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Biosorption of Heavy Metals by four acclimated microbial species, *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp. and *Aspergillus niger*

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ABSTRACT

The four acclimated microorganisms viz. *Bacillus* spp. *Pseudomonas* spp., *Staphylococcus* spp., and *Aspergillus niger*, were isolated from soil and sludge undergone enrichment process. They were allowed to grow in synthetic media amended with heavy metal solution. The waste samples both solid and liquid were collected and chemical parameters was checked viz. pH, BOD, COD, chloride, fluoride, TDS and TH. The heavy metals Mn, Cd, Cr, Cu, Zn and Pb were determined in liquid waste and industrial wastewater, while other metals viz. Cr, Co, Zn, Mn and Ni were measured in leachate (solid waste). The acclimated microorganisms were used to remediate the waste by biosorption process in a Batch culture. *Pseudomonas* sp. and *Bacillus* sp. reduced Cu 4.165mgl^{-1} and 3.332 mgl^{-1}(68% and 56%) and Ni 5.015 mgl^{-1} and 3.8 mgl^{-1} (65% and 48%) respectively. *Aspergillus niger* reduced Cd 0.267 mgl^{-1} (50%) and Zn 5.988 mgl^{-1}(58%) whereas *Staphylococcus* sp. reduced Cr 4.108 mgl^{-1}(45%), Cu 2.615 mgl^{-1}(42%) and Pb 0.813 mgl^{-1} (93%). The results showed that *Pseudomonas* sp. reduced heavy metals more than other microbes but *Staphylococcus* sp. reduced lead 93% which was surprising and very much amount of lead uptake by *Staphylococcus* sp.

Key Words: Heavy metals, solid and liquid waste, acclimated microorganisms, biosorption, *Pseudomonas* sp.

INTRODUCTION

The speedy development and increasing sophistication of various industries in the past century has remarkably increased the amount and complexity of toxic waste effluents, which may be bioremediated by appropriate plants and microbes, either natural occurring or tailor-made for the specific purpose. This technology is termed as bioremediation. The bioremediation and natural attenuation area has both basic research and field application foci for the environmental biotechnology. The basic research foci are co-metabolism, bio-treatability, biotransformation kinetics, and modeling of biogeochemical processes. The field application foci are co-metabolic techniques, biogeochemical assessment techniques, and modeling of attenuation and environmental fate (Kumar et al 2010a). Bioremediation can be defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition. Biosorption can be defined as the selective sequestering of metal soluble species that result in the immobilization of the metals by microbial cells. Metal sequestering by different arts of the cell can occur via various processes: complexation, chelation, coordination, on exchange, precipitation, reduction. Biosorption is a process with some unique characteristics. It can effectively sequester dissolved metals from very dilute complex solutions with high efficiency. This makes biosorption an ideal candidate for the treatment of high volume low concentration complex waste-waters.

Arbitrary and hysterical discharge of industrial and urban wastes into the environmental sink has become an issue of major global concern (Hernandez et al., 1998; Gupta and Mahapatra, 2003; Strong and Burgess, 2008). Intensification of agriculture and manufacturing industries has resulted in increased release of a wide range of xenobiotic compounds to the environment. Excess loading of hazardous waste has led to scarcity of clean water and disturbances of soil thus limiting crop production (Kamaludeen et al., 2003). Although enactment of stringent regulation has led to less indiscriminate disposal of organic and inorganic wastes (Kamaludeen et al., 2003), challenges remain that require other interventions. Compared to other methods, bioremediation is a more promising and less expensive way for cleaning up contaminated soil and water (Eccles and Hunt, 1986; Kamaludeen et al., 2003). Bioremediation uses biological agents, mainly microorganisms, yeast, fungi or bacteria to clean up contaminated soil and water (Strong and Burgess, 2008). This technology relies on promoting the growth of specific microflora or microbial consortia that are indigenous to the contaminated sites that are able to perform desired activities (Agarwal, 1998). Establishment of such microbial consortia can be done in several ways, e.g. by promoting growth through addition of nutrients, by adding terminal electron acceptor or by controlling moisture and temperature conditions, among

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others (Hess et al., 1997; Agarwal, 1998; Smith et al., 1998). In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources (Hess et al., 1997; Agarwal, 1998; Tang et al., 2007).

Metals play an integral role in the life processes of living organisms. Heavy metals are metals with densities higher than 5 g/cm^3. Some metals (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni and Zn) are essential, serve as micronutrients and are used for redox-processes, to stabilize molecules through electrostatic interactions; as components of various enzymes; and regulation of osmotic pressure. While many other metals (Ag, Al, Cd, Au, Pb, and Hg) have no biological role and they are nonessential. They are potentially toxic to living organism specially microorganisms. Toxicity of nonessential metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions. Heavy metals in wastewater come from industries and municipal sewage, and they are one of the main causes of water and soil pollution (Lloyd et al. 2001). Accumulation of these metals in wastewater depends on many local factors such as type of industries in region, people's way of life and awareness of the impacts done to the environment by careless disposal of wastes. Therefore the presence of heavy metals in wastewater is not only of great environmental concern but also strongly reduces microbial activity, as a result adversely affecting biological wastewater treatment processes.

Natural organisms, either indigenous or extraneous are the prime agents used for bioremediation (Prescott et al., 2002). The organisms that are utilized vary, depending on the chemical nature of the polluting agents and are to be selected carefully as they only survive within a limited range of chemical contaminants (Prescott et al., 2002; Dubey, 2004). Since numerous types of pollutants are to be encountered in a contaminated site, diverse types of microorganisms are likely to be required for effective mediation (Watanabe et al., 2001). The first patent for a biological remediation agent was registered in 1974, being a strain of *Pseudomonas putida* (Prescott et al., 2002) that was able to degrade petroleum. In 1991, about 70 microbial genera were reported to degrade petroleum compounds (U.S Congress, 1991) and almost an equal number has been added to the list in the successive two decades. Bioremediation can occur naturally or through intervention processes (Agarwal, 1998). Natural degradation of pollutants relies on indigenous microflora that is effective against specific contaminants and it usually occurs at a slow rate. With intervention processes, the rate of biodegradation is aided by encouraging growth of microorganisms, under optimized physico-chemical conditions (Blackburn and Hafker, 1993; Bouwer et al., 1998; Smith et al., 1998).

Industrialization is accelerating the deposition of heavy metals in soil and water bodies. In some ecosystems these metals can be easily incorporated by organic and inorganic fractions of the soil and by sediments. The extent of this incorporation depends on the concentration of metals and on characteristic biotic and abiotic factors. Nevertheless, in water bodies or soil, metals can be remobilized, acting as toxic elements. This way, it is essential to minimize deleterious effects of dispersion in natural waters, through the use of suitable technology-based techniques. The purpose of the present work was to investigate the ability of four acclimated microorganisms to accumulate the heavy metals and to use as bioremediating agent in situ. The objective is the selection of the best microbe to be used in association with waste materials to reduce the heavy metals, with the advantage of best agent for bioremediation.

MATERIALS AND METHODS

Collection of Samples

The liquid and solid waste samples were collected from landfills and industries of Doon Valley Uttarakhand viz. New Tehri, Chamba, University campus Badshahithaul, Srinagar, Pauri, Rishikesh, Haridwar, Dehradun, Mussorie, Kotdwar, Rudraprayag, Devprayag, Nainital, Haldwani, Lakshar and Uttarkashi. Sample collection was performed by following the Standard Methods APHA, 1995. Samples were collected in the pre sterilized glass bottles covered with aluminum foil to protect from contamination. All samples were collected and stored at 4^0C for further analysis.

Measurement of Physico-Chemical Parameter of the waste Samples

The physico-chemical parameters were measured in all samples for screening of the samples. The pH of samples was measured by digital pH meter. The temperature, chloride, fluoride, TDS and total hardness of samples were measured by thermometer, Ion selective electrode (ISE), Volhard's method and titration with EDTA (complexometric titration) respectively (Kumar et al 2010b). BOD and COD were measured by LaMonte Dissolved Oxygen Test Kit Code 5856 according the procedure given in manual provided with kit.

The chemicals used for analysis were AR grade. The estimation of heavy metals was done by Inductive coupled plasma mass spectrometry (ICP-MS).

Measurement of Metal concentration in Liquid waste

Liquid samples were filtered through Whatman filter paper 42, residue discarded and supernatant was used to determine the total metal in it by ICP-MS.

Measurement of Metal concentration in Solid waste

Metal concentration cannot be measured directly in solid. Therefore, 10% solid waste prepared as leachate to determine the heavy metals concentration. The leachate from solid waste was prepared according to the method described by Standard method given by Ferrari et al., 1999 and Srivastava et al., 2005. The leachate prepared as: 100 g of solid waste was added to 1000 ml of distilled water, which was then kept on a rotary shaker at 180 rpm at 30 $\pm 1^0$C for 24 hr for continuous shaking. The suspension was first course filtered by glass wool and then by Whatman filters paper No. 42. To remove the fine suspended particles it was centrifuged at 3000 rpm for 15 min and the supernatant was used after making test dilutions (2.5%, 5.0% and 10%) with double distilled water. The 10% leachate was used for chemical analysis. After metal screening, it was decided to carry out the bioremediation process with four samples viz. HR, DD, MR and RK only due to high content of heavy metals.

Microbial strains

In the present study four microbial species were used: *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp. and *Aspergillus niger*. All the four microbes were isolated from the soil and sludge by serial dilution and pour plating method (Figure 1). Strains were maintained in agar slants containing nutrient broth. They were characterized morphologically and on the basis of biochemical reactions (Table 1).They were transferred weekly to new medium in order to keep metabolic activity and checked for purity by microscopic examination (Figure 2).

Culture medium and Heavy metal exposure

Cells were cultured in nutrient broth with the following composition: beef extract (3.0 g), peptone (10.0 g), dissodium phosphate (1.0 g), sodium chloride (5.0 g), dissolved in one liter of distilled water. Final pH was around 7.4-7.6. The medium was autoclaved at 121ºC for 20 minutes. Cultures were maintained in agar slants (nutrient broth plus 30 g/L agar). They were allowed to grow in the synthetic media having different heavy metal solutions to make capable of heavy metal resistant. The concentration of metals Zn, Mn, Mg, Cu, Cr, Co, Cd Ni and Pb were 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml. Cells were inoculated in nutrient broth (100 mL/flask) and kept under agitation in a rotary shaker, at 80 rpm, for 48 hours at 35 ± 2ºC. Cells to be used in biosorption experiments were separated by centrifugation.

Experiments of biosorption

Experiments of heavy metals biosorption were done in Erlenmeyer flasks containing 150 mL of each samples and 15.0 ± 1.0 mg of cells. To ensure equilibrium, cells and waste were maintained in contact for 48 hours, under constant agitation, at 30-35^{0}C ± 2ºC. In all experiments, cells were obtained from only one cultivation and collected from the same flask at the same growth stage. After 48 hours, cells were separated from the medium and residual metal concentrations were monitored by ICP-MS. Experiments were done in triplicate. The optimum pH and temperature maintained for the growth of microorganisms in the batch culture (Cybulski et al, 2003, Hietala and Roane 2009). The pH and temperature were recorded daily.

Statistical Analysis

The data were statistically analyzed by using one way analysis of variance (ANOVA) at $p = 0.05$. Before statistical analyses were performed, data were tested for normality, if needed. The obtained data had a normal distribution and were not distorted before statistical analysis (Ashok et al 2010). All statistical analyses were performed with Statistical Analysis System programs (SAS 2001, Snedecor and Cochran 1982).

RESULTS

Recent studies have demonstrated that microbes might be used to remediate metal contamination by removing metals from contaminated water or waste streams, sequestering metals in soils and sediments or solubilizing metals to aid in their extraction. This is primarily accomplished either by biosorption of metals or enzymatically catalyzed changes in the metal redox state. Bioremediation of metals is still primarily a research problem with little large-scale application of this technology (Chatterjee et al 2008, Vidali 2001). The figure 1 and 2 depicts the cultures and biochemical tests for characterization of acclimated microbes.

Figure 1(a) and (b). Showing cultures and working in LAF

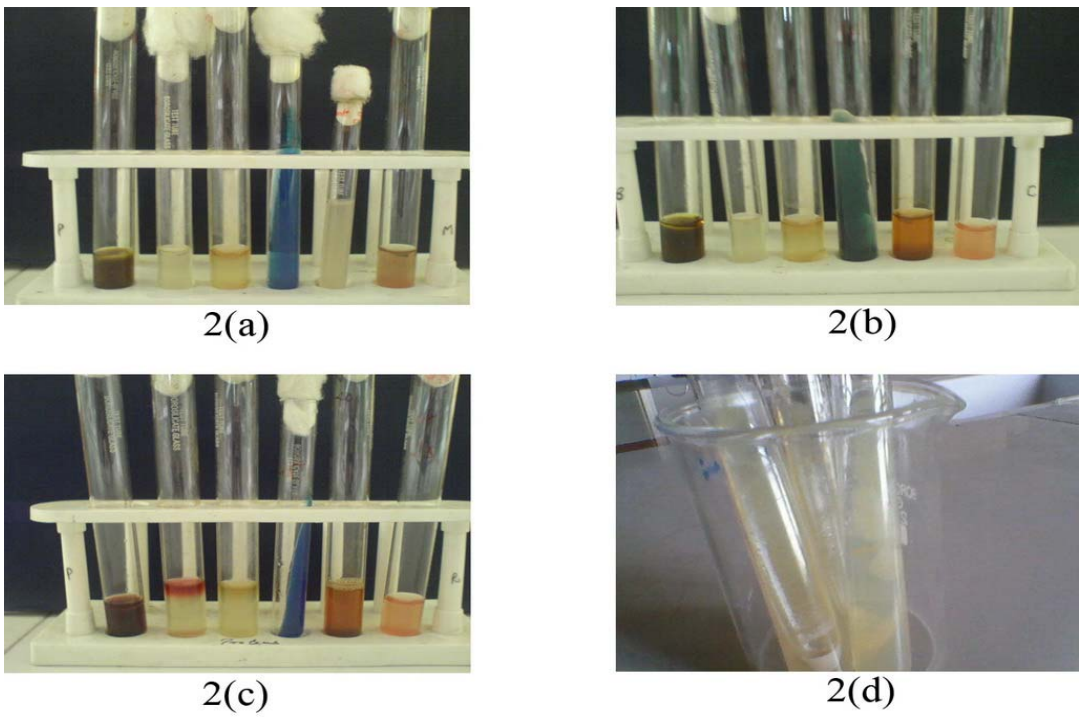

Figure 2. Showing Biochemical tests for *Pseudomonas* spp. (a), *Bacillus* spp. (b) *Staphylococcus* spp. (c) and *A. niger* (d)

Table 1. Biochemical test of acclimated microorganisms

S No	Indole	MR	VP	Citrate utilization	Urease test	Nitrate Reduction	Catalase	Oxidase	Identified Organism/s
1.	_	+	-	+	-	+	+	+	*Staphylococcus* sp.
2.	+	+	_	_	+	+	_	_	*Bacillus* sp.
3.	_	_	+	+	_	+	+	+	*Pseudomonas* sp.

Note: *A. niger* was identified by microscopic examination.

Physico-chemical Parameters of Samples

The physical and chemical parameters viz. temperature, pH, BOD, COD, TH, TDS, fluoride and chloride listed in table 2. The pH ranged from 3.1 to 9.6 and temperature ranged from 18 to 34^0C. The BOD is the degree of pollution. More BOD, more polluted the water/sample. BOD ranged from 8.5 to 53 mg/l, COD ranged form 98 to 780mg/l, total hardness ranged from 145 to 340mg/l and total dissolved solids found in the range of 105 to 850mg/l. The calcium and Magnesium ions ranged from 45mg/l to 280mg/l and 45mg/l to 230mg/l respectively. The fluoride and chloride was estimated and found that they were more than acceptable limit. Maximum permissible limit of chloride is 1000 mg/L and desirable limit of chloride is 250 mg/L as per Indian standards. Desirable limit of fluoride is 1.0 mg/L and permissible limit is 1.5 mg/L as per Indian standards. This indicates that wastes contain many pollutants and it should not be disposed off directly to the water bodies.

Table 2. Physico-chemical analyses of waste samples: (mgl^{-1} except pH and temperature)

S No	Sites/Code	Sample	pH	Temp	BOD	COD	TH	TDS	Mg	Ca	F^-	Cl^-
1	**New Tehri**	**Solid***	6.8	27	26	485	150	155	50	120	2.30	345
	NT	**Liquid**	6.5	22.4	15	162	154	412	69	98	2.86	125
2	**Chamba**	**Solid**	6.7	28.2	53	355	175	150	120	79	3.82	238
	CB	**Liquid**	8.2	22	22	552	156	178	98	45	2.73	321
3	**Badshahitahul**	**Solid**	7.5	28	43	532	340	410	79	120	2.55	254
	BT	**Liquid**	6.5	25	12	180	220	155	45	210	3.45	236
4	**Srinagar**	**Solid**	6.2	32	33	780	200	142	120	159	2.08	252
	SN	**Liquid**	6.0	24	27	368	175	145	110	175	2.15	220
5	**Pauri**	**Solid**	6.5	29	15.4	188	156	302	95	135	3.45	110
	PG	**Liquid**	6.8	26	22	126	340	155	115	120	3.85	450
6	**Rishikesh**	**Solid**	7.2	30	15	430	220	140	180	230	2.59	424
	RK	**Liquid**	7.1	23	17.5	265	200	421	120	130	2.30	234
7	**Haridwar**	**Solid**	7.3	31	21	680	340	580	155	160	2.85	120
	HR	**Liquid**	6.5	24	11.5	485	220	490	168	105	3.56	358
8	**Dehradun**	**Solid**	6.3	31	18.4	662	200	356	190	120	2.30	512
	DD	**Liquid**	6.9	25	12.5	655	175	850	230	280	2.55	550
9	**Mussorrie**	**Solid**	9.6	27	38.2	235	156	334	210	295	3.45	315
	MR	**Liquid**	6.3	18.5	20.4	178	340	677	215	232	2.50	235
10	**Kotdwar**	**Solid**	8.4	34	28.3	165	340	432	190	200	2.30	285
	KD	**Liquid**	5.3	26	16.5	98	220	435	180	135	2.85	210
11	**Rudraprayag**	**Solid**	4.1	29.9	16	178	200	157	190	120	3.85	198
	RG	**Liquid**	7.4	24.5	8.5	145	175	342	159	115	2.13	321
12	**Devprayag**	**Solid**	3.1	29	18.5	162	156	155	175	180	2.15	325
	DG	**Liquid**	4.8	22.4	12.5	116	340	420	135	120	4.45	132
13	**Haldwani**	**Solid**	5.8	30	18.9	552	220	155	120	155	2.80	150
	HD	**Liquid**	6.2	26.2	14.2	243	200	124	115	168	1.85	421
14	**Nainital**	**Solid**	3.8	22	34	312	340	152	130	120	2.03	234
	NL	**Liquid**	7.4	18	18	230	150	140	160	90	2.85	240
15	**Lakshar**	**Solid**	3.1	34	35	485	154	105	105	98	3.45	124
	LS	**Liquid**	4.8	26	17.5	162	175	215	110	79	3.05	234
16	**Uttarkashi**	**Solid**	5.8	28	23	155	156	105	98	45	2.55	175
	UK	**Liquid**	6.8	22	11.5	122	145	149	95	60	3.50	126

*The parameters measured in Leachate form.

Total Metal Analysis of the Samples and Selection of samples for Bioremediation

The total metals analysis was done by ICP-MS and the metals Mn, Cd, Cr, Cu, Zn, As and Pb were determined in liquid waste (sludge) and industrial wastewater, while other metals viz. Cr, Co, Zn, Mn and Ni were measured in solid waste (Table 3). Hg and As were not detected in all the samples. The concentration of zinc ranged from 0.76-9.98mg/l, manganese ranged 0.98-45.75mg/l, nickel 0.032-7.60mg/l, copper 0.015-5.95mg/l, chromium 0.018-8.56mg/l, cadmium 0.001-0.523mg/l, lead 0.010-0.865mg/l and cobalt ranged 0.002-0.905mg/l. Desirable limit for chromium, cadmium, lead, zinc is 0.05 mg/l, 0.01 mg/l, 0.05 mg/l, 5.0 mg/l respectively according to Indian standards. This indicates that wastes contain many heavy metals; it needs to remediate before disposing off. The four samples HR, DD, MR and RK, out of sixteen, were selected for this study due to high content of heavy metals (Table 3).

Table 3. Metal Screening in the samples (mgl^{-1})

S No	Sites	Sample	Zn	Mn	Ni	Cu	Cr	Cd	Pb	Hg	Co	As
1	**New Tehri NT**	Solid*	ND	**1.50**	**0.06**	ND	ND	ND	ND	ND	**0.045**	ND
		Liquid	**5.989**	**7.80**	ND	**0.015**	ND	**0.001**	**0.010**	ND	ND	ND
2	**Chamba CB**	Solid	ND	**3.60**	**0.40**	ND	ND	ND	ND	ND	**0.079**	ND
		Liquid	**6.840**	**6.50**	ND	**0.89**	**0.098**	**0.003**	**0.101**	ND	ND	ND
3	**Badshahitahul BT**	Solid	ND	**8.95**	**0.76**	ND	ND	ND	ND	ND	**0.040**	ND
		Liquid	**0.760**	**4.76**	ND	**2.08**	**0.054**	**0.004**	**0.150**	ND	ND	ND
4	**Srinagar SN**	Solid	ND	ND	**0.78**	ND	ND	ND	ND	ND	**0.120**	ND
		Liquid	**6.980**	**10.65**	ND	**0.45**	**0.07**	**0.002**	**0.230**	ND	ND	ND
5	**Pauri PG**	Solid	ND	**12.50**	**0.85**	ND	**0.09**	ND	ND	ND	**0.230**	ND
		Liquid	**4.560**	**15.90**	ND	**1.09**	**0.03**	**0.007**	**0.830**	ND	ND	ND
6	**Rishikesh RK**	Solid	**7.980**	**11.45**	**2.60**	ND	**0.96**	ND	ND	ND	**0.420**	ND
		Liquid	**8.500**	**13.67**	ND	**1.80**	**1.45**	**0.020**	**0.865**	ND	ND	ND
7	**Haridwar HR**	Solid	**7.830**	**10.90**	**5.30**	ND	**2.09**	ND	ND	ND	**0.550**	ND
		Liquid	**8.227**	**20.50**	ND	**3.90**	**8.56**	**0.523**	**0.858**	ND	ND	ND
8	**Dehradun DD**	Solid	**7.908**	**30.20**	**5.50**	ND	**3.34**	ND	ND	ND	**0.605**	ND
		Liquid	**9.980**	**45.75**	ND	**4.78**	**1.85**	**0.405**	**0.560**	ND	ND	ND
9	**Mussorrie MR**	Solid	**8.455**	**12.97**	**7.60**	ND	**0.56**	ND	ND	ND	**0.905**	ND
		Liquid	**8.120**	**34.80**	ND	**5.95**	**1.05**	**0.210**	**0.202**	ND	ND	ND
10	**Kotdwar KD**	Solid	**6.458**	**11.90**	**5.95**	ND	**0.45**	ND	ND	ND	**0.430**	ND
		Liquid	**5.435**	**12.40**	ND	**3.78**	**0.76**	**0.100**	**0.322**	ND	ND	ND
11	**Rudra prayag RG**	Solid	ND	ND	**6.50**	ND	**0.32**	ND	ND	ND	**0.450**	ND
		Liquid	**4.550**	**9.87**	ND	**1.65**	ND	**0.012**	**0.025**	ND	ND	ND
12	**Devprayag DG**	Solid	**3.455**	**4.58**	**3.30**	ND	**0.05**	ND	ND	ND	**0.230**	ND
		Liquid	**4.350**	**8.90**	ND	**0.79**	ND	**0.015**	**0.054**	ND	ND	ND
13	**Haldwani HD**	Solid	**2.305**	**5.45**	**1.95**	ND	**0.56**	ND	ND	ND	**0.087**	ND
		Liquid	**4.550**	ND	ND	**0.320**	**0.87**	**0.010**	**0.120**	ND	ND	ND
14	**Nainital NL**	Solid	ND	**9.50**	**1.29**	ND	ND	ND	ND	ND	**0.089**	ND
		Liquid	**2.320**	**4.60**	ND	**0.110**	ND	**0.008**	**0.010**	ND	ND	ND
15	**Lakshar LS**	Solid	ND	ND	**0.65**	ND	**0.018**	ND	ND	ND	**0.076**	ND
		Liquid	**3.450**	**3.07**	ND	**0.078**	**0.020**	**0.005**	**0.011**	ND	ND	ND
16	**Uttarkashi UK**	Solid	ND	**0.980**	**0.03**	ND	ND	ND	ND	ND	**0.002**	ND
		Liquid	**3.120**	ND	ND	**0.065**	ND	**0.003**	**0.013**	ND	ND	ND

*The parameters measured in Leachate form.

ND= Not Detected

***Biosorption of metal by Bacillus* sp.**

The heavy metals reduced by *Bacillus* sp. at pH 6.2 and temperature 35^0C is listed in Table 4. The nickel and copper were sorbed by *Bacillus* spp. The average Ni reduction was 48% and Cu reduction was recorded as 65%. The pH increases from 6.2 to 7.4 and temperature from 35^0C to 38^0C.

Table 4. Biosorption with *Bacillus* sp. at pH 6.2 and temperature 35 ^{0}C

S No.	Site	Sample	pH	Temp	Ni (Before)	Ni (After)	Cu (Before)	Cu (After)
1	**HR**	Solid*	**6.5**	**37**	5.30	2.32	ND	ND
		Liquid	**7.4**	**38**	ND	ND	3.90	1.755
2	**DD**	Solid	**6.8**	**36**	5.50	2.75	ND	ND
		Liquid	**7.2**	**37**	ND	ND	4.78	2.055
3	**MR**	Solid	**6.2**	**35**	7.60	3.80	ND	ND
		Liquid	**6.8**	**37**	ND	ND	5.95	2.618
4	**RK**	Solid	**7.4**	**38**	2.60	1.43	ND	ND
		Liquid	**7.2**	**37**	ND	ND	1.80	0.810

ND= Not Detected

***Biosorption of Metal by Pseudomonas* sp.**

The heavy metals reduced by *Pseudomonas* sp. at pH 6.5 and temperature 35^{0}C was listed in Table 5. The average Ni reduction was 56% and Cu reduction was recorded as 68%. The pH increases from 6.5 to 8.5 and temperature from 35^{0}C to 39^{0}C.

Table 5. Biosorption with *Pseudomonas* sp. at pH 6.5 and temperature 35 ^{0}C

S No.	Site	Sample	pH	Temp (^{0}C)	Ni (Before)	Ni (After)	Cu (Before)	Cu (After)
1	**HR**	Solid*	7.2	36	5.30	1.560	ND	ND
		Liquid	7.5	38	ND	ND	3.90	1.247
2	**DD**	Solid	7.8	35	5.50	1.925	ND	ND
		Liquid	7.9	39	ND	ND	4.78	1.530
3	**MR**	Solid	8.5	37	7.60	2.585	ND	ND
		Liquid	8.4	39	ND	ND	5.95	1.785
4	**RK**	Solid	8.5	38	2.60	0.962	ND	ND
		Liquid	7.8	35	ND	ND	1.80	0.615

ND= Not Detected

***Biosorption of Metal by Staphylococcus* sp.**

The heavy metals reduced by *Staphylococcus* sp. at pH 6.5 and temperature 37^{0}C was listed in Table 6. The average Cu reduction was 42%, Cr reduction 45% and most reduction was recorded in case of Pb and it was 93%. The pH increases from 6.5 to 7.4 and temperature from 35^{0}C to 39^{0}C.

Table 6. Heavy metal concentration after Biosorption with *Staphylococcus* sp. at pH 6.5 and temperature 35 ^{0}C

S No.	Site	Sample	pH	Temp (^{0}C)	Cu (Before)	Cu (After)	Cr (Before)	Cr (After)	Pb (Before)	Pb (After)
1	**HR**	Solid*	6.4	35	ND	ND	2.09	1.128	ND	ND
		Liquid	6.6	37	3.90	2.260	8.56	4.452	0.858	0.60
2	**DD**	Solid	6.5	36	ND	ND	3.34	1.804	ND	ND
		Liquid	6.8	38	4.78	2.725	1.85	1.018	0.560	0.038
3	**MR**	Solid	7.4	36	ND	ND	0.56	0.325	ND	ND
		Liquid	6.7	39	5.95	3.335	1.05	0.578	0.202	0.016
4	**RK**	Solid	6.5	35	ND	ND	0.96	0.547	ND	ND
		Liquid	7.2	39	1.80	1.08	1.45	0.798	0.865	0.052

ND= Not Detected

Biosorption of Metals by Aspergillus niger

The heavy metals reduced by *Aspergillus niger* at pH 7 and temperature 30^{0}C was listed in Table 7. *Aspergillus niger* reduced the zinc and cadmium only. The average Zn reduction was 58%, and Cd reduction was recorded as 50%. The pH increases from 7 to 8.0 and temperature from 30^{0}C to 35^{0}C in this case.

Table 7. Heavy metal concentration after Biosorption with *Aspergillus niger* at pH 7.0 and temperature 30 ^{0}C

S No.	Site	Sample	pH	Temp (^{0}C)	Zn (Before)	Zn (After)	Cd (Before)	Cd (After)
1	**HR**	Solid*	7.2	32	7.830	3.367	ND	ND
		Liquid	7.2	37	8.227	3.455	0.523	0.256
2	**DD**	Solid	7.4	31	7.908	3.401	ND	ND
		Liquid	7.5	29	9.980	3.992	0.405	0.202
3	**MR**	Solid	7.8	34	8.455	3.551	ND	ND
		Liquid	8.0	32	8.120	3.410	0.210	0.107
4	**RK**	Solid*	7.8	36	7.980	3.431	ND	ND
		Liquid	7.2	31	8.500	3.485	0.020	0.010

ND= Not Detected

DISCUSSION

The primary goal of metal remediation is to remove the metal from the waste or to decrease metal mobility and toxicity within the sample. Numerous microbially-mediated reactions can achieve these goals, including metal methylation, oxidation–reduction reactions and metal complexation. The diverse nature of microbial metabolic activities has long been exploited for human purposes, for example in extraction of precious metals from ores in bioleaching. Understanding metal–microbe relationships has led to advances in bioremediation (Malik 2004; Bruins et al. 2000). Metals are toxic to all biological systems from microbial to plant and animal, with microorganisms affected more so than other systems, due, in part, to their small size and direct involvement with their environment (Patel et al. 2007; Sarret et al. 2005; Giller et al. 1999). Metal toxicity negatively impacts all cellular processes, influencing metabolism, genetic fidelity and growth. Loss of bacterial populations in metal-contaminated soils impacts elemental cycling, organic remediation efforts, plant growth and soil structure.

For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. Renella et al (2003; 2005) found that certain metal combinations, e.g., Cu + Zn and Ni + Cd, in soils resulted in decreased soil microbial activities due to bacterial diversity changes. Stefanowicz et al. (2008) found that metals influence fungal and bacterial communities differently. In their study, lead and zinc soil contamination from mining activities resulted in decreased bacterial functional diversity, whereas fungal functional diversity increased amongst the culturable organisms. Some evidence indicates that microbial community activities recover upon metal remediation. Numerous microbially mediated transformations of metals have been identified (Roane and Pepper 2000; Nies 1999), many of which may immobilize or mobilize metals in the environment. Microbial transformations of metals are often the result of metal resistance mechanisms that include complexation and precipitation mechanisms as well as solubilization mechanisms that offer bioremediation strategies. The use of biological materials for effective removal of heavy metals contaminant from waste water has emerged as a potential alternative method to conventional treatment technique (Zouboulis et al 2003).

The control and optimization of bioremediation processes is a complex system of many factors. These factors include: the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population; the environment factors such as temperature, pH, the presence of oxygen or other electron acceptors and nutrients. In present study for remediation purpose, four metal resistant microbial strains were isolated from soil and sludge and on the basis of morphological, cultural and biochemical characteristics were tentatively identified as *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp. and *Aspergillus niger*. They were allowed to grow in the media containing heavy metals concentration. In the supplemented media, they were adopted for the concerned metals. They were used for biosorption. The heavy metal chromium reduced by *Staphylococcus* sp., lead reduced by *Staphylococcus* sp., cadmium by *Aspergillus niger*, copper by *Pseudomonas* sp., *Bacillus* sp. and *Staphylococcus* sp. Nickel reduced by *Pseudomonas* sp. and *Bacillus* sp. and zinc reduced by *Aspergillus niger* and *Bacillus* sp. in the present study. The pH and temperature changed during the biosorption process due to metabolic activity. The biosorption of heavy metal depends on the higher heavy metal solution taken for the study. Some basic points about the surface structures of Gram-positive and Gram-negative bacteria should be briefly presented. A characteristic

component of Gram-positive cells are teichoic acids and acids associated to the cell wall, whose phosphate groups are key components for the uptake of metals. The literature reports several studies on the interaction of heavy metals with bacterial surfaces, but just a few works consider these interactions at the molecular level (Beveridge 1989; Da Costa 1999). Thus, a detailed investigation of the chemical structures of bacterial cells and the understanding of the mechanism involved in the interaction is still missing in the study of the bioaccumulation process. Carboxyl groups are the main agents in the uptake of heavy metals. The sources of these carboxyl groups are the teichoic acids, associated to the peptidoglycan layers of the cell wall. Microbial biomass offers an economical option for removing heavy metals by the phenomenon of biosorption (Gupta and Mahapatra 2003), therefore bacterial species are tested for heavy metal removal and it is found that *Pseudomonas* sp. is able to remove copper and nickel better than others and lead is removed better by *Staphylococcus* sp. Wong and So (1993) also reported the similar result with *Pseudomonas* sp. for removal of Cu^{2+}. Lopez et al. (2000) reported that *Pseudomonas fluorescence* 4F39 accumulated heavy metals very rapidly in the order Cu>Cd>Co>Cr>Pb.

Microbial growth and activity are readily affected by pH, temperature and moisture. Although microorganisms have been also isolated in extreme conditions, most of them grow optimally over a narrow range, so that it is important to achieve optimal conditions (Vidali 2001). In the present study, the remediation of lead, cadmium, nickel, copper, zinc and chromium occurred with four acclimated microorganisms. The temperature and pH affect the biosorption process. Therefore, the temperature and pH was adjusted for the optimal growth of concerned organisms. The metal precipitation occurs during the biosorption study. The better remediation found with *Pseudomonas* sp. for copper and nickel, when pH was in range of 7.2-8.5. Sze et al. (1996) also proved that pH range 5-8 is good for heavy metal removal. It may be because a higher concentration of H^+ competed with heavy metal at low pH and hence the removal capacity of cell decreased. Cell age is considered as an important microbial factor that affects metal accumulation. Maximum heavy metal uptake by bacterial strains occurred after three days incubation these results are in conformity with the findings (Mondal, et al 2008). This is possibly due to the presence of many highly active enzymes at this growth phase, during which cells are at their most metabolically active stage. In order to select a suitable acclimated microorganism for further studies, a simple mathematical analysis was performed with the overall results obtained in the four groups of experiments. They were compared and the most suitable strain to accumulate the metals at each concentration level was detected. Due to the poor performance of *A. niger* will not be included in future experiments. The *Pseudomonas* spp. and *Staphylococcus* spp. can be used, in the future, for heavy metals removal from the waste.

CONCLUSION

The microbes play a vital role in the remediation of heavy metals and other pollutants. In the present study, the four samples out of sixteen were screened for biosorption study and four microbial strains *Pseudomonas* sp., *Staphylococcus* sp., *Bacillus* sp. and *Aspergillus niger*. After treatment, *Pseudomonas* sp. and *Bacillus* sp. reduced Cu 4.165mgl^{-1} and 3.332 mgl^{-1}(68% and 56%) and Ni 5.015 mgl^{-1} and 3.8 mgl^{-1} (65% and 48%) respectively. *Aspergillus niger* reduced Cd 0.267 mgl^{-1} (50%) and Zn 5.988 mgl^{-1}(58%) whereas *Staphylococcus* sp. reduced Cr 4.108 mgl^{-1}(45%), Cu 2.615 mgl^{-1}(42%) and Pb 0.813 mgl^{-1} (93%). The results showed that *Pseudomonas* sp. reduced heavy metals more than other microbes but *Staphylococcus* sp. uptake the lead in very significant amount, it was measured that 93% of lead was reduced by *Staphylococcus* spp. Consequently, the *Pseudomonas* sp. which was isolated form soil was more potent bioremediation agent than other three microbes. It may be concluded that microbes can tolerate against the heavy metals and they are armed with various resistance and catabolic potentials. This catabolic potential of microbes is enormous and is advantageous to mankind for a cleaner and healthier environment through bioremediation.

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