008, 2009).

Conventional methods consume high amounts of energy and large quantities of chemical reagents. It is well known that bioremediation of toxic pollutants has advantages over other techniques as it is cheap, non-destructive and contamination remains localized (Rise-Roberts, 1998; Rehman *et al.,* 2009). The use of metal-resistant microorganisms (Shakoori *et al.,* 2004; Rehman *et al.,* 2008, 2009) can help to remove metal from contaminated environments. Understanding the regulation of heavy metal

resistance could be useful for biological waste treatment and estimating the impact that industrial activity may have on natural ecosystems (Permina *et al.,* 2006). Microorganisms have a high surface area-to-volume ratio (Bagot *et al.,* 2006) and therefore provide a large contact area that can interact with metals in the surrounding environment (Huang, 2005). Detoxification of the polluted water and soil involves the concentration of the metal, or binding it in a way that makes it biologically inert (Ron *et al.,* 2004).

Mortuza *et al.* (2005) reported that *Paramecium bursaria* accumulated 1.72 to 15.5 pg Cr/cell in a time and concentration–dependent manner. These microorganisms actively contribute to the amelioration of the effluent quality, since the majority of them feed upon dispersed bacteria (Madoni, 2000). In one of the previous reports from this laboratory *Euplotes mutabilis* grown in the medium containing Cu^{2+} (5 µg/ml) has been reported to reduce 60% of copper from the medium after 48 hours, 82% after 72 hours and 95% after 96 hours (Rehman *et al*., 2006). It could also reduce 67% Hg^{2+} after 48 hours, 75% after 72 hours, and 82% after 96 hours from the medium containing Hg^{2+} at a concentration of 10 µg/ml. Likewise, the ciliate could uptake 85% Zn^{2+}, 84% of Cd^{2+}, and 87% of Ni^{2+} after 96 hours of inoculation of growth medium containing 10 µg/ml of Zn^{2+} and 5 µg/ml of Cd^{2+} and Ni^{2+}, with actively growing ciliates. After 6 days of incubation the ciliate removed 87% Cd^{2+}, 92% Ni^{2+}, and 93% Zn^{2+} from the wastewater (Rehman *et al*., 2009).

In the present study *O. fallax* decreased 91% and 90% of Zn^{2+} and Hg^{2+} after 96 hours of incubation in a culture medium containing10 µg/ml of each metal. The ciliate could also remove 94% of Cu^{2+} and Cd^{2+} and 88% of Ni^{2+} from the medium containing 5 µg/ml of each metal after 96 hours, respectively. *P. caudatum* could remove 82% (Cd^{2+}), 78% (Hg^{2+}), 95% (Zn^{2+}), 94% (Cu^{2+}), and 76% (Ni^{2+}) from the medium after 96 hours of incubation. This clearly indicates that the ciliates actively take up the heavy metals. Metal bioaccumulation has also been reported to be the main mechanism of resistance to heavy metals in ciliates by others (Martin-Gonzalez *et al.,* 2006; Diaz *et al.,* 2006).

CONCLUSIONS

O. fallax showed tolerance to heavy metals in the given order Hg^{2+}= Ni^{2+}>Zn^{2+}>Cu^{2+}=Cd^{2+} at a concentration range of 16-18 µg/ml whereas *P. caudatum* showed this tolerance in the order Ni^{2+}>Hg^{2+}>Zn^{2+}>Cu^{2+}>Cd^{2+} at a concentration range of 14-18 µg/ml. *O. fallax* decreased 91% of Zn^{2+}, 90% of Hg^{2+}, 94% of Cu^{2+} and Cd^{2+} and 88% of Ni^{2+} from the medium after 96 hours of incubation. *P. caudatum* removed 95% of Zn^{2+}, 78% of Hg^{2+}, 94% of Cu^{2+}, 82% of Cd^{2+} and 76% of Ni^{2+} from the medium during the same period. These results indicate that the *O. fallax* and *P. caudatum* may have potential application in bioremediation of wastewaters containing heavy metals. Further studies are needed on these ciliates to understand the mechanism of resistance and high ability to accumulate metals.

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