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Induction of Bacterial and Yeast Recombinants and Their Decontaminated Factory Effluents

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Abstract: Factory effluents is one of the main sources of pollution to ground water amd river water. In this article ten bacterial strains and two *Saccharomyces cerevisiae* strains were used in this work. These parental strains were tested for their tolerance to fertilizer factory effluents. All yeast and bacterial strains appeared high tolerance to 100% effluent concentration. Bacterial strains were marking using 19 antibiotics to be use as a selectable marker in conjugation process. The available markers obtained were used in 14 matinj,10 of them were success, two transconjugants from each conjugation were selected to be use in biosorption experiments. Two *Saccharomyces cerevisiae* strains were mated and the hybrids were isolated to be use in uptake experiments. This indicated that the microbial world could adapt to Factory efflluents leading us to developing biotechnology for use in pollution control of hazardous wastes. Plasmids seem to play a major role in the adaptation of bacteria to xenobiotic and in the acquisition of new genetic traits due to pollution. A particularly important aspect is the occurrence of some broad host range plasmids specialized in the degradation of synthetic chemicals. Modern ecological biotechnology attempts to solve the problems of pollution by screening for and molecularly breeding microbial strains that are capable of degrading recalcitrant. Research in this direction is in good shape. This investigation aimed to apply microbial genetic technique to induce recombinants from bacteria and yeast to be testing for maximal accumulation of heavy metals from factory effluents to improve the quality of drinking and irrigated water in industrial regions. This enhancement the biosorption which shall resulting in a decrease of environmental loading, i.e., in lesser contamination of groundwater and also receiving surface waters.

Key words: Biosorption, conjugation, factory effluents, genetic markers, heavy metals, plasmid transfer.

INTRODUCTION

Heavy-metal pollution represents an important environmental problem due to the toxic effects of metals, and their accumulation throughout the food chain leads to serious ecological and health problems. The main sources of heavy metal pollution are mining, milling and surface finishing industries, discharging a variety of toxic metals such as Cd, Cu, Ni, Co, Zn and Pb into the environment. Biosorption is a term that describes the removal of heavy metals by the passive binding to non-living biomass from an aqueous solution. This implies that the removal mechanism is not metabolically controlled. In contrast, the term bioaccumulation describes an active process whereby removal of metals requires the metabolic activity of a living organism. High metal-binding capacities of several biological materials have already been identified in part. Among the biosorbents, there are marine algae, bacteria, yeasts, fungi and waste mycelia from the fermentation and food industry (reviewed in Kadukova and Vircikova, 2005). Metal uptake capacity by various biosorbents (algae/fungi/yeasts) has been evaluated using biosorption isotherm curves derived from equilibrium batch sorption experiments, and the effect of various process parameters such as pH, biomass loading, biomass pretreatments, etc. has been studied extensively. In this report, we show the useful method of research development using biosorption techniques. To develop the techniques of bioremediation using biosorption method from heavy metal-contaminated soil or water, we have characterised the heavy metal resistant bacterium to heavy metal-contaminated effluents.

"Heavy metals" are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4°C (39°F). Simply stated, specific gravity is a measure of density of a given amount of a solid substance when it is compared to an equal amount of water. Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic, 5.7; cadmium, 8.65; iron, 7.9; lead, 11.34; and mercury, 13.546 (Kadukova and Vircikova, 2005).

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Heavy metals are elements having atomic weights between 63.546 and 200.590 and a specific gravity greater than 4.0. Living organisms require trace amounts of some heavy metals, including cobalt, copper, iron, manganese, molybdenum, vanadium, strontium, and zinc. Excessive levels of essential metals, however, can be deterimental to the organism. Non-essential heavy metals of particular concern to surface water systems are cadmium, chromium, mercury, lead, arsenic, and antimony Kadukova and Vircikova, 2005.

Bioremediation of industrial wastes containing heavy metals has been demonstrated by several biotechnology companies employing bioaccumulation (Macaskie and Dean. 1984). Biosorption, bioprecipitation, and uptake by purified biopolymers derived from microbial cells provide alternative and/or additive processes for conventional physical and chemical methods (Silver, 1991). Intact microbial cells, live or dead, and their products can be highly efficient bioaccumulators of both soluble and particulate forms of metals (Silver, 1991). The cell surfaces of all microorganisms are negatively charged owing to the presence of various anionic structures. This gives bacteria the ability to bind metal cations. Various microbial species, mainly *Pseudomonas,* have been shown to be relatively efficient in bioaccumulation of uranium, copper, lead, and other metal ions from polluted effluents, both as immobilized cells and in the mobilized state (Macaskie and Dean. 1984). The accumulation of heavy metals in soils adds an environmental dimension to the problem. All of these issues have a public profile and there is a significant amount of detailed information from credible sources that is very accessible to the public.

Water is the most vital element among the natural resources, and is crucial for the survival of all living organisms including human, food production, and economic development. Today, nearly 40 percent of the world's food supply is grown under irrigation, and a wide variety of industrial processes depends on water (BCAS, 2000). Moreover, in Egypt the environment, economic growth, and developments are all highly influenced by water - its regional and seasonal availability. In terms of quality, the surface water of the country is vulnerable to pollution from untreated industrial effluents and municipal wastewater, runoff from chemical fertilizers and pesticides, and oil and tube spillage in the coastal area from the operation of sea and river ports. Water quality also depends on effluent types and discharge quantity from different type of industries, types of agrochemicals used in agriculture, and seasonal water flow and dilution capability by the river system (DHV, 1998).

The wastes, effluents and agrochemicals contain heavy metals, toxic substances, germs and nitrogen containing toxic substances. They pollute the natural system of water. Table 1 appeared the standards of drinking water (DoE, 1997).

Table 1: Standards for drinking water (DoE, 1997)

Sl. No.	Parameter	Unit	Standards
1.	Aluminum	mg/l	0.2
2.	Ammonia (NH_3)	,,	0.5
3.	Arsenic	,,	0.05
6.	BOD520°C	,,	0.2
7.	Boron	,,	1.0
8.	Cadmium	,,	0.005
9.	Calcium	,,	75
10.	Chloride	,,	150 – 600
16.	Chromium (total)	,,	0.05
17.	COD	,,	4
20.	Color	Hazen	15
21.	Copper	mg/l	1
24.	DO	,,	6
26.	Hardness (as $CaCO_3$)	,,	200 – 500
27.	Iron	,,	0.3 – 1.0
28.	Kjeldhl Nitrogen (total)	,,	1
29.	Lead	,,	0.05
30.	Magnesium	,,	30 – 35
31.	Manganese	,,	0.1
34.	Nitrate	,,	10
36.	Odor	,,	Odorless
38.	pH	,,	6.5 – 8.5
40.	Phosphate	,,	6
41.	Phosphorus	,,	0
42.	Potassium	,,	12
47.	Sodium	,,	200
48.	Suspended particulate matters	,,	10
50.	Sulfate	,,	400
51.	Total dissolved solids	,,	1000
52.	Temperature	°C	20-30
55.	Zinc	mg/l	5

MATERIALS AND METHODS

Ten bacterial strains and seven *Saccharomyces cerevisiae* strains (Table 2) were used in this study, they are kindly obtained from National Center for Agriculture Utilization Research,, USA. One of *Saccharomyces cerevisiae* strains (NBIMCC 82) was kindly providing from National Bank for Industrial Microorganisms and Cell Cultures, Bulgaria, Sofia. All strains used in this study are wild type strains.

Table 2: Bacteria and yeast strains used in this study.

No.	Strains	Designation	Origin
1	Citrobacter amalonaticus	NRRL B-41228	USA
2	*Citrobacter freundii*	NRRL B-2643	USA
3	*Bacillus subtilis* var *niger*	NRRL NRS-213	USA
4	*Bacillus subtilis*	NRRL B-642	USA
5	Bacillus licheniformis	NRRL B-571	USA
6	*Bacillus licheniformis*	NRRL B-1584	USA
7	*Bacillus licheniformis*	NRRL NRS-1264	USA
8	*Bacillus licheniformis*	NRRL B-358	USA
9	*Micrococcus luteus*	NRRL B-287	USA
10	*Kocuria rhizophila*	NRRL B-4375	USA
11	*Saccharomyces cerevisiae*	NRRL Y – 12632	USA
12	*Saccharomyces cerevisiae*	NRRL Y - 11562	USA
13	*Saccharomyces cerevisiae*	NBIMCC 82	Bulgaria (National Bank for industrial microorganisms and cell cultures), sofia
14	*Saccharomyces cerevisiae*	NRRL Y - 12619	USA
15	*Saccharomyces cerevisiae*	NRRL Y – 136	USA
16	*Saccharomyces cerevisiae*	NRRL Y – 137	USA
17	*Saccharomyces cerevisiae*	NRRL Y - 1370	USA

Factory effluents:

The present study was undertaken using the wastewaters resulted from ammonia unit of Fertilizer Factory (FF). Polluted water was collected from the main pipe of the factory before being mixed with water in the river. This collection was done in October 2007. A specific problem associated with heavy metals in the environment is accumulation in the food chain and persistence in the environment.

Media:

Bacterial strains were grown as described previously by Horikoshi *et al.* (1981). However, yeast strains were grown on yeast extract peptone dextrose (YEPD) medium.

Methodology:

Antibiotic susceptibility assays:Antibiotic susceptibility was measured by plate diffusion method, according to Collins and Lyne (1985) with cultures grown to logarithmic growth phase in nutrient agar medium for each microbe. All antibiotics were used at a concentration of 100 mg/ml, according to Roth and Sonti (1989).The selectable markers were identified as antibiotic resistance and or sensitive genes as listed in the Table of conjugation (Table 4). Antibiotic designation was listed in Table 3.

Table 3: Antibiotics and their abbreviations used for genetic marking against different bacterial strains.

No	Antibiotics	Designation
1	Flucamox	*flu*
2	Streptomycin	*Str*
3	Tetracycline	*Tc*
4	Neomycinsulphate	*Nm*
5	Ampicillin	*Ap*
6	Erythromycin	*Erth*
7	Amoxycillin and flucloxacillin	*Am-Fluc*
8	Rifampicillin	*Rf*
9	Ibiamox	*Ibim*
10	Amoxycillin	*Amoxy*
11	Ibidroxil	*Ibid*
12	Haiconcil	*Hico*
13	Velosef	*Velo*
14	Epicocillin	*Epico*
15	Nystatin	*Nyst*
16	Epicocillin	*Epico*
17	Erythrocin	*Ery*
18	Duricef	*Duri*
19	Pencillin	*pen*

Conjugation:

Nutrient broth cultures, in the late-exponential growth phase were used. Quantitative spot mating of conjugal transfer was carried out according to Lessel *et al*. (1993) by inoculating 10 ml samples of the donor culture onto the surface of selective medium, previously seeded with 100 ml of the recipient culture. A single colony of transconjugants was picked up and transferred to slant nutrient agar medium. Conjugation was carried out between strains carrying the opposite genetic markers as shown in Table 4. From each mating, two different isolates were selected to be used in pollutants uptake experiments.

Table 4: Mating between bacterial strains that having the opposite genetic markers.

No. of mating	Mating	Revelant geneotype of mating
1	NRRL B-571 X NRRL B-1584	*Erth*$^+$, *Ap*$^+$, *Ibim*$^+$, *Amoxy*$^+$, *Hico+, Epico*$^+$,*Cp*$^-$ X *Erth*$^-$, *Ap*$^+$, *Ibim*$^-$, *Amoxy*$^-$, *Hico*$^-$, *Epico*$^-$,Cp+
2	NRRL B-571 X NRRL B-358	*Erth*$^+$, *flu*$^+$, *Hico+ Epico*$^+$,*Cp*$^-$ X *Erth*$^-$, *Flu*$^-$, *Hico*$^-$, *Epico*$^-$,*Cp+*
3	NRRL B-571 X NRRL B-2643	*Erth*$^+$, *flu*$^+$, *Epico*$^+$, *Velo*$^-$, *Duri*$^-$, *Cp*$^-$, *Ibid*$^-$ X *Erth*$^-$, *flu*$^-$ *Epico*$^-$, *Velo*$^+$, *Duri*$^+$, *Cp*$^+$, *Ibid*$^+$
4	NRRL B-571 X NRRL B-41228	*Erth*$^+$, *flu*$^+$, *Ap*$^+$, *Epico*$^+$,*Cp*$^-$ X *Erth*$^-$, *flu*$^-$, *Ap*$^-$, *Epico*$^-$,*Cp*$^+$
5	NRRL B-1584 X NRRL B-41228	*Ap*$^+$, *Ibid*$^-$, *Amoxy*$^-$, *Ibim*$^-$ X *Ap*$^-$, *Ibid*$^+$, *Amoxy*$^+$, *Ibim*$^+$
6	NRRL B-1584 X NRRL B-642	*Ap*$^+$, *Cp*$^+$, *Am-Fluc*$^+$, *pen*$^+$, *Hico*$^-$, *Epico*$^-$ X *Ap*$^-$, *Cp*$^-$, *Am-Fluc*$^-$, *pen*$^-$, *Hico*$^+$, *Epico*$^+$
7	NRRL B-1584 X NRRL NRS-213	*Ap*$^+$, *Cp*$^+$, *Am-Fluc*$^+$, *pen*$^+$, *Amoxy*$^-$ X *Ap*$^-$, *Cp*$^-$, *Am-Fluc*$^-$, *pen*$^-$, *Amoxy*$^+$
8	NRRL NRS-1264 X NRRL B-2643	*Erth*$^+$, *Tc*$^+$, *Ibim*$^+$, *flu*$^+$, *Ibid*$^-$, *Velo*$^-$, *Duri*$^-$ *X Erth*$^-$, *Tc*$^-$, *Ibim*$^-$, *flu*$^-$, *Ibid*$^+$, *Velo*$^+$, *Duri*$^+$
9	NRRL B-358 X NRRL B-642	*Ap*$^+$, *Cp*$^+$, *Am-Fluc*$^+$, *pen*$^+$, *Ibim*$^+$, *Amoxy*$^+$, *Hico*$^-$, *Epico*$^-$ X *Ap*$^-$, *Cp*$^-$, *Am-Fluc*$^-$, *pen*$^-$, *Ibim*$^-$, *Amoxy*$^-$ *Hico*$^+$, *Epico*$^+$
10	NRRL B-2643 X NRRL B-642	*Ap*$^+$, *Cp*$^+$, *Am-Fluc*$^+$, *pen*$^+$, *Ibim*$^+$, *Amoxy*$^+$, *Ibid*$^+$, *Velo*$^+$, *Duri*$^+$, *Epico*$^-$ *X Ap*$^-$, *Cp*$^-$, *Am*-Fluc$^-$, *pen*$^-$, *Ibim*$^-$, *Amoxy*$^-$, *Ibid*$^-$, *Velo*$^-$, *Duri*$^-$, *Epico*$^+$
11	NRRL B-41228 X NRRL B-642	*Cp*$^+$, *Am-Fluc*$^+$, *pen*$^+$, *Ibim*$^+$, *Amoxy*$^+$, *Epico*$^-$ *X Cp*$^-$, *Am-Fluc*$^-$, *pen*$^-$, *Ibim*$^-$, *Amoxy*$^-$ *Epico*$^+$
12	NRRL B-642 X NRRL B-4375	*Hico+, Epico*$^+$, *Am-Fluc*$^-$, *pen*$^-$ X *Hico-, Epico*$^-$, *Am-Fluc*$^+$, *pen*$^+$
13	NRRL B-642 X NRRL NRS-213	*Hico+, Epico*$^+$, *Amoxy*$^-$ *X Hico-, Epico*$^-$, *Amoxy*$^+$
14	NRRL B-4375 X NRRL NRS-213	*Am-Fluc*$^+$, *pen*$^+$, *Amoxy*$^-$ x *Am-Fluc*$^-$, *pen*$^-$, *Amoxy*$^+$

Uptake experiments:

In the heavy metals uptake test, precultured cells were suspended in 250 ml conical flasks containing 150 ml minimal medium and another using nutrient broth (1.5 g peptone and 0.5 g beef extract per litre) for bacteria and YEPD (0.5 g yeast extract 1.0 g peptone and 1.0 g glucose per litre) medium for yeast, each supplemented with factory effluents and incubated under a static conditions at 30°C for 48 h. Thereafter, the cells were collected by filtration on membrane filter (pore size 0.45 mm). Amounts of metals taken up by the cells were determined according to Nakajima and Sakaguchi (1986).

In the next step, overnight cultures yeast and bacteria were harvested, washed twice with distilled water, and resuspended in 150 ml factory effluents supplemented with 1 mg glucose / 10 ml wastewater, glucose was used as a sole source of carbon . After 48 hours of incubation at 30 C the cells were removed and the filtrate was used to determine the amount of heavy metals using atomic absorption spectrophotometry. Chemistry, Dept., Faculty of science, Hamsoura University.

Metal biosorption:

Metal biosorption experiments were carried out in a 250 ml flask at 30 ∘C without shaking. The flask was filled with 150 ml of previously prepared media containing factory effluents without any dilution.. Each experiment was conducted for 48 h, which was enough time to achieve steady state biosorption. The pH was uncontrolled throughout the experiment.

Dry cell weight:

Dry cell weight measurements were carried out by passing a volume of 50 ml cell culture through a previously weighted Millipore filters (Watman No. 1). Cell pellets were also washed twice with filtered deionized/distilled water to remove non-biomass ash. Filtered and collected cells were dried in an oven set at temperature 110 ∘C and weight for every 24 h until constant weight was obtained.

Determination of heavy metals concentration:

The samples were collected and filtered using Millipore filters of 0.22 _m. The filtrate was collected for heavy metals analysis. The concentration of heavy metals in solution was determined using atomic absorption spectrophotometer (Pantech Instruments, Victoria, Australia) at the Atomic Absorption Unit,Department of

Chemistry, Faculty of Science, Mansoura University. Heavy metals under investigation in this study were as follows ; Lead, Cadmium, Nickel, Platinum, Copper, Cobalt, Iron, Manganese, Molybdenum, Vanadium, strontium, Zinc, Chromium, Antimony, Mercury and Arsenic.

Data evaluation (Langmuir isotherms):

The uptake of the metals (in mg of metal/g of dry cell weight) was calculated according to (Liu *et al*. 2004) using the following formula: $Q = v(C_i-C_f)/m$

Where Q is the metal uptake (mg metal per g biosorbent), v the liquid sample volume (ml), C_i the initial concentration of the metal in the solution (mg/L), C_f the final (equilibrium) concentration of the metal in the solution (mg/L) and m the amount of the added biosorbent on the dry basis (mg).

RESULTS AND DISCUSSION

Testing the tolerance of bacterial strains to factory effluents:

All bacterial and yeast strains used in this study were tested for their ability to grow on minimal and or complete medium containing 100% factory effluents. All of them were well grown on the media containing 100% factory effluents (Figures 1, 2 and 3).It is well recognized that microorganisms have a high affinity for metals and can accumulate both heavy and toxic metals by a variety of mechanisms (Silver,1991). Microorganisms highly effective in sequestering heavy metals include bacteria, fungi, algae, and actinomycetes (Wong, and So. 1993). These have been used to remove metals from polluted industrial and domestic effluent on a large scale. Wong *et al*. 1993 isolated *Pseudomonas putida* II-11, from electroplating effluent, showing that it accumulated Cu(II), up to 6.5% dry weight, from a Cu(II) containing solution. Other investigators have demonstrated the capabilities of several bacteria in removing uranium, Cd, Pb, and other toxic metals from polluted effluents (Silver, S. 1991). Safety aspects of the the organism used for bioremediation are being evaluated. If the organism is determined to be a pathogen, steps to permit biological control (i.e., insertion of a suicide gene) or transferring the genetic properties to a non-pathogen species will be considered.

Attempts for metal removal by bacterial biopolymers could be divided into two approaches. One approach is to express metal-binding biopolymers including metalbinding proteins (MBPs) that are encoded in bacterial genome DNA. For example, it is well-known that activated sludge bacteria can produce metal-binding biopolymers. A marked reduction in the concentration of soluble metals occurs during the activated sludge process (Lawson *et al*., 1984). Bacteria have developed resistant mechanisms to toxic metals to make them innocuous. Bacteria with unique abilities of metal adsorption, accumulation or resistance can be searched for among naturally occurring bacteria. The synthesis of MBPs such as metallothioneins is one of the bacterial defense systems, because the toxicity of heavy metals to bacteria is reduced by complexing metals with bacterial extracellular biopolymers. Many researchers have reported that quantities of heavy metals removed from the solution were enhanced by these characteristics of bacteria (Fukushi *et al*., 1996). Fukushi *et al*. (2001) reported that some amount of copper in growth media stimulated the production of novel proteins that had high enough capacity to bind copper ions. However, since these MBPs have not been sufficiently characterized, molecular biological analysis of MBPs, such as amino acid sequence and structure analysis, would be necessary for further development of the metal removal technology.

All bacterial strains used in this study appeared high tolerance to factory effluents at the concentration of 100%, all of them were grown very well as seen before in a previous Figures (1, 2 and 3).

Genetic marking of different bacterial strains to be used in conjugation:

Bacterial strains were exposed to different antibiotics to determine their resistance and sensitivity. These were used as a genetic markers in conjugation between different bacterial strains harboring the opposite genetic markers. The following Figures 4, 5 and 6 appeared the resistance and sensitivity of bacterial strains to antibiotics used in this study.

Bacterial resistance to antimicrobial agents is a serious problem worldwide, and understanding of the molecular basis of how resistance genes are acquired and transmitted may contribute to the creation of new antimicrobial strategies. One efficient mechanism for the acquisition and dissemination of resistance determinants is their transmission through mobile genetic elements. It has been proposed that promiscuous plasmids, conjugative transposons, and transposons carried by conjugative plasmids are responsible for the horizontal spread of resistance genes throughout bacteria. Recently, naturally occurring gene expression elements called "integrons" have been described as vehicles for the acquisition of resistance genes carried by mobile elements. These structures have also been found to be involved in the genetic reassortment of resistance determinants frequently observed in multiple-antibiotic-resistant bacterial pathogens (Swartz 1997).

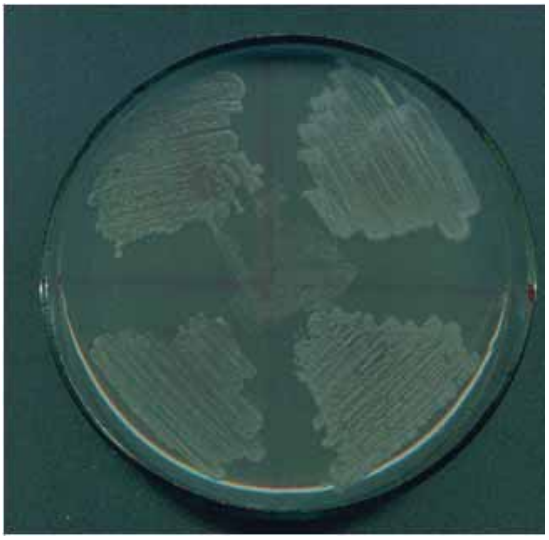

Figure 1: Strains number 5, 6, 7, 8 grown on minimal medium.

Figure 2: Bacterial strains No. 12, 19, 18 and 20 grown on complete medium

One way of grouping plasmids is by their ability to transfer to other bacteria. *Conjugative* plasmids contain so-called *tra-genes*, which perform the complex process of conjugation, the transfer of plasmids to another bacterium. Non-conjugative plasmids are incapable of initiating conjugation, hence they can only be transferred with the assistance of conjugative plasmids, by 'accident'. An intermediate class of plasmids are mobilizable, and carry only a subset of the genes required for transfer. They can 'parasitise' a conjugative plasmid, transferring at high frequency only in its presence. Plasmids are now being used to manipulate DNA and may possibly be a tool for curing many diseases.

The results obtained in this study are in agreement with Onaolapo 1994, who asserted that kanamycin resistance genes are harmless as kanamycin is not used much today. This argument ignores the fact that Kanamycin belongs to a group of antibiotics, within which there is considerable cross resistance. This study indicates that the Kanamycin gene, used as a marker in genetic engineering, confers cross resistance against other clinically important Kanamycin-related antibiotics

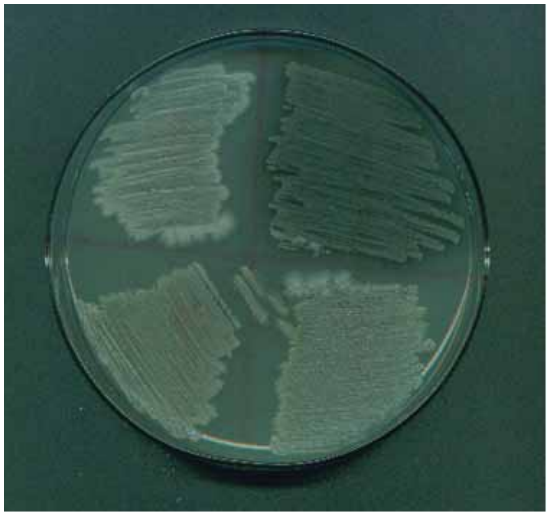

Figure 3: Bacterial strains number 5, 6, 7 and 8 grown on complete medium

Figure 4: Resistance of *Bacillus subtilis* NRRL B-642 to nystatin.

Conjugation between different bacterial strains:

Mobile genetic elements, especially plasmids, and their horizontal transfer play an important role in the evolution and adaptability of prokaryotes (Davison, 1999). Plasmids have been studied extensively as vehicles that promote the dissemination of antibiotic-resistance determinants (Davison, 1999). More recent work has shown that they also facilitate the adaptation of bacteria to environments contaminated with toxic xenobiotics by exchanging genes and entire operons that code for the degradation of these pollutants (Top *et al*., 2002). Detailed analyses of antibiotic-resistance plasmids isolated from clinical and environmental bacteria [reviewed by Davison (1999) revealed that several of these plasmids belong to the IncP-1 incompatibility group and serve as vectors for the horizontal mobility of the encoded accessory genes (Heuer *et al*., 2002). IncP-1 plasmids are very promiscuous, as they are able to self-transfer and be stably maintained in a wide range of Gram-negative bacteria (Thomas & Smith, 1987). The prototype IncP-1 resistance plasmid R751 contains the resistance genes *dhfrIIc* encoding a dihydrofolate reductase for trimethoprim resistance and *qacE* encoding a small exporter protein mediating resistance to quaternary ammonium compounds and disinfectants. Two transposable elements, the cryptic Tn*4321* and the integron-containing Tn*402*/*5090* are inserted downstream of the replication gene *trfA1* and the conjugative transfer gene *traC* of R751, respectively (Thorsted *et al*., 1998).

Bacteria can acquire antibiotic resistance genes from other bacteria in several ways. By undergoing a simple mating process called "conjugation," bacteria can transfer genetic material, including genes encoding resistance to antibiotics (found on plasmids and transposons) from one bacterium to another. Viruses are another mechanism for passing resistance traits between bacteria. The resistance traits from one bacterium are packaged into the head portion of the virus. The virus then injects the resistance traits into any new bacteria it attacks. Bacteria also have the ability to acquire naked, "free" DNA from their environment.

Figure 5: Sensitivity of *Bacillus licheniformis* NRRL NRS-1264 to streptomycin.

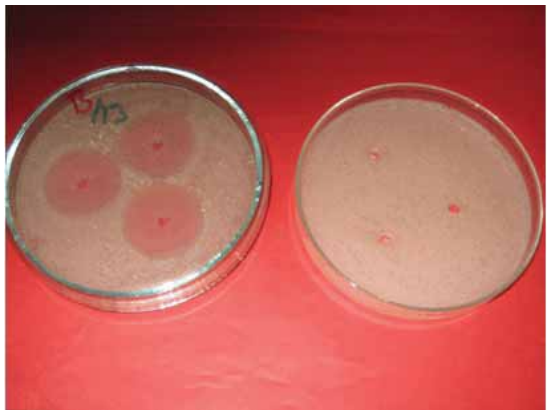

Figure 6: Sensitivity (left) and resistance (right) of *Saccharomyces cerevisiae* NBIMCC 82 grown on YEPD medium against antibiotics.

Any bacteria that acquire resistance genes, whether by spontaneous mutation or genetic exchange as seen in Table 10 with other bacteria, have the ability to resist one or more antibiotics. Because bacteria can collect multiple resistance traits over time, they can become resistant to many different families of antibiotics.

Table 4: Diameter (cm) of inhibition zones due to sensitivity of bacterial strains to different antibiotics and Crystal violet.

Bacterial strains	Diameter (cm) of inhibition zones										
	Erth	*Str*	*Ap*	*Cp*	*Am-Fluc*	*Tc*	*pen*	*Nm*	*Ibim*	*Amoxy*	*flu*
NRRL B-41228	1.2	3.0	1.5	0.0	0.0	2.8	0.0	0.8	0.0	0.0	1.2
NRRL B-2643	2.3	2.5	0.0	0.0	0.0	2.2	0.0	2.7	0.0	0.0	1.2
NRRL NRS-213	4.1	2.3	3.9	2.2	1.8	3.3	4.0	1.8	4.0	0.0	4.3
NRRL B-642	2.8	2.7	2.0	2.5	1.8	1.5	3.2	1.2	1.3	3.0	1.7
NRRL B-571	0.0	3.3	0.0	2.4	0.0	0.7	0.0	1.6	0.0	0.0	0.0
NRRL B-1584	4.2	2.3	0.0	0.0	0.0	1.2	0.0	1.2	2.7	2.0	2.8
NRRL NRS-1264	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
NRRL B-358	4.0	1.5	0.0	0.0	0.0	1.3	0.0	2.0	0.0	0.0	2.2
NRRL B-4375	5.0	2.6	2.6	2.1	0.0	2.0	2.0	4.0	2.2	1.6	3.2
NRRL B-287	2.8	4.4	1.6	3.2	1.0	3.0	3.3	2.8	1.0	1.0	5.7
F-test	0	N.S	0.0	N.S	N.S	N.S	N.S	N.S	N.S	N.S	0
L.S.D 5%	0.44		0.5								0.91
1%	0.6		0.7								1.24

Table 4: Continued.

Bacterial strains	Diameter (cm) of inhibition zones							
	Ibid	*Hico*	*Ery*	*Velo*	*Duri*	*Epico*	*Nyst*	Cry. Vio.
NRRL B-41228	2.2	0.0	2.6	3.5	2.6	0.5	0.0	1.2
NRRL B-2643	0.0	0.0	1.8	0.0	0.0	1.0	0.0	1.2
NRRL NRS-213	3.8	1.2	1.2	3.7	4.8	1.2	0.0	4.3
NRRL B-642	2.8	0.0	2.7	2.5	3.0	0.0	0.0	1.7
NRRL B-571	2.3	0.0	3.0	2.8	2.4	0.0	0.0	3.0
NRRL B-1584	3.5	2.2	1.3	12.0	2.8	2.8	0.0	2.8
NRRL NRS-1264	2.1	0.0	2.8	2.2	1.2	0.0	0.0	3.3
NRRL B-358	2.9	1.2	2.7	3.5	3.2	2.1	0.0	2.2
NRRL B-4375	3.3	2.2	2.2	4.3	3.2	1.8	0.0	3.2
NRRL B-287	1.3	1.0	4.0	2.0	1.2	3.0	0.0	3.3
F-test	0	0	N.S	N.S	N.S	0	N.S	**
L.S.D 5%	0.49	0.28				0.42		1.24
1%	0.67	0.38				0.58		1.70

NS, *, ** = Insignificant, significant at 0.05 and 0.01 propability levels, respectivily.

Table 5: Resistance (+) and sensitivity (-) of bacterial strains to different antibiotics.

Bacterial strains	Resistance (+) and sensitivity (-)										
	Erth	*Str*	*Ap*	*CP*	*Am-Fluc*	*Tc*	*pen*	*Nm*	*Ibim*	*Amoxy*	*flu*
NRRL B-41228	-	-	-	+	+	-	+	-	+	+	-
NRRL B-2643	-	-	-	+	+	-	+	-	+	+	-
NRRL NRS-213	-	-	-	-	-	-	-	-	-	+	-
NRRL B-642	-	-	-	-	-	-	-	-	-	-	-
NRRL B-571	+	-	+	-	+	-	+	-	+	+	+
NRRL B-1584	-	-	+	+	+	-	+	-	-	-	-
NRRL NRS-1264	+	-	+	+	+	+	+	-	+	+	+
NRRL B-358	-	-	+	+	+	-	+	-	+	+	-
NRRL B-4375	-	-	-	-	+	-	-	-	-	-	-
NRRL B-287	-	-	-	-	-	-	-	-	-	-	-

Table 5: Continued.

Bacterial strains	Resistance (+) and sensitivity (-)								
	flu	*Ibid*	*Hico*	*Ery*	*Velo*	*Duri*	*Epico*	*Nyst*	Cy.vio.
NRRL B-41228	-	-	+	-	-	-	-	+	-
NRRL B-2643	-	+	+	-	+	+	-	+	-
NRRL NRS-213	-	-	-	-	-	-	-	+	-
NRRL B-642	-	-	+	-	-	-	+	+	-
NRRL B-571	+	-	+	-	-	-	+	+	-
NRRL B-1584	-	-	-	-	-	-	-	+	-
NRRL NRS-1264	+	-	-	-	-	-	+	+	-
NRRL B-358	-	-	-	-	-	-	-	+	-
NRRL B-4375	-	-	-	-	-	-	-	+	-
NRRL B-287	-	-	-	-	-	-	-	+	-

Table 6: Resistance (+) and sensitivity (-) of *Saccharomyces cerevisiae* to different antibiotics.

Antibiotics	*Saccharomyces cerevisiae* strains						
	NRRL Y-12632	NRRL Y-11562	NBIMCC 82	NRRL Y-12619	NRRL Y-136	NRRL Y-137	NRRL Y-1370
Streptomycin	-	+	+	+	+	+	+
Ampicillin	+	+	-	+	+	+	+
Ibiamox	+	-	-	+	+	+	+
Velosef	-	-	-	+	+	+	+
Flucamox	+	-	-	+	+	+	+
Neomycin sulphate	+	-	-	+	+	+	+
Erthyromycin	-	-	-	+	+	+	+
Ibidroxil	-	-	-	+	+	+	+
Ceporex	-	-	-	+	+	+	+
Rifampicillin	-	-	-	+	+	+	+
Tetracycline	-	-	-	+	+	+	+
Chloramphenicol	-	-	-	+	+	+	+
Gentamycin	-	-	-	+	+	+	+
Pencillin	+	+	+	+	+	+	+

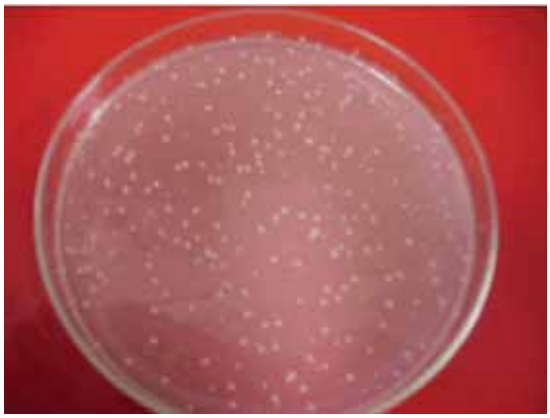

Figure 7: Mating No. 11 between bacterial strains ; NRRL B-41228 X NRRL B-642.

Testing the tolerance of bacterial transconjugants to factory effluents : As shown from Figures No. 8 and 9 that bacterial transconjugants and their parents were grown on nutrient broth containing factory effluents and the growth of most transcongugants may be efficient than the parental strains.

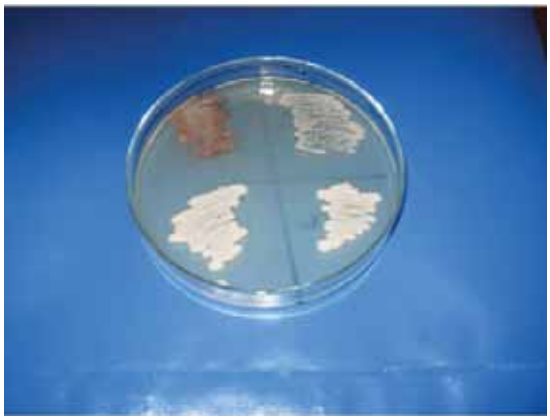

Figure 8: Illustrated the growth of two transconjugants (below) compared to their parental strains (above) (mating No. 6) growing on nutrient agar broth (0.5 % g peptone, 0.3 % g beef extract lab lemco powder) supplemented with factory effluents.

Figure 9: Illustrated the growth of two transconjugants (below) compared to their parental strains (above) (mating No. 8) growing on nutrient agar broth (0.5 % g peptone, 0.3 % g beef extract lab lemco powder) supplemented with factory effluents.

Bacterial conjugation was carried out in this proposal to obtain a recombinants may having higher uptake of pollutants, it is the transfer of genetic material between bacteria through direct cell-to-cell contact (Holmes and Jobling 1996). Discovered in 1946 by Joshua Lederberg and Edward Tatum conjugation as a mechanism of horizontal gene transfer-as are transformation and transduction - although these mechanisms do not involve cell-to-cell contact (*Griffiths et al 1999)*. Bacterial conjugation shown in Figure 7 is often incorrectly regarded as the bacterial equivalent of sexual reproduction or mating. It is not actually sexual, as it does not involve the fusing of gametes and the creation of a zygote, nor is there equal exchange of genetic material. It is merely the transfer of genetic information from a donor cell to a recipient. In order to perform conjugation, one of the bacteria, the *donor*, must play host to a conjugative or mobilizable genetic element, most often a conjugative or mobilizable plasmid or transposon (Ryan and Ray 2004). Most conjugative plasmids have systems ensuring that the recipien*t* cell does not already contain a similar element.

The genetic information transferred is often beneficial to the recipient cell. Benefits may include ; antibiotic resistance, heavy metals uptake, other xenobiotic tolerance, or the ability to utilize a new metabolite (Holmes and Jobling 1996). Such beneficial plasmids may be considered bacterial endosymbionts. Some conjugative elements may also be viewed as genetic parasites on the bacterium, and conjugation as a mechanism was evolved by the mobile element to spread itself into new hosts. Five single colonies from that appeared on the figures presented herein were picked up and transferring to a nutrient agar slant, each colony may significantly differ than other ones on the same plate resulted from the same mating in harboring genetic background. This because these are recombinations, each recombination resulted from the mating between two bacterial cells.

Uptake of heavy metals by bacterial cells using minimal medium supplemented with wastewaters:

Microbial biomass can be used to decontaminate metal bearing wastewaters as well as to concentrate metals. The nature of biological surfaces is such that different functional groups form complexes with metal ions (Huang *et al*., 1994), resulting in chemical complexation as an uptake mechanism. Metal uptake can also be due to physical sorption or bioaccumulation. The concentrations of these metals need to be reduced to meet ever-changing legislative standards. According to the World Health Organization (WHO, 1984), the metals of most immediate concern are cadmium, chromium, cobalt, copper, lead, nickel, mercury and zinc. The presence of such metals (>5 g cm3, Mahavi, 2005) in aquatic environments cause severe damage to aquatic life, killing microorganisms during biological water purification process. Moreover, these metals have exacting consequences on humans such as brain damage, reproductive failures, nervous system failures, tumour formation, etc (Mahavi, 2005). Biological methods such as biosorption or bioaccumulation strategies for the removal of metals ions may provide an attractive alternative to existing technologies (Preetha and Viuthagiri, 2005). Brierley *et al*. (1986) has suggested that a metal loading capacity greater than 15% of biomass could be used as an economic threshold for practical applications of biosorption as compared with alternative techniques.

The results reported in Table 11 provided as a source of information about the efficiency of bacterial strains and their transconjugants on heavy metals uptake from wastewaters resulted from fertilizer factories. It is intended as a the importance of biosorption of heavy metal contaminants in fertilizer wastes. It also address the introduction of these materials into plants using in biological control of pollutants. The focus of these results is on their application to biocontrol of heavy metal pollutants in agricultural lands, ground water and drinking water. In addition, some information can be obtained from wastewater treated by bacterial strains and their transconjugants on the use of recycled industrial by-products as fertilizers and also in irrigation ; however, an in-depth investigation of these recycling practices was beyond the scope of this study.

These results focus on the efficieny of bacterial transconjugants in heavy metals uptake from wastewaters resulted from fertilizer fatory, as shown herein Tr1 resulted from the mating between NRRL B-571 X NRRL B-1584 and NRRL B-1584 X NRRL NRS-213 was more efficient in biosorption of Cu, Co, Fe, Cd and Pb than the parental strains. However Tr2 resulted from the matings between NRRL NRS-1264 X NRRL B-2643 and NRRL B-642 X NRRL B-4375 appeared the same trend in biosorption efficiency of Cu, Co, Fe, Cd and Pb than the parental strains. On the other hand, both Tr1 and Tr2 resulted from the mating between NRRL B-41228 X NRRL B-642 were more efficient in biosorption efficiency of Cu, Co, Fe, Cd and Pb than their parental strains. Strong biosorbent behaviour of certain bacterial transconjugants towards metallic ions is a function of the chemical make-up of the microbial cells. These biosorption experiments have focused attention on waste materials, which are by-products or the waste materials from large-scale industrial operations. The results showed that the transconjugant biomass resulted from some mating done in this study had not a net preference for heavy metals uptake. The mechanism of biosorption is complex, mainly ion exchange, chelation, adsorption by physical forces, entrapment in inter and intrafibrilliar capillaries and spaces of the structural polysaccharide network as a result of the concentration gradient and diffusion through cell walls and membranes.

The results obtained in this study are in agreement with those obtained by Norton *et al*. 2003, who used dewatered waste activated sludge from a sewage treatment plant for the biosorption of zinc from aqueous solutions. The adsorption capacity was determined to be 0.564 mM/g of biosolids. The use of biosolids for zinc adsorption was favourable compared to the bioadsorption rate of 0.299 mM/g by the seaweed Durvillea potatorum . Keskinkan *et al*. 2003 studied the adsorption characteristics of copper, zinc and lead on submerged aquatic plant Myriophyllum spicatum. The adsorption capacities were 46.69 mg/g for lead, 15.59 mg/g for zinc and 10.37 mg/g for copper.

The application of biosorption in environmental treatment has become a significant research area in the past ten years. Heavy metal ions are reported as priority pollutants, due to their mobility in natural water ecosystems and due to their toxicity (Volesky and Holan, 1995). The discharge of heavy metals into surface waters has become a matter of concern in Pakistan over the last two decades. These contaminants are introduced into surface waters through various industrial operations. The pollutants of concern include lead, chromium, zinc, and copper. Heavy metals such as zinc, lead and chromium have number of applications in basic engineering works, paper and pulp industries, leather tanning, petrochemicals fertilizers, etc. The hexavalent and trivalent chromium is often present in electroplating wastewater (Kratochvil *et al*. 1998). Other sources of chromium pollution are leather tanning, textile, metal processing, paint and pigments, dyeing and steel fabrication. Lead is used as industrial raw material in the manufacture of storage batteries, pigments, leaded glass, fuels, photographic materials, matches and explosives (Raji and Anirudhan, 1997).

Lead and chromium are toxic metal contaminants in water. According to Pakistan standards the maximum discharge limits for lead and chromium in wastewater are respectively 0.5 mg l^{-1} and 1.0 mg l^{-1}. Maximum limit in drinking water is 0.05 mg l^{1} for both metals. In fact there is no safe level of these metals in drinking water and even a very dilute content can cause adverse health effects. Lead is toxic to living organisms and if released into the environment can bio accumulate and enter the food chain. Lead is known to cause mental retardation, reduces haemoglobin production necessary for oxygen transport and it interferes with normal cellular metabolism. Lead has damaging effects on body nervous system. It reduces I.Q level in children. Strong exposure of hexavalent chromium causes cancer in the digestive tract and lungs and may cause gastric pain, nausea, vomiting, severe diarrhoea, and haemorrhage (Mohanty *et al*. 2005).

On the other hand, both transconjugants (Tr 1 and Tr 2) resulted from the following matings ; NRRL B-1584 X NRRL B-642, NRRL B-1584 X NRRL NRS-213, NRRL NRS-1264 X NRRL B-2643, NRRL B-41228 X NRRL B-642, NRRL B-4375 X NRRL NRS-213, as well as, Tr1 resulted from NRRL B-2643 X NRRL B-642 and Tr2 resulted from NRRL B-642 X NRRL B-4375, were more efficient than their parental strains in the uptake of ; Hg, As, Mn, Pt and Mo.

In addition, the same trend was also appeared in Zn, Cr, V, Sr, Sb and Ni uptake by both Tr1 and Tr2 resulted from the following matings ; NRRL B-1584 X NRRL B-642, NRRL B-1584 X NRRL NRS-213, NRRL NRS-1264 X NRRL B-2643, NRRL B-41228 X NRRL B-642, NRRL B-4375 X NRRL NRS-213, as well as, Tr1 resulted from NRRL B-2643 X NRRL B-642 and Tr2 resulted from NRRL B-642 X NRRL B-4375. This indicated that heavy metal contamination of soils and waters poses a major environmental and human health problem. Polluted water may be recuperated by various strategies of which on -site bioremediation is a preferable and low-cost strategy with a low impact on the ecosystem. Efficient transconjugant associations have therefore often been considered to be a major importance in bioremediation. This are in agreement with Barker *et al*. 1998, who are found that intracellular U uptake by *P. membranacea,* localized mainly within cellular organelles known as ‘‘concentric bodies’’. The functional role of these proteinaceous organelles is presently unclear, but they appear to strongly accumulate U, Os, Pb, and possibly other heavy metals (Peveling *et al*. 1985). these organelles strongly accumulate transition and heavy elements suggests that they may assist in cellular storage of micronutrients, and may therefore contain metallophilic compounds similar to siderophores. The identity(ies) of observed U-bearing phase(s) formed within *P. membranacea* concentric bodies, on cell walls and in extracellular exudates remains unclear. Do analogous phases form after the uptake of other heavy elements on cell walls, in extracellular gels, or in concentric bodies? How does this process vary among lichen taxa ? At present it is not possible to directly compare the bioaccumulative properties of a wide variety of lichen, fungal and microbial taxa with respect to a broad range of metals, because the required experimental data are limited or unavailable. Such data could greatly facilitate studies of heavy metal and radionuclide speciation and transport at or near the Earth’s surface.

The resultes obtained in this study compared with traditional approaches for removing or recovering metals, such as precipitation, oxidation/reduction, ion exchange, filtration, electrochemical processes, membrane separations, and evaporation, all exhibit several disadvantages, such as high cost, incomplete removal, low selectivity, high energy consumption, and generation of toxic slurries that are difficult to be eliminated (Celaya *et al*, 2000). Therefore, much attention has been paid to the removal of metal ions by microorganisms and their

transconjugants due to its potential applications in environmental protection, and recovery of toxic or strategic heavy metals (Chang *et al*, 1997). Certain types of microbial biomass as we seen in this proposal are considered to retain relatively high quantities of metals by means of passive process known as biosorption. The process is relatively fast and the fact that it is a surface phenomenon facilitates the removal of metal ions from solutions and the subsequent application of the material as biosorbent (Celaya *et al*, 2000). Biosorption which we are seen in this proposal is either metabolism independent, such as physical or chemical sorption onto the microbial cell walls, or metabolism associated, such as transport, internal compartmentalization, and extracellular precipitation by metabolites (Gadd, 1988). In addition, an important aspect of biosorption is that it can be carried out either with metabolically active or inactive cells. Many microorganisms have been intensively examined for their abilities to be applied in biosorption of heavy metals, such as bacteria, fungi, yeast, and algae (Chen *et al*, 2000). Both chemical pretreatments, such as contacting cells with acids, alkali, and organic compounds (Galun 1983), and physical pretreatments, such as heat treatment, autoclaving, freeze drying, and boiling [Huang *et al* 1988], showed enhancement in metal biosorption by microorganisms.

As shown from the results presented in Table 12 which leading to report that this study describe the potential of bacterial transconjugants and *Saccharomyces cerevisiae* strains and their hybrids in biosorbing of heavy metals from chemical fertilizer manufacturing industrial effluent. The results indicating that many of bacterial strains and their transconjugants appeared higher percentage rates of biosorping activity. This recovery of heavy metals was increased above of 50 % from the initial concentration. Some of bacterial transconjugants appeared recovery percentage more than the mid parents, however others giving recovery percentage more than the better parent. In addition, both bacterial strains and their transconjugants giving recovery of heavy metals more than 50 %. Heavy metals released by a number of industrial processes are major pollutants in marine, ground, industrial and even treated wastewaters (Martins *et al*. 2006). Lead is widely used in many industrial applications such as storage battery manufacturing, printing, pigments, fuels, photographic materials and explosive manufacturing. Lead is highly toxic as its presence in drinking water above the permissible limit (5 ng/mL) causes adverse health effects such as anemia, encephalopathy, hepatitis and nephritic syndrome (Martins *et al*. 2006).

Table 11: Heavy meatals uptak (mg metai par g biosrbent) by parental strains of bacteria and their transconjugants growing on minimal medium supplemented with wastewaters.

Biocontrol agents		ppm				
		Cu	Co	Fe	Cd	Pb
NRRL B-571 X NRRL B-1584	571	793	724	13793	862	758
	1584	631	368	7017	368	736
	M. P.	712	546	10405	615	747
	Tr1	1085	1200	22685	1028	1771
	Tr2	403	456	7070	403	543
NRRL B-571 X NRRL B-2643	571	793	724	13793	862	758
	2643	549	167	6061	244	76
	M. P.	671	445	9927	553	417
	Tr1	371	268	4123	185	432
	Tr2	351	120	4362	241	307
NRRL B-571 X NRRL B-41228	571	793	724	13793	862	758
	41228	514	495	7561	533	666
	M. P.	653	609	10677	697	712
	Tr1	234	71	4153	224	428
	Tr2	675	525	10000	450	775
NRRL B-1584 X NRRL B-642	1584	631	368	7017	368	736
	642	301	198	2830	188	179
	M. P.	466	283	4923	278	457
	Tr1	1531	1319	17319	638	1191
	Tr2	808	584	9146	494	764
NRRL B-1584 X NRRL NRS-213	1584	631	368	7017	368	736
	213	653	428	8163	428	387
	M. P.	642	398	7590	398	561
	Tr1	1600	1050	20150	1100	1250
	Tr2	677	225	13000	677	1161
NRRL NRS-1264 X NRRL B-2643	1264	326	456	8630	456	391
	2643	549	167	6061	244	76
	M. P.	437	311	7345	350	233

Table 11: Continued.

	Tr1	612	193	12806	483	903
	Tr2	1600	1300	20150	1300	1550
NRRL B-358 X NRRL B-642	358	590	180	6557	245	590
	642	301	198	2830	188	179
	Tr1	426	280	5373	266	520
	Tr2	39	33	839	43	75
NRRL B-2643 X NRRL B-642	2643	549	167	6061	244	76
	642	301	198	2830	188	179
	M. P.	425	182	4445	216	127
	Tr1	225	258	3166	175	325
	Tr2	298	168	5220	194	324
NRRL B-41228 X NRRL B-642	41228	514	495	7561	533	666
	642	301	198	2830	188	179
	M. P.	407	346	5195	360	422
	Tr1	10800	10800	160000	8800	8800
	Tr2	2750	5250	100000	3750	7750
NRRL B-642 X NRRL B-4375	642	301	198	2830	188	179
	4375	335	167	4188	188	293
	M.P.	318	182	3509	188	236
	Tr1	108	206	3970	177	108
	Tr2	490	290	7272	363	400
NRRL B-642 X NRRL NRS-213	642	301	198	2830	188	179
	213	653	428	8163	428	387
	M. P.	477	313	5496	308	283
	Tr1	410	205	5038	230	25
	Tr2	1250	1750	33333	1833	3250
NRRL B-4375 X NRRL NRS-213	4375	335	167	4188	188	293
	213	653	428	8163	428	387
	M.P.	494	297	6175	308	340
	Tr1	597	675	10311	597	1246
	Tr2	640	420	7860	320	760

Table 11: continued

Biocontrol agents		ppb		ppm		
		Hg	As	Mn	Pt	Mo
NRRL B-571 X NRRL B-1584)	571	124	158	1275	1482	4482
	1584	93	80	350	526	1754
	M.P.	108	119	812	1004	3118
	Tr1	11	91	2457	1714	7428
	Tr2	82	80	877	298	2578
NRRL B-571 X NRRL B-2643)	571	124	158	1275	1482	4482
	2643	35	9	595	870	2244
	M.P.	79	83	935	1176	3363
	Tr1	47	37	103	309	1340
	Tr2	50	39	219	692	1615
NRRL B-571 X NRRL B-41228)	571	124	158	1275	1482	4 4 8 2
	41228	81	30	761	704	1904
	M.P.	102	94	1018	1093	3193
	Tr1	54	36.7	102	173.5	1020.4
	Tr2	105	65	425	425	3250
NRRL B-1584 X NRRL B-642)	1584	93	80	350	526	1754
	642	30	24	405	660	1698
	M.P.	61	52	377	593	1726
	Tr1	170	153	1829	3276	5 5 3 1
	Tr2	103	80	1056	1528	2471
NRRL B-1584 X NRRL NRS-213	1584	93	80	350	526	1754
	213	65	32	877	1163	2244
	M.P.	79	56	613	844	1999
	Tr1	165	130	2000	3500	6500
	Tr2	151	116	1064	1838	3548
NRRL NRS-1264 X NRRL B-2643	1264	76	78	1087	1369	2826
	2643	35	9	595	870	2244
	M.P.	55	43	841	1119	2535
	Tr1	125	116	1387	2483	4741
	Tr2	210	80	2500	3150	7350
NRRL B-358 X NRRL B-642	358	85	59	442	1262	2131
	642	30	24	405	660	1698

Table 11: Continued.

	M.P.	57	41	423	961	1914
	Tr1	66	61	626	1026	3000
	Tr2	104	75	208	479	1666
NRRL B-2643 X NRRL B-642)	2643	35	9	595	870	2244
	642	30	24	405	660	1698
	M.P.	32	16	500	765	1971
	Tr1	416	300	3916	4750	9166
	Tr2	33	33	714	740	1428
NRRL B-41228 X NRRL B-642	41228	81	30	761	704	1904
	642	30	24	405	660	1698
	M.P.	55	27	583	682	1801
	Tr1	1320	1040	17200	22800	65200
	Tr2	1125	650	10000	7500	15000
NRRL B-642 X NRRL B-4375)	642	30	24	405	660	1698
	4375	51	27	104	387	1151
	M.P.	40	25	254	523	1424
	Tr1	24	15	394	492	1448
	Tr2	80	47	181	781	2672
NRRL B-642 X NRRL NRS-213	642	30	24	405	660	1698
	213	65	32	877	1163	2244
	M.P.	47	28	641	911	1971
	Tr1	19	7	474	897	2089
	Tr2	391	216	3916	3083	8333
NRRL B-4375 X NRRL NRS-213	4375	51	27	104	387	1151
	213	65	32	877	1163	2244
	M.P.	58	29	490	775	1697
	Tr1	137	93	1116	1298	3376
	Tr2	108	72	660	1660	2600

Table 11: continued.

Biocontrol agents		ppb					
		Zn	Cr	V	Sr	Sb	Ni
NRRL B-571 X NRRL B-1584	571	4034	6517	5862	6379	9310	4551
	1584	1754	1578	1929	2280	4035	1929
	M.P.	2894	4047	3895	4329	6672	3240
	Tr1	7028	4571	4000	4571	12114	7142
	Tr2	2280	2631	2280	2807	4193	2315
NRRL B-571 X NRRL B-2643	571	4034	6517	5862	6379	9310	4551
	2643	1816	2290	1832	2626	4106	2274
	M.P.	2925	4403	3847	4502	6708	3412
	Tr1	927	1546	1443	1948	1958	1628
	Tr2	1098	2230	1978	1340	1868	1494
NRRL B-571 X NRRL B-41228	571	4034	6517	5862	6379	9310	4551
	41228	2285	3314	3238	3371	5123	2590
	Tr1	918	1775	1938	816	2244	1500
	Tr2	2425	4725	4500	3725	5450	3300
NRRL B-1584 X NRRL B-642	1584	1754	1578	1929	2280	4035	1929
	642	1160	1783	1698	1811	1132	1518
	M.P.	1457	1680	1813	2045	2583	1723
	Tr1	5234	4255	5531	7063	10170	5914
	Tr2	2853	2696	2921	3775	5348	3033
NRRL B-1584 X NRRL NRS-213	1584	1754	1578	1929	2280	4035	1929
	213	2510	3061	3469	3632	5428	2836
	Tr1	6000	4500	5500	8450	11800	6700
	Tr2	3645	3871	3225	5161	7580	4290
NRRL NRS-1264 X NRRL B-2643	1264	2826	4413	2608	3782	5804	3087
	2643	1816	2290	1832	2626	4106	2274
	M.P.	2321	3351	2220	3204	4955	2680
	Tr1	3967	3225	4193	5451	8677	4483
	Tr2	6500	6500	9000	9000	13550	5500
NRRL B-358 X NRRL B-642	358	1754	2852	2786	2377	3803	2262
	642	1160	1783	1698	1811	1132	1518
	M.P.	1457	2317	2242	2094	2467	1890
	Tr1	1693	1866	1600	2093	3146	1986
	Tr2	1875	3937	3750	3062	4541	3020
NRRL B-2643 X NRRL B-642	2643	1816	2290	1832	2626	4106	2274
	642	1160	1783	1698	1811	1132	1518

Table 11: Continued.

	M.P.	1488	2036	1765	2218	2619	1896
	Tr1	10583	16916	12500	14166	20833	9083
	Tr2	1753	3194	2987	1818	2818	1779
NRRL B-41228 X NRRL B-642	41228	2285	3314	3238	3371	5123	2590
	642	1160	1783	1698	1811	1132	1518
	M.P.	1722	2548	2468	2591	3127	2054
	Tr1	49200	44000	56000	59200	91600	56800
	Tr2	30000	50750	47500	37250	54250	34500
NRRL B-642 X NRRL B-4375	642	1160	1783	1698	1811	1132	1518
	4375	942	837	1466	1047	2387	1361
	M.P.	1051	1310	1582	1429	1759	1439
	Tr1	1182	1182	1477	1379	2236	1399
	Tr2	1636	2363	2545	1636	3981	2181
NRRL B-642 X NRRL NRS-213	642	1160	1783	1698	1811	1132	1518
	213	2510	3061	3469	3632	5428	2836
	M.P.	1835	2422	2583	2721	3280	2177
	Tr1	1500	1666	1923	2115	3397	1769
	Tr2	10583	18083	17500	12666	19000	11333
NRRL B-4375 X NRRL NRS-213	4375	942	837	1466	1047	2387	1361
	213	2510	3061	346	3632	5428	2836
	M.P.	1726	1949	906	2339	3907	2098
	Tr1	3194	4155	4155	4727	6961	3039
	Tr2	2260	4060	3400	3340	5300	3040

Tr = Transconjugants , M.P. = Mid parents

Conventionally, the following methods are employed for the removal of heavy metals from effluents: oxidation and reduction, precipitation, filtration, electrochemical treatment and evaporation (Baik *et al*. 2002). Physicochemical methods have several disadvantages such as unpredictable metal ion removal, high reagent requirements and formation of sludge and its disposal, in addition to high installation and operational costs (Deepa *et al*. 2006). Search for newer treatment technologies for removal of toxic metals from wastewaters as in use in this proposal has directed attention to biosorption (Veglio and Beolchini, 1997). It can be considered as an alternative technology for industrial wastewater treatment (Martins *et al*. 2006). The phenomenon of biosorption is defined as a metabolism independent adsorption of pollutants based on the partition process on a microbial biomass (Ringot *et al*. 2006). It is a passive non-metabolically - mediated process of metal binding by biosorbent (Davis *et al*. 2003). Bacteria, yeasts, fungi and algae have been used as biosorbents of heavy metals. Among these, yeasts are known to be selective metal biosorbents as compared to fungi, actinomycetes and bacteria (Zouboulis *et al*. 1999). Metal uptake by biosorption is reported to occur through interactions with functional groups native to the biomass cell wall (Goksungur *et al*. 2005). It is metabolism independent and proceeds rapidly within several minutes by any one or a combination of the following metal binding mechanisms: coordination, complexation, ion exchange, physical adsorption (*e.g.* electrostatic) or inorganic microprecipitation. The mechanism of metal biosorption is complicated and not fully understood.

Table 12: Percentage of heavy metals uptake by bacterial strains and their transconjugants growing in MM suplemented with factory effluents.

Mating	Parental strains and resulted transconjugants	Heavy metals uptake %				
		Pb	Cd	Fe	Co	Cu
NRRL B-571(P1) X NRRL B-1584) (P2)	P1	34	76	98	58	58
	P2	65	64	98	58	90
	M.P	49.5	70	98	58	74
	Tr1	48	55	97	58	48
	Tr2	48	70	98	72	58
NRRL B-571 (P1) X NRRL B-2643 (P2)	P1	34	76	98	58	58
	P2	8	48	97	31	90
	M.P	21	62	97.5	44.5	74
	Tr1	65	55	98	72	90
	Tr2	43	67	97	31	80
NRRL B-571 (P1) X NRRL B-41228(P2)	P1	34	76	98	58	58
	P2	54	85	97	72	68
	M.P	44	80.5	97.5	65	63
	Tr1	65	67	99	19	58
	Tr2	48	55	98	58	68
NRRL B-1584(P1) X NRRL B-642(P2)	P1	65	64	98	58	90

Table 12: Continued.

	P2	29	61	73	58	80
	M.P	47	62.5	85.5	58	85
	Tr1	43	45	99	86	90
	Tr2	52	67	99	72	90
NRRL B-1584 X NRRL NRS-213	P1	65	64	98	58	90
	P2	29	64	98	58	80
	M.P	47	64	98	58	85
	Tr1	38	67	98	58	80
	Tr2	55	64	98	19	53
NRRL NRS-1264 X NRRL B-2643	P1	28	64	97	58	38
	P2	8	48	97	31	90
	M.P	18	56	97	44.5	64
	Tr1	43	45	97	17	48
	Tr2	48	79	98	72	80
NRRL B-358 X NRRL B-642	P1	55	45	98	31	90
	P2	29	61	73	58	80
	M.P	42	53	86	45	85
	Tr1	48	55	97	58	48
	Tr2	55	64	98	44	48
NRRL B-2643 X NRRL B-642)	P1	8	48	97	31	90
	P2	29	61	73	58	80
	M.P	18.5	54.5	85	44.5	85
	Tr1	60	64	93	86	68
	Tr2	38	45	98	36	58
NRRL B-41228 X NRRL B-642	P1	54	85	97	72	68
	P2	29	61	73	58	80
	M.P	41.5	73	85	65	74
	Tr1	34	67	98	75	68
	Tr2	48	45	98	58	28
NRRL B-642 X NRRL B-4375)	P1	29	61	73	58	80
	P2	43	55	98	44	80
	M.P	36	58	85.5	51	80
	Tr1	17	55	98	58	28
	Tr2	34	61	98	44	68
NRRL B-642 X NRRL NRS-213	P1	29	61	73	58	80
	P2	29	64	98	58	80
	M.P	29	62.5	85.5	58	80
	Tr1	3	55	96	44	80
	Tr2	60	67	98	58	38
NRRL B-4375 X NRRL NRS-213	P1	43	55	98	44	80
	P2	29	64	98	58	80
	M.P	36	59.5	98	51	80
	Tr1	74	70	97	72	58
	Tr2	58	48	96	58	80
Micrococcus luteus(NRRL B-287)		22	45	96	72	68

Table 12: continued

Mating	Parental strains and resulted transconjugants	Heavy metals uptake %				
		Mo	Pt	Mn	As	Hg
NRRL B-571(P1) X NRRL B-1584) (P2)	P1	57	39	62	70	53
	P2	43	27	33	70	78
	M.P	50	33	48	70	66
	Tr1	57	27	72	24	3
	Tr2	64	15	83	70	69
NRRL B-571 (P1) X NRRL B-2643 (P2)	P1	57	39	62	70	53
	P2	64	52	65	9	34
	M.P	60.5	45.5	63.5	39.5	43.5
	Tr1	57	27	17	55	68
	Tr2	64	57	33	55	68
RRL B-571 (P1) X NRRL B-41228(P2)	P1	57	39	62	70	53
	P2	43	34	67	24	63
	M.P	50	36.5	64.5	47	58
	Tr1	43	15	17	55	78
	Tr2	57	15	28	39	62
NRRL B-1584(P1) X NRRL B-642(P2)	P1	43	27	33	70	78
	P2	78	64	72	39	47
	M.P	60.5	45.5	52.5	54.5	62.5

Table 12: Continued.

Tr1	57	70	72	55	59	
	Tr2	48	62	78	55	68
NRRL B-1584 X NRRL NRS-213	P1	43	27	33	70	78
	P2	48	52	72	24	47
	M.P	45.5	39.5	52.5	47	62.5
	Tr1	57	64	67	39	49
	Tr2	48	52	55	55	69
NRRL NRS-1264 X NRRL B-2643	P1	57	57	83	55	51
	P2	64	52	65	9	34
	M.P	60.5	54.5	74	32	42.5
	Tr1	64	70	72	55	57
	Tr2	64	57	83	24	62
NRRL B-358 X NRRL B-642	P1	57	70	45	55	76
	P2	78	64	72	39	47
	M.P	67.5	67	58.5	47	61.5
	Tr1	98	70	78	70	74
	Tr2	35	21	17	55	74
NRRL B-2643 X NRRL B-642)	P1	64	52	65	9	34
	P2	78	64	72	39	47
	M.P	71	58	68.5	24	40.5
	Tr1	48	52	78	55	74
	Tr2	48	52	92	39	38
NRRL B-41228 X NRRL B-642	P1	43	34	67	24	63
	P2	78	64	72	39	47
	M.P	60.5	49	69.5	31.5	55
	Tr1	71	52	72	39	49
	Tr2	26	27	67	39	66
NRRL B-642 X NRRL B-4375)	P1	78	64	72	39	47
	P2	48	34	17	39	72
	M.P	63	49	44.5	39	59.5
	Tr1	64	45	67	24	37
	Tr2	64	39	17	39	65
NRRL B-642 X NRRL NRS-213)	P1	78	64	72	39	47
	P2	48	52	72	24	47
	M.P	63	58	72	31.5	47
	Tr1	71	64	62	9	22
	Tr2	43	34	78	39	69
NRRL B-4375 X NRRL NRS-213		P1	48	34	17	3972
	P2	48	52	72	24	47
	M.P	48	43	44.5	31.5	59.5
	Tr1	57	45	72	55	78
	Tr2	57	75	55	55	79
Micrococcus luteus(NRRL B-287)	Wild type	48	39	78	24	3

Table 12: continued

Mating	Parental strains and resulted transconjugants	Heavy metals uptake %					
		Ni	Sb	Sr	V	Cr	Zn
NRRL B-571(P1) X NRRL B-1584) (P2)	P1	73	96	88	61	73	73
	P2	61	82	62	35	35	61
	M.P	67	89	75	48	54	67
	Tr1	69	76	38	17	31	69
	Tr2	73	85	76	43	58	73
NRRL B-571 (P1) X NRRL B-2643 (P2)	P1	73	96	88	61	73	73
	P2	83	96	82	39	58	83
	M.P	78	96	85	50	65.5	78
	Tr1	88	68	90	48	58	88
	Tr2	76	61	58	65	78	76
NRRL B-571 (P1) X NRRL B-41228(P2)	P1	73	96	88	61	73	73
	P2	76	96	84	61	67	76
	M.P	74.5	96	86	61	70	74.5
	Tr1	82	79	38	70	67	82
	Tr2	73	78	71	65	73	73
NRRL B-1584(P1) X NRRL B-642(P2)	P1	61	82	62	35	35	61
	P2	89	43	91	65	73	89
	M.P	75	62.5	76.5	50	54	75
	Tr1	77	85	79	43	38	77
	Tr2	75	85	80	43	46	75

Table 12: Continued.

NRRL B-1584 X NRRL NRS-213	P1	61	82	62	35	35	61
	P2	77	95	85	61	58	77
	M.P	69	88.5	73.5	48	46.5	69
	Tr1	74	84	80	35	35	74
	Tr2	74	84	76	30	46	74
NRRL NRS-1264 X NRRL B-2643	P1	79	95	83	39	78	79
	P2	83	96	82	39	58	83
	M.P	81	95.5	82.5	39	68	81
	Tr1	77	96	80	43	38	77
	Tr2	61	97	86	65	50	61
NRRL B-358 X NRRL B-642	P1	77	83	69	61	67	77
	P2	89	43	91	65	73	89
	M.P	83	63	80	63	70	83
	Tr1	83	84	75	39	54	83
	Tr2	81	78	70	65	73	81
NRRL B-2643 X NRRL B-642)	P1	83	96	82	39	58	83
	P2	89	43	91	65	73	89
	M.P	86	69.5	86.5	52	65.5	86
	Tr1	61	89	81	52	78	61
	Tr2	76	78	67	87	95	76
NRRL B-41228 X NRRL B-642	P1	76	96	84	61	67	76
	P2	89	43	91	65	73	89
	M.P	82.5	69.5	87.5	63	70	82.5
	Tr1	79	82	70	48	42	79
	Tr2	77	78	71	70	78	77
NRRL B-642 X NRRL B-4375)	P1	89	43	91	65	73	89
	P2	72	81	48	48	31	72
	M.P	80.5	62	69.5	56.5	52	80.5
	Tr1	79	81	67	52	46	79
	Tr2	67	78	43	48	50	67
NRRL B-642 X NRRL NRS-213)	P1	89	43	91	65	73	89
	P2	77	95	85	61	58	77
	M.P	83	69	88	63	65.5	83
	Tr1	77	95	79	52	50	77
	Tr2	76	81	72	78	83	76
NRRL B-4375 X NRRL NRS-213	P1	72	81	48	48	31	72
	P2	77	95	85	61	58	77
	M.P	74.5	88	66.5	54.5	44.5	74.5
	Tr1	65	96	87	57	62	65
	Tr2	84	95	80	61	78	84
Micrococcus luteus(NRRL B-287)	Wild type	71	76	48	17	35	70

In conclusion, biosorption of heavy metals from aqueous solutions is a relatively new technology for the treatment of industrial wastewater. Biosorbent materials can be used for the effective removal and recovery of heavy metal ions from wastewater streams. The major advantages of biosorption technology are its effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbent materials (Kratochvil and Volesky, 1998). Removing metals from wastewater requires development of new biosorbents.

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