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Enhancement Biosorption of Heavy Metals from Factory Effluents via Recombinants Induced in Yeast and Bacteria

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Abstract: This investigation aimed to apply microbial genetic techniques to induce recombinants in bacteria and yeast to be used for maximal accumulation of heavy metals from factory effluents. This also leading to improve the quality of drinking and irrigated water in industrial regions. In this study ten bacterial strains and seven *Saccharomyces cerevisiae* strains were used. Bacterial strains were marking using 19 antibiotics to be use as a selectable markes in conjugation process. The available markers obtained were used in 14 mating, 10 of them were success, two transconjugants from each mating were selected to be use in biosorption experiments. Two from *Saccharomyces cerevisiae* strains were mated and the hybrids were isolated to be use in uptake experiments . Modern ecological biotechnology attempts to solve the problems of pollution by screening for and molecularly breeding microbial strains that are capable of degrading recalcitrant. 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. The results appeared that the biosorption capacities for all heavy metals determined in this study was higher for some metals than others. The maximum capacities of biosorption were higher by some of the parental strains than their transconjugants in some of matings, in contrast with other matings which appeared the biosorption capacities of transconjugants were higher than that in their parental strains. This indicated that the total amount of metal biosorption in a multiple metal system is lower than that in a single metal system. Most bacterial strains and their transconjugants in all matings appeared more than 50% removal for each one of heavy metal ions determined in this Study. The mechanism of metal sorption by *Saccharomyces cerevisiae* NRRL Y – 11562 shows superior properties in maintaining high uptake of heavy metal ions. Many of yeast strains and their hybrids appeared more than 50% removal in heavy metals uptake. This indicated that *Saccharomyces cerevisiae* are extremely effective in concentrating metals.

Key words: Biosorption, conjugation, factory effluents, heavy metals, pollutants uptake.

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. 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,200). Moreover, in Egypt, the environment, economic growth, and developments are all highly influenced by water - its regional and seasonal availability, and the quality of surface and groundwater. 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 lube 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).

Biosorption of metal ions usually can be classified as two types : the Freundlich model, in which the amount of metal uptake by the biomass increases with time, and the Langmuir model, in which the amount

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of metal uptake by the biomass reaches equilibrium (Chang and Hong 1994).

Therefore, both adsorption and desorption are independent of the total number of sites occupied. Adsorption is considered as a state of dynamic equilibrium, in which the rate at which metals are adsorbed equals the rate at which metals are desorbed. In the early stage, the rate of biosorption is fast since most of the binding sites on cell surface are freely available, whereas the rate of biosorption decreases when the cell surface is occupied with bound metal molecules. In other words, the rate of biosorption decreases with decreasing accessible surface area on the cell walls.

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). Conventional processes for removal of metals from industrial wastewaters include chemical precipitation, oxidation- reduction, filtration, electrochemical techniques and other sophisticated separation procedures using membranes. These processes are expensive when metals are found in relatively moderate concentrations, such as 1 - 100 mg/L. 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). So far, the biomass from filamentous fungi such as *Aspergillus niger* and *Rhizopus oryzae*, yeast-like *Saccharomyces cerevisiae*, algae such as *Chlorella regularis* and unicellular bacteria such as *Zoogloea ramigera* and *Pseudomonas aeruginosa,* have demonstrable capability for the uptake or binding of several metal ions. This study aimed to overcome heavy metals pollution using the lower addition (0.01 %) of carbon source added to factory effluents. to imftove the guality of wast waters.

MATERIALS AND METHODS

Ten bacterial strains and seven *Saccharomyces cerevisiae* strains (Table 1) 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 obtained from National Bank for Industrial Microorganisms and Cell Cultures, Bulgaria, Sofia. All strains used in this investigation are wild type strains.

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.

II. 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 Table 3. Antibiotic designation was listed in Table 2.

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 10. From each mating, two different isolates were selected to be used in pollutants uptake experiments. The genetic information transferred (Table 3) 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 in each conjugation were picked up and transferring to a nutrient agar slant, each colony may 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.

Table 1. Bacterial 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 Bankfor 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

Table 2. Antibiotics and their abbreviations used for genetic marking against different bacterial strains

Antibiotics	Designation
Flucamox	*flu*
Streptomycin	*Str*
Tetracycline	*Tc*
Neomycinsulphate	*Nm*
Ampicillin	*Ap*
Erythromycin	*Erth*
Amoxycillin and flucloxacillin	*Am-Fluc*
Rifampicillin	*Rf*
Ibiamox	*Ibim*
Amoxycillin	*Amoxy*
Ibidroxil	*Ibid*
Haiconcil	*Hico*
Velosef	*Velo*
Epicocillin	*Epico*
Nystatin	*Nyst*
Epicocillin	*Epico*
Erythrocin	*Ery*
Duricef	*Duri*
Pencillin	*pen*

Table 3: 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*$^+$

Table 3: Contineud.

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, overnight cultures form yeast and bacteria grown in nutrient broth for bacteria and YEPD for yeast were harvested, washed twice with distilled water, and resuspended in 250 ml conical flasks each containing 150 ml factory effluents supplemented with 1 mg glucose / 10 ml wastewater, glucose was used as a sole source of carbon. The flasks were 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). After the cells were removed the filtrate was used to determine the amount of heavy metals using atomic absorption spectrophotometry. Amounts of metals taken up by the cells were determined according to Nakajima and Sakaguchi (1986).

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 at the Atomic Absorption Unit, Department of Chemistry, Faculty of Science, Mansoura University. Heavy metals under investigation in this study included 16 heavy metals ions, which 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

Factory effluents is one of the main sources of pollution to ground water amd river water. The microbial world could adapt the new chemical 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 via inducing recombinant microbial strains from yeast and bacteria that are capable in uptake of heavy metals. Research in this direction is a good shape in reducing environmental pollution.

Uptake of heavy metals by bacterial cells using wastewaters supplemented with 0.01% glucose as a carbon source: As shown in Table 4, the biosorption capacities for all heavy metals determined in this study was higher for some metals than others. The maximum capacities of biosorption were higher by some of the parental strains than their transconjugants in some of matings, in contrast with other matings which appeared the biosorption capacities of transconjugants were higher than that in their parental strains. The present results

are in harmony with Liu *et al* 2004, who found that the maximum capacities for Zn(II) biosorption were 95.24 and 172.4 mg/g at 30 and 40 ∘C, respectively, and those for Cu(II) biosorption were 32.36 and 39.84 mg/g at 30 and 40 ∘C, respectively. Although, the same authors found that temperature effect was not significant on the maximum capacity for Cu (II) biosorption, the amount of Cu(II) adsorbed at lower initial Cu(II) concentrations was increased at higher temperature. Although higher temperature increases both the adsorption and desorption rates according to the Arrhenius equations, the equilibrium concentration of Langmuir isotherms still shift to a higher value since the adsorption rate is accelerated much more than the desorption rate.

These results obtained in our study are in good agreement with the previous reports showing that the total amount of metal biosorption in a multiple metal system is lower than that in a single metal system. (Utgikar *et a*l 200). The interference phenomenon for metal biosorption from binary mixture has been observed by many researchers. For example, Chang and Chen (1998). have reported that Cd(II) affects the uptake of Fe(II) by non-living biomass of *Sargassum fluitans*, and vice versa. Chang and Chen 1998 have found similar interference in metal uptake study involving *Pseudomonas aeruginosa* PU21 (RIP64) in a ternary system of Cu(II), Pb(II) and Cd(II). Although some microorganisms showed a slightly preference for Cu(II) adsorption over Zn (II) (Chang and Chen (1998)), our results showed that most of bacterial strains and their transconjugants were much more in favor of heavy metals uptake. It indicates that the specific characteristics of the metal binding sites and the functional groups responsible for metal interaction on the cell walls of the microorganisms play amajor role in determining the selectivity of metal biosorption.

The results presented in this study indicated that 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, resulting in chemical complexation as an uptake mechanism. Metal uptake can also be due to physical sorption or bioaccumulation.

Our results show that bacterial strains used in this study and their transconjugants has biosorption capability, by being able to sequester subtantial amounts of heavy metals from factory effluents. It is difficult to say whether these heavy metals were biodegraded. However, their accumulation in the bacterial biomass suggests that bacteria was able to entrap the heavy metals as they occur in the aqueous phase. Our results could establish a basis for evaluating the role of bacteria in the search for an environmentally friendly approach to dealing with pollutants in aqueous phase.

Table 4: Heavy metals uptake from wastewaters (containing 0.01% glucose as a carbon source) treated by parental strains of bacteria and their transconjugants.

Biocontrol agents	Transconjugant, and their Parcnt,	Heavy metals uptake (ppm)				
		Cu	Co	Fe	Cd	Pb
NRRL B-571 X NRRL B-1584	571	135	157	163	59	71
	1584	190	203	243	71	67
	M.P.	162	180	203	65	69
	Tr1	60	85	95	33	37
	Tr2	141	141	177	71	46
NRRL B-571 X NRRL B-2643	571	135	157	163	59	71
	2643	52	69	71	23	33
	Tr1	94	113	117	41	52
	Tr2	78	75	84	30	37
NRRL B-571 X NRRL B-41228	571	60	71	73	26	33
	41228	135	157	163	59	71
	M.P.	87	128	114	45	51
	Tr1	111	142	139	52	61
	Tr2	183	194	199	92	97
NRRL B-1584 X NRRL B-642	1584	166	173	203	75	62
	642	190	203	243	71	67
	M.P.	106	112	130	45	51
	Tr1	148	157	186	58	59
	Tr2	162	164	209	75	81
NRRL B-1584 X NRRL NRS-213	1584	108	105	52	45	50
	213	190	203	243	71	67
	M.P.	127	150	165	67	63
	Tr1	158	176	204	69	65
	Tr2	48	55	58	25	21
NRRL NRS-1264 X NRRL B-2643	1264	87	96	99	37	36
	2643	62	60	81	30	20
	M.P.	52	69	71	23	33
	Tr1	57	65	76	27	26
	Tr2	98	113	114	43	39

Table 4: Continued.

NRRL B-358 X NRRL B-642		358	127	173	157	62	32
		642	60	66	65	28	0
		M.P.	106	112	130	45	16
		Tr1	83	89	97	36	20
		Tr2	123	174	158	67	15
NRRL B-2643 X NRRL B-642		2643	122	153	121	56	39
		642	52	69	71	23	33
		M.P.	106	112	130	45	51
		Tr1	79	91	101	34	42
		Tr2	85	99	86	35	21
NRRL B- 41228 X NRRL B-642		41228	70	77	85	36	16
		642	87	128	114	45	51
		M.P.	106	112	130	45	51
		Tr1	96	120	122	45	51
		Tr2	87	88	69	36	16
NRRL B-642 X NRRL B-		4375	642	73	82	75	368
		4375	106	112	130	45	51
		M.P.	68	91	101	42	33
		Tr1	87	102	116	43	42
		Tr2	175	188	185	89	0
NRRL B-642 X NRRL NRS-213		642	6	237	297	126	39
		213	106	112	130	45	51
		M.P.	127	150	165	67	63
		Tr1	116	131	147	56	57
		Tr2	92	116	129	45	38
NRRL B- 4375 X NRRL NRS-213		4375	232	284	329	122	98
		213	68	91	101	42	33
		M.P.	127	150	165	67	63
		Tr1	97	121	133	54	48
		Tr2	68	81	92	30	34

Biocontrol agents		Ppb		ppm		
		Hg	As	Mn	Pt	Mo
NRRL B-571 X NRRL B-1584)	571	108	56	83	147	236
	1584	145	65	33	134	223
	M.P.	126	60	58	140	229
	Tr1	63	36	64	54	131
	Tr2	118	70	134	77	232
NRRL B-571 X NRRL B-2643	571	108	56	83	147	236
	2643	26	20	34	45	36
	M.P.	67	38	58	96	136
	Tr1	56	31	69	51	126
	Tr2	53	30	35	79	126
NRRL B-571 X NRRL B-41228	571	108	56	83	147	236
	41228	35	33	68	94	77
	M.P.	71	44	75	120	156
	Tr1	155	81	88	155	428
	Tr2	141	72	111	139	384
NRRL B-1584 X NRRL B-642	571	143	74	109	194	312
	642	83	41	66	60	153
	M.P.	113	57	88	127	232
	Tr1	132	66	106	149	322
	Tr2	90	39	46	104	196
NRRL B-1584 X NRRL NRS-213	1584	145	65	33	134	223
	213	112	58	73	83	216
	M.P.	128	61	53	109	220
	Tr1	40	18	32	55	92
	Tr2	71	40	72	61	206
NRRL NRS-1264 X NRRL B-2643	1264	51	26	30	43	83
	2643	26	20	34	45	36
	M.P.	38	23	32	44	60
	Tr1	81	45	80	86	235
	Tr2	110	64	102	177	141
NRRL B-358 X NRRL B-642	358	47	25	49	70	110
	642	83	41	66	60	153
	M.P.	65	33	58	65	131
	Tr1	116	62	103	142	163
	Tr2	106	56	100	129	161
NRRL B-2643 X NRRL B-642	2643	26	20	34	45	36

Table 4: Continued.

	642	83	41	66	60	153
	M.P.	54	31	50	52	95
	Tr1	8	40	65	95	120
	Tr2	61	35	57	83	126
NRRL B-41228 X NRRL B-642	41228	35	33	68	94	77
	642	83	41	66	60	153
	M.P.	59	37	67	77	115
	Tr1	66	34	69	100	166
	Tr2	56	28	47	95	169
NRRL B-642 X NRRL B-4375	642	83	41	66	60	153
	4375	64	37	62	84	158
	M.P.	73	39	64	72	155
	Tr1	86	61	116	156	396
	Tr2	141	84	204	276	538
NRRL B-642 X NRRL NRS-213	642	83	41	66	60	153
	213	112	58	73	83	216
	M.P.	97	49	70	72	185
	Tr1	40	33	84	126	179
	Tr2	128	107	220	320	439
NRRL B-4375 X NRRL NRS-213	4375	64	37	62	84	158
	213	112	58	73	83	216
	M.P.	88	48	67	84	187
	Tr1	55	32	63	94	137
	Tr2	68	36	38	111	159

Biocontrol agents	Transconjugant, and their Parcnt,	Heavy metals uptake (ppm)					
		Zn	Cr	V	Sr	Sb	Ni
NRRL B-571 X NRRL B-1584	571	155	285	185	219	420	258
	1584	245	397	134	156	486	283
	M.P.	200	341	160	188	453	270
	Tr1	105	140	131	184	235	140
	Tr2	285	273	250	325	250	259
NRRL B-571 X NRRL B-2643	571	155	285	185	219	420	258
	2643	29	95	117	15	165	133
	M.P.	92	190	151	117	292	195
	Tr1	126	52	164	147	112	148
	Tr2	134	82	152	49	49	141
NRRL B-571 X NRRL B-41228	571	155	285	185	219	420	258
	41228	96	180	141	38	90	232
	M.P.	126	232	163	129	255	245
	Tr1	363	486	412	67	162	391
	Tr2	320	348	367	412	367	348
TR from NRRL B-1584 X NRRL B-642	1584	245	397	134	156	486	283
	642	90	89	127	167	216	216
	M.P.	168	243	130	161	351	250
	Tr1	315	315	324	360	437	217
	Tr2	234	290	242	280	273	211
NRRL B-1584 X NRRL NRS-213	1584	245	397	134	156	486	283
	213	108	200	261	83	368	288
	M.P.	177	298	198	120	427	286
	Tr1	68	107	120	135	117	92
	Tr2	74	172	196	74	196	165
NRRL NRS-1264 X NRRL B-2643	1264	112	45	90	60	155	122
	2643	00	80	102	102	36	121
	M.P.	56	63	96	81	96	122
	Tr1	100	254	230	264	241	213
	Tr2	283	253	279	387	247	247
NRRL B-358 X NRRL B-642	358	97	116	98	107	187	115
	642	90	89	127	167	216	216
	M.P.	94	103	113	137	201	166
	Tr1	169	294	292	394	200	249
	Tr2	135	253	242	272	261	250
NRRL B-2643 X NRRL B-642	2643	29	95	117	15	165	133
	642	90	89	127	167	216	216
	M.P.	60	92	122	91	190	174
	Tr1	76	166	76	215	197	109
	Tr2	17	128	125	89	153	71
NRRL B-41228 X NRRL B-642	41228	96	180	141	38	90	232
	642	90	89	127	167	216	216

Table 4: Continued.

	M.P.	93	134	134	103	153	224
	Tr1	131	133	169	214	181	102
	Tr2	109	107	155	182	176	83
NRRL B-642 X NRRL B-4375	642	90	89	127	167	216	216
	4375	134	182	162	109	251	124
	M.P.	112	135	145	138	234	170
	Tr1	219	356	386	409	337	207
	Tr2	180	495	483	541	610	589
NRRL B-642 X NRRL NRS-213	642	90	89	127	167	216	216
	213	108	200	261	83	368	288
	M.P.	99	144	194	125	292	252
	Tr1	38	180	267	222	218	249
	Tr2	213	427	427	567	427	588
NRRL B-4375 NRRL NRS-213	4375	134	182	162	109	251	124
	213	108	200	261	83	368	288
	M.P.	121	191	212	96	310	206
	Tr1	76	132	112	161	214	109
	Tr2	129	129	156	165	266	146

As shown from the results presented in Table 5 the treatment of wastewater is necessary to protect the environment and public health. Wastewater carries many of heavy metals as shown in this study which are harmful to humans and wildlife; removal of these heavy metals is necessary. Most bacterial strains and their transconjugants in all matings appeared more than 50% removal for each one of heavy metal ions determined in this study. This are in agreement with Brierley *et al* (1986), who 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.

Metals considered highly toxic include: arsenic, beryllium, cadmium, chromium, lead, mercury, and nickel . Many are potent neurotoxins (acute and chronic exposure), e.g., lead . Some inorganics are considered human carcinogens . For this the removal of heavy metal ions from wastewater is very important to overcome envirnmental pollution, this because water is one of the most important natural resources of mankind . The development of water resources for crop cultivation, or irrigation project, is considered quite important and highly beneficial for the vast majority of people living in the rural areas, since water enables them to farm their lands throughout the year.

The results obtained in this work are in harmony with that obtained by the following authors:

- Abdul and Shakoori (2004), who found that the reduction in the amount of Cd2+ after 7, 14, 21 and 28 days of culture was 76, 80, 88 and 96%, respectively. *Chlorella* could also remove 78% Ni+2 after 7 days, 82% after 14 days, 88% after 21 days and 94% after 28 days from the medium. The resistance of algae against heavy metals present in industrial effluents indicated that the algae has acquired efficient means of resisting, tolerating or processing metal ions. The heavy metal uptake ability of *Chlorella* can be exploited for metal detoxification and environmental clean-up operations.
- Abou-Shanab *et al*. (2004), who found that heavy metal-contaminated land is an important environmental, health, economic, and planning issue in Egypt. Phytoextraction involves use of plants to remove metals from soil. In a greenhouse experiment, *Zea mays*, *Helianthus annuus* and *Sorghum bicolor* plants were grown in tannery effluent polluted soils and non-polluted reference soils. After 8 weeks of growth, the plants were harvested and the dry weight and the content of Cr were determined. The relationship between mycorrhizas and plants indicates that the percentage of mycorrhizal colonization in all plant species grown in unpolluted soils were higher than plants grown in polluted soil. Roots of all three plant species growing on both soils possessed arbuscular mycorrhizal (AM) colonization in their roots and AM propagules in the associated rhizospheres. High Cr contents adversely affected the number and diversity of AM species. Five AM fungi belonged to the *Glomus* genera and one species belonged to *Acaulospora* genus. The order of Cr foliar accumulation was *Z. mays* > *S. bicolor* > *H. annuus*. The effect of AM fungi on heavy metal uptake is dependent upon the initial soil metal concentration. The uptake of heavy metals by *Z. mays*, *H. annuus* and *S. bicolor* was affected by the colonization of roots with AM fungi .
- Al Ramalli *et al*. (2005), who demonstrated that the non-living, dried roots of the water hyacinth plant [*Echhornia craissipes* (Mart.) Solms] can rapidly remove arsenic from water. Atomic absorption spectrometry was used to demonstrate that more than 93% of arsenite (As(III)) and 95% of arsenate (As(v)) were removed from a solution containing 200 mu g As 1(-1) within 60 minutes of exposure to

a powder produced from dried roots. No difference in removal efficiency was observed between the two oxidation states of As studied. The amount of arsenic remaining in solution was found to be less than 10 mu g 1(-1) which is the WHO guideline limit value for As in drinking water. The presence of arsenic in drinking water in a number of countries in the developing world has been found to be much higher than the WHO level, affecting the health of millions of people. In this project, we show that a biomaterial is found in abundant supply in many parts of the world, can provide a simple, effective and yet cheap method for removing heavy metal ions from contaminated water.

- Arao and Ishikawa (2006), who investigated the genotypic differences in seed cadmium (Cd) concentration in soybean and rice, 17 soybean and 49 rice varieties were cultivated in Cd-polluted soils or water culture containing Cd. Significant differences in seed Cd concentration were found among soybean and rice varieties. A high level of inheritance of the seed Cd concentration was revealed for soybean. The physiological mechanism underlying the Cd translocation to shoots and seeds in soybean was involved in Cd retention in the roots. The commercial rice varieties (e.g., Koshihikari) were categorized into the low grain Cd group. On the other hand, several indica or indica-japonica rice varieties accumulated considerably high Cd concentrations in grains as well as straws, when they were cultivated under upland conditions, suggesting that these varieties would be most responsive to phytoremediation of Cd-polluted paddy fields. There was no correlation of the Cd concentration between younger shoots and mature seeds in the rice cultivars, so it may be impossible to use rice for evaluating the genotypic variation in seed Cd concentration using relatively younger shoots. On the other hand, a positive correlation between them was found in the soybean cultivars, so it may be possible to evaluate the genotypic variation in soybean seed Cd concentration using relatively younger soybean shoots . Interactions between Cd and other metals (Cu, Fe, Mn, and Zn) in terms of their uptake and translocation to shoots were found among the rice and soybean cultivars .
- Asma *et al*. (2005) who treated tannery effluents with hydrophytes: *Chara intermedia*, *Typha angustifolia*, *Hemarthria compressa*, *Pistia stratioties*, *Marsilea minuta* and *Salvinia natans* resulted to reduction in heavy metal concentration of the effluents. It was found that the performance of *T. angustifolia* was superior followed by *H. compressa*. These plants did not only tolerated the heavy metal concentrations but also reduced the chromium content of the tannery effluents. The other species were sensitive to high heavy metal concentrations and did not survive long during the study period.
- Axtell *et al*. (2003), who reported that aquatic plants can remove heavy metal contamination from the surrounding water. Their study examined the ability of Microspora (a macro-alga) and *Lemna minor* (an aquatic plant) to remove soluble lead and nickel under various laboratory conditions. Microspora was tested in a batch and semi-batch process for lead removal. *L. minor* was tested in a batch process with lead and nickel to examine the potential competition between metals for adsorption. The Microspora was exposed to 39.4 mg/l of lead over 10 days. Results show up to 97% of the lead was removed in the batch process and 95% in the semibatch process. Initial concentrations below 50 mg/l (a dose that kills the algae) had no effect on the final concentration. The *L. minor* was exposed to lead and nickel using a full 3(2) factorial experimental design (nine experiments, plus replications). Initial lead concentrations were 0.0, 5.0, and 10.0 mg/l, and nickel concentrations were 0.0, 2.5, and 5.0 mg/l in the experiment. Overall, *L. minor* removed 76% of the lead, and 82% of the nickel. No synergistic/antagonistic effect was noted for the multiple metal experiments, in terms of metal removal.

Table 5: Percentage of heavy metals uptake from wastewaters (containing 0.01% glucose as a carbon source) treated by parental strains of bacteria and their transconjugants.

Biocontrol agents		ppm				
		Cu	Co	Fe	Cd	Pb
NRRL B-571 X NRRL B-1584	571	86	85	75	78	59
	1584	91	83	84	71	42
	M.P.	89	84	79	74	51
	Tr1	69	83	78	78	56
	Tr2	85	72	76	89	37
NRRL B-571 X NRRL B-2643	571	86	85	75	78	59
	2643	77	86	75	71	63
	M.P.	82	85	75	74	61
	Tr1	98	79	75	78	61
	Tr2	78	78	68	71	56
NRRL B-571 X NRRL B-41228	571	86	85	75	78	59
	41228	73	91	68	78	56

Table 5: Continued.

	M.P.	80	88	72	78	58
	Tr1	85	76	66	89	59
	Tr2	84	74	73	78	41
NRRL B-1584 X NRRL B-642	1584	91	83	84	71	42
	642	89	80	78	78	56
	M.P.	90	81	81	74	49
	Tr1	82	70	75	78	54
	Tr2	83	68	28	71	51
NRRL B-1584 X NRRL NRS-213	1584	91	83	84	71	42
	213	82	82	76	89	54
	M.P.	87	82	80	80	48
	Tr1	85	81	73	89	49
	Tr2	88	83	72	78	48
NRRL NRS-1264 X NRRL B-2643	1264	88	73	83	89	37
	2643	77	86	75	71	63
	M.P.	83	80	79	80	50
	Tr1	86	84	72	78	45
	Tr2	77	89	68	78	25
NRRL B-358 X NRRL B-642	358	86	79	66	82	
	642	89	80	78	78	56
	M.P.	88	80	72	80	28
	Tr1	73	87	67	82	11
	Tr2	82	86	58	78	34
NRRL B-2643 X NRRL B-642	2643	77	86	75	71	63
	642	89	80	78	78	56
	M.P.	83	83	77	74	60
	Tr1	84	83	61	71	27
	Tr2	85	79	74	89	25
NRRL B- 41228 X NRRL B-642	41228	73	91	68	78	56
	642	89	80	78	78	56
	M.P.	81	85	73	78	56
	Tr1	91	78	52	78	23
	Tr2	88	84	65	89	13
NRRL B-642 X NRRL B-4375	642	89	80	78	78	56
	4375	73	84	78	93	46
	M.P.	81	82	78	86	51
	Tr1	89	81	68	93	0
	Tr2	2	72	76	93	18
NRRL B-642 X NRRL NRS-213	642	89	80	78	78	56
	213	82	82	76	89	54
	M.P.	85	81	77	83	55
	Tr1	77	83	78	78	42
	Tr2	82	85	83	89	45
NRRL B- 4375 X NRRL NRS-213	4375	73	84	78	93	46
	213	82	82	76	89	54
	M.P.	77	83	77	91	50
	Tr1	85	85	82	78	55
	Tr2	73	87	82	89	

		Ppb		ppm		
Biocontrol agents		Hg	As	Mn	Pt	Mo
NRRL B-571 X NRRL B-1584)	571	89	83	58	62	58
	1584	90	73	18	43	42
	M.P.	90	78	38	53	50
	Tr1	93	95	80	41	58
	Tr2	92	98	88	31	54
NRRL B-571 X NRRL B-2643	571	89	83	58	62	58
	2643	50	70	55	44	21
	M.P.	69	76	56	53	40
	Tr1	90	90	94	42	61
	Tr2	89	93	51	69	64
NRRL B-571 X NRRL B-41228	571	89	83	58	62	58
	41228	38	65	62	52	25
	M.P.	63	74	60	57	42
	Tr1	93	88	45	48	77
	Tr2	92	85	61	46	75
NRRL B-1584 X NRRL B-642	571	89	83	58	62	58
	642	90	80	61	34	50
	M.P.	90	81	59	48	54

Table 6: Continued.

	Tr1	86	78	59	50	63
	Tr2	89	70	39	53	58
NRRL B-1584 X NRRL NRS-213	1584	90	73	18	43	42
	213	93	88	52	36	54
	M.P.	92	80	35	39	48
	Tr1	90	73	61	64	63
	Tr2	93	95	80	41	81
NRRL NRS-1264 X NRRL B-2643	1264	93	85	47	41	46
	2643	50	70	55	44	21
	M.P.	72	78	51	43	33
	Tr1	92	93	76	50	80
	Tr2	86	90	68	71	33
NRRL B-358 X NRRL B-642	358	86	83	76	66	60
	642	90	80	61	34	50
	M.P.	88	81	69	50	55
	Tr1	89	85	67	56	38
	Tr2	92	88	73	57	42
NRRL B-2643 X NRRL B-642	2643	50	70	55	44	21
	642	90	80	61	34	50
	M.P.	70	75	58	39	35
	Tr1	10	93	71	62	46
	Tr2	94	98	75	66	59
NRRL B-41228 X NRRL B-642	41228	38	65	62	52	25
	642	90	80	61	34	50
	M.P.	64	73	62	43	38
	Tr1	90	83	80	70	68
	Tr2	88	78	62	76	79
NRRL B-642 X NRRL B-4375	642	90	80	61	34	50
	4375	89	93	73	61	66
	M.P.	90	86	67	47	58
	Tr1	57	73	65	53	78
	Tr2	65	70	80	66	75
NRRL B-642 X NRRL NRS-213	642	90	80	61	34	50
	213	93	88	52	36	54
	M.P.	92	84	56	35	52
	Tr1	43	65	78	71	58
	Tr2	58	88	85	75	60
NRRL B-4375 X NRRL NRS-213	4375	89	93	73	61	66
	213	93	88	52	36	54
	M.P.	91	90	62	48	60
	Tr1	89	93	86	78	66
	Tr2	88	83	41	74	61

Biocontrol agents		Heavy metals uptake (ppm)					
		Zn	Cr	V	Sr	Sb	Ni
NRRL B-571 X NRRL B-1584	571	54	70	44	57	96	77
	1584	65	74	24	30	84	64
	M.P.	59	72	34	43	90	70
	Tr1	66	62	56	86	97	75
	Tr2	94	64	56	79	54	73
NRRL B-571 X NRRL B-2643	571	54	70	44	57	96	77
	2643	24	54	64	9	87	91
	M.P.	39	62	54	33	91	84
	Tr1	86	25	76	74	50	86
	Tr2	96	42	74	26	23	86
NRRL B-571 X NRRL B-41228	571	54	70	44	57	96	77
	41228	44	58	44	13	27	91
	M.P.	49	64	44	35	61	84
	Tr1	92	88	71	13	27	85
	Tr2	88	68	69	84	66	82
TR from NRRL B-1584 X NRRL B-642	1584	65	74	24	30	84	64
	642	42	29	40	57	65	85
	M.P.	53	52	32	44	75	74
	Tr1	87	62	61	73	79	51
	Tr2	98	86	69	87	75	76
NRRL B-1584 X NRRL NRS-213	1584	65	74	24	30	84	64
	213	38	50	63	22	85	87
	M.P.	51	62	43	26	84	75
	Tr1	65	73	78	96	73	75
	Tr2	41	68	74	30	71	78

NRRL NRS-1264 X NRRL B-2643	1264	87	25	48	35	79	81
	2643	0	46	56	61	19	83
	M.P.	44	35	52	48	49	82
	Tr1	48	87	75	94	76	87
	Tr2	94	60	63	95	54	70
NRRL B-358 X NRRL B-642	358	75	64	52	61	95	76
	642	42	29	40	57	65	85
	M.P.	59	47	46	59	80	81
	Tr1	55	68	64	94	42	69
	Tr2	49	65	60	73	62	78
NRRL B-2643 X NRRL B-642	2643	24	54	64	9	87	91
	642	42	29	40	57	65	85
	M.P.	33	42	52	33	76	88
	Tr1	41	63	28	86	70	50
	Tr2	65	60	56	43	66	40
NRRL B-41228 X NRRL B-642	41228	44	58	44	13	27	91
	642	42	29	40	57	65	85
	M.P.	43	44	42	35	46	88
	Tr1	75	54	66	91	68	50
	Tr2	72	50	70	89	76	47
NRRL B-642 X NRRL B-4375	642	42	29	40	57	65	85
	4375	79	76	65	48	97	63
	M.P.	61	53	53	52	81	74
	Tr1	61	70	73	84	62	49
	Tr2	35	69	64	78	78	98
NRRL B-642 X NRRL NRS-213	642	42	29	40	57	65	85
	213	38	50	63	22	85	87
	M.P.	40	40	51	39	75	86
	Tr1	18	59	84	76	66	98
	Tr2	41	58	56	81	54	97
NRRL B-4375 X NRRL NRS-213	4375	79	76	65	48	97	63
	213	38	50	63	22	85	87
	M.P.	59	63	64	35	91	75
	Tr1	52	64	52	81	96	64
	Tr2	71	50	58	67	95	68

Uptake of heavy metals by Saccharomyces cerevisiae using wastewaters supplemented with 0.01% glucose as a carbon source:

The results presented in Table 6 appeared the uptake of heavy metal ions by *Saccharomyces cerevisiae* strains and their hybrids. It can be found that *Saccharomyces cerevisiae* NRRL Y – 11562 appeared a good uptake of heavy metal ions than both *Saccharomyces cerevisiae* NRRL Y – 12632 and the hybrids obtained. This work highlights the potential of yeast *Saccharomyces cerevisiae* NRRL Y – 11562 in uptake of heavy metals. The results indicated that bioremediation of heavy metal pollution remains a major challenge in environmental biotechnology. The mechanism of metal sorption by yeast cells gave good fits for Freundlich and Langmuir models. Characteristic of a good and useful biosorbent is its ability to be utilized as a fixed or expanded bed for continuous system. This yeast biomass was shown to be suitable for use in column reactor. The mechanism of metal sorption by *Saccharomyces cerevisiae* NRRL Y – 11562 shows superior properties in maintaining high uptake of heavy metal ions . However, heavy metals released by a number of industrial processes are major pollutants in marine, ground, industrial and even treated wastewaters, the use of microbial cells as biosorbents as shown in this study for heavy metals offers a potentially inexpensive alternative compared to conventional methods of heavy metal decontamination from a variety of industrial aqueous process conventional treatment methods include low cost, high efficiency of metal removal from dilute solution, minimization of chemical and/or biological sludge, no additional nutrient requirement, regeneration of biosorbent and the possibility of metal recovery [Veglio *et al*, 1997]. Bacteria [Gadd and White, 1993], fungi [Volesky., 1987], marine algae[Volesky. and Holan, 1995], etc. have been studied before for their heavy metal uptake capacities and suitability to be used as development of biosorbents. Biomass cell walls, consisting mainly of polysaccharides, proteins and lipids, offer many functional groups that can bind metal ions such as carboxylate, hydroxyl, sulphate, sphosphate and amino groups.

The investigation of bioaccumulation / biosorption, which have been used in this study for the removal of heavy metal ions by microorganisms, has become an attractive subject. In particular, *Saccharomyces cerevisiae* is the most popular biomass investigated as a useful biosorbent as seen in this study . This also are in agreement with Jung *et al* 1998, who reported that on the basis of the above results and discussions, a reliable mechanism of Pb21 accumulation in *S.cerevisiae* has been produced. The first step of this mechanism

is a rapid binding to the cell wall and a passive transport of Pb21 through the cell wall for a short time within 3, 5 min, and this process is metabolism-independent. The second step is the penetration through the cell membrane and into the cytoplasm, but this step cannot be clearly labeled as metabolism-dependent or -independent. Cationic ion exchange between Pb21 and potassium-magnesium occurred through the first and second steps. A much slower process that is obviously independent of metabolism and cation exchange follows the first and second steps. The third step is the Pb21 accumulation into the cell cytoplasm even though the cells have already entered a dead phase after 24 h. It can be concluded that, because the mode of Pb21 accumulation is closely related to the cell dry weight and initial Pb21 concentration, careful consideration should be taken to determine the time needed to reach an equilibrium state.

Table 6: Heavy metals uptake from wastewaters (containing 0.01% glucose as a carbon source) treated by parental strains of *Saccharomyces cerevisiae* and their hybrids.

Biocontrol agents	ppm				
	Cu	Co	Fe	Cd	Pb
Heavy metal ions concentration in wastewaters (without glucose)	1.4	1.1	1.5	0.60	0.71
Heavy metal ions concentration in wastewaters (with glucose)	0.93	1.1	1.3	0.45	0.71
Saccharomyces cerevisiae NRRL Y – 12632	160	176	199	67	81
Saccharomyces cerevisiae NRRL Y - 11562	469	526	634	183	217
M. P.	315	351	417	125	149
Hybrid No. 1	106	142	164	60	42
Hybrid No. 2	163	151	205	76	50
Hybrid No. 3	149	154	191	76	45
Hybrid No. 4	165	176	218	88	50
Hybrid No. 5	118	136	158	54	61
Saccharomyces cerevisiae NBIMCC 82	121	131	145	53	51
Saccharomyces cerevisiae NRRL Y - 12619	260	295	345	117	108
Saccharomyces cerevisiae NRRL Y – 136	67	80	94	33	51
Saccharomyces cerevisiae NRRL Y – 137	80	95	112	39	61
Saccharomyces cerevisiae NRRL Y - 1370	129	153	180	62	98
Biocontrol agents	ppb			ppm	
	Hg	As	Mn	Pt	Mo
Heavy metal ions concentration in wastewaters (without glucose)	0.88	0.50	0.90	1.60	2.50
Heavy metal ions concentration in wastewaters (with glucose)	0.72	0.40	0.85	1.40	2.40
Saccharomyces cerevisiae NRRL Y – 12632	32	20	10	41	20
Saccharomyces cerevisiae NRRL Y – 11562	331	251	326	663	857
M.P.	182	136	168	352	439
Hybrid No. 1	133	75	136	241	377
Hybrid No. 2	136	92	130	245	325
Hybrid No. 3	131	89	134	198	354
Hybrid No. 4	140	100	153	222	431
Hybrid No. 5	98	66	104	146	302
Saccharomyces cerevisiae NBIMCC 82	83	63	94	124	171
Saccharomyces cerevisiae NRRL Y - 12619	181	143	219	314	444
Saccharomyces cerevisiae NRRL Y – 136	36	34	57	86	110
Saccharomyces cerevisiae NRRL Y – 137	54	41	66	108	135
Saccharomyces cerevisiae NRRL Y - 1370	80	64	104	158	257
Biocontrol agents	ppm				
	Zn	Cr	V	Sr	Sb	Ni
Heavy metal ions concentration in wastewaters (without glucose)	1.7	2.4	2.5	2.3	2.6	2.0
Heavy metal ions concentration in wastewaters (with glucose)	1.6	2.5	2.4	2.5	2.8	2.2
Saccharomyces cerevisiae NRRL Y – 12632	207	284	329	444	523	404
Saccharomyces cerevisiae NRRL Y – 11562	611	880	1091	1063	1480	697
M, P,	409	582	710	754	1002	550
Hybrid No, 1	109	234	312	226	408	265
Hybrid No, 2	266	352	388	249	358	367
Hybrid No, 3	285	312	285	218	381	339
Hybrid No, 4	318	370	310	331	251	320
Hybrid No, 5	136	294	336	294	123	251
Saccharomyces cerevisiae NBIMCC 82	134	280	142	280	273	242
Saccharomyces cerevisiae NRRL Y – 12619	339	662	517	577	773	526
Saccharomyces cerevisiae NRRL Y – 136	86	147	113	128	169	143
Saccharomyces cerevisiae NRRL Y – 137	72	148	153	133	185	115
Saccharomyces cerevisiae NRRL Y – 1370	121	218	248	194	330	259

It can be concluded that *Saccharomyces cerevisiae* can remove toxic metals, recover precious metals and clean radio-nuclides from aqueous solutions to various extents. *S. cerevisiae* is not only a by-product of established fermentation processes, but also can be easily obtained in considerably substantial quantities at low costs (Goksungur *et al*. 2005). Often, the economics of the process can be improved by using waste biosorbent instead of cultured biosorbent (Marques *et al*. 2000). The application of *S. cerevisiae* as a biosorbent not only removes metals from wastewaters but also eases the burden of disposal costs associated with the waste (Ting and Sun, 2000).

As shown from the results presented in Table 7 that many of yeast strains and their hybrids appeared more than 50% removal in heavy metals uptake. This indicated that *Saccharomyces cerevisiae* are extremely effective in concentrating metals . Research on biosorption is revealing that it is sometimes a complex phenomenon where the metallic species could be deposited in the solid biosorbent through various sorption processes, such as ion exchange, complexation, chelation, microprecipitation, etc . In general, biosorption of toxic metals and radionuclides is based on non-enzymatic processes such as adsorption. Adsorption is due to the non-specific binding of ionic species to polysaccharides and proteins on the cell surface or outside the cell (Mullen *et al*, 1989). Bacterial cell walls and envelopes, and the walls of fungi, yeasts and algae, are efficient metal biosorbents that bind charged groups. The cell walls of gram-positive bacteria bind larger quantities of toxic metals and radionuclides than the envelopes of gram-negative bacteria.

These results indicating the advantages of biosorption, biosorption is highly competitive with the presently available technologies like ion exchange, electrodialysis, reverse osmosis, etc. Some of the key features of biosorption compared to conventional processes include: competitive performance, heavy metal selectivity, cost-effectiveness, regenerative, no sludge generation.

Biosorption is particularly economical and competitive for environmental applications in detoxifying effluents from, for example: metal plating and metal finishing operations, mining and ore processing operations, metal processing, battery and accumulator manufacturing operations, thermal power generation (coal-fired plants in particular), nuclear power generation. In conclusion there appear to be many modes of non-active metal uptake by microbial biomass. Any one or a combination of them can be functional in immobilizing metallic species on biosorbents. A number of anionic ligands participate: phosphoryl, carbonyl, sulfhydryl and hydroxyl groups can all be active to various degrees in binding the metal.

Many scientific studies are currently underway to provide a deeper understanding of biosorption and to support its effective application. Some pollution seems inevitable, and one might wonder what should be done to minimize it. Human populations need methods and technologies to clean waters and diminish the environmental dangers related to technological progress. Biosorption can be one such solution to clean up heavy metal contamination as seen in these study. This study is very important in cleaning wastewaters from heavy metals.

Cost-effectiveness is the main attraction of metal biosorption. This cost-effectiveness can be maintained by using the microbial biomass directly where possible. In addition, biosorbents derived from microbial biomass through a simple process are expected to be the lowest-priced and most-economical for metal removal.

It has been suggested that numerous chemical groups contribute to biosorption metal binding, by either whole organisms such as algae and bacteria or by molecules such as biopolymers. These include hydroxyl, carbonyl, carboxyl, sulfhydryl, thioether, sulfonate, amine, imine, amide, imidazole, phosphonate, and phosphodiester groups. The importance of any given group for biosorption of a certain metal by a certain biomass depends on such factors as the number of sites in the biosorbent material, the accessibility of the sites, the chemical state of the sites (i.e., availability), and the affinity between the site and the metal (*i.e.,* binding strength). For covalent metal binding, even an occupied site is theoretically available; the extent to which the site can be used by a given metal depends on its binding strength and concentration compared to the metal already occupying the site.

The current work focus around the use of bacteria and yeast biomass to remove water pollution by heavy metals. Nonliving biomass of many microbioal species is an excellent sorber of metal ions. We are investigating the uptake of metal ions in the laboratory by using yeast and bacterial biomass. We are studying the concentration of heavy metals prior to metal adsorption experiments.

In conclusion, biosorption is being demonstrated as a useful alternative to conventional systems for the removal of toxic metals from industrial effluents. The development of the biosorption processes requires further investigation in the direction of modeling, of regeneration of biosorbent material with industrial effluents. Due to the extensive research and significant economic benefits of biosorption, some new biosorbent materials are poised for commercial exploitation. Our results show that bacterial cells and their transconjugants, yeast strains and their hybrids has biosorption capability, by being able to sequester subtantial amounts of heavy metals

Table 7: Percentage of heavy metals uptake from wastewaters (containing 0.01% glucose as a carbon source) treated by parental strains of *Saccharomyces cerevisiae* and their hybrids.

Biocontrol agents	Heavy metals uptake (ppm)				
	Cu	Co	Fe	Cd	Pb
Saccharomyces cerevisiae NRRL Y – 12632	77	81	85	78	61
Saccharomyces cerevisiae NRRL Y - 11562	82	77	84	78	59
M. P.	80	79	85	78	60
Hybrid No. 1	85	79	75	73	56
Hybrid No. 2	88	84	85	71	54
Hybrid No. 3	77	88	87	82	51
Hybrid No. 4	75	85	84	89	39
Hybrid No. 5	91	72	82	89	37
Saccharomyces cerevisiae NBIMCC 82	88	77	81	93	35
Saccharomyces cerevisiae NRRL Y - 12619	85	76	80	93	34
Saccharomyces cerevisiae NRRL Y – 136	83	81	79	78	56
Saccharomyces cerevisiae NRRL Y – 137	91	84	78	82	51
Saccharomyces cerevisiae NRRL Y - 1370	88	85	84	82	48
Biocontrol agents	ppb			ppm	
	Hg	As	Mn	Pt	Mo
Saccharomyces cerevisiae NRRL Y – 12632	58	85	61	69	58
Saccharomyces cerevisiae NRRL Y – 11562	72	78	76	68	65
M.P.	65	81	69	68	62
Hybrid No, 1	76	95	74	77	67
Hybrid No, 2	78	98	81	64	77
Hybrid No, 3	71	95	80	61	82
Hybrid No, 4	67	83	74	54	78
Hybrid No, 5	58	85	72	48	46
Saccharomyces cerevisiae NBIMCC 82	57	88	75	56	54
Saccharomyces cerevisiae NRRL Y – 12619	47	93	86	70	59
Saccharomyces cerevisiae NRRL Y – 136	65	95	85	75	61
Saccharomyces cerevisiae NRRL Y – 137	58	90	82	67	73
Saccharomyces cerevisiae NRRL Y – 1370	71	93	80	76	68
Biocontrol agents	Heavy metals uptake (ppm)					
	Zn	Cr	V	Sr	Sb	Ni
Saccharomyces cerevisiae NRRL Y – 12632	64	56	68	88	92	90
Saccharomyces cerevisiae NRRL Y – 11562	67	62	80	74	93	55
M. P.	65	59	74	81	92	73
Hybrid No. 1	45	62	86	60	97	80
Hybrid No. 2	87	74	85	52	67	87
Hybrid No. 3	98	69	65	48	75	85
Hybrid No. 4	95	71	62	63	43	70
Hybrid No. 5	56	77	91	77	29	75
Saccharomyces cerevisiae NBIMCC 82	59	79	42	79	69	77
Saccharomyces cerevisiae NRRL Y – 12619	67	84	68	73	87	75
Saccharomyces cerevisiae NRRL Y – 136	74	81	65	71	83	90
Saccharomyces cerevisiae NRRL Y – 137	53	69	74	62	77	60
Saccharomyces cerevisiae NRRL Y – 1370	54	63	75	56	85	85

from effluents. Previously, the ability of fungi to degrade recalcitrant pollutants has been demonstrated. This ability is probably largely due their elaborate ligninolytic enzymes. It is difficult to say whether these heavy metals were biodegraded. However, their accumulation in the bacteria and yeast biomass suggests that the these cells is able to entrap the heavy metals as they occur in the aqueous phase. Our results could establish a basis for evaluating the role bacteria and yeast in the search for an environmentally friendly approach to dealing with pollutants in aqueous phase.

This study showed that yeast can efficiently remove heavy metals from chemical fertilizer manufacturing industrial effluents. This study also emphasizes the importance and need for carrying out extended testing for the compatibility of biosorption to a specific industrial effluent. The findings of the study indicate that biosorption is a promising technology for removal of heavy metals in manufacturing effluent. However, further studies with respect to metal-biosorbent specificity, applicability to various other types of metal-laden effluents and large scale studies will help fine-tune the biosorption technology for large-scale application. From an overview of microbial sorbents and biowaste as sorbent candidate, it can be concluded that laboratory trials do show their potential for commercialization since it is technically feasible, ecofriendly with good metal-binding capacity. Besides that, being composed entirely of agricultural and fishing industry waste, it helps in

reduction of waste generation. The adsorbent can be regenerated using higher pH buffer and reused up to 8 times without any loss in metals binding capacity. This adsorbent can be a good candidate for adsorption of not only chromium ions but also other heavy metal ions in wastewater stream.

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