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Bioremediation of Zn, Cu, Mg and Pb in Fresh Domestic Sewage by *Brevibacterium* sp.

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ABSTRACT: The study applied an isolated Brevibacterium sp. (MTCC 10313) for bioremediation of Zn, Cu, Mg and Pb in domestic sewage. Batch culture experiments were performed on both the fresh and stale sewage samples with glucose supplementation of 1-8g/l. Nutrient broth medium was prepared, sterilized and pH adjusted to 6.5-6.8. 1% of the Brevibacteria sp. stock was inoculated into the broth and maintained at 370C for 24hrs in shaker incubator at 120 rpm. Another 1% of fresh grown sub-culture of broth was inoculated into supplemented and sterilized samples. Optical Density was taken at 600nm, growth monitored over 12 days, cultured samples denatured with TCA and centrifuged, supernatants filtered and analyzed with AAS, Settled pellets oven dried, subjected to SEM analysis for morphology and constituents determination. Fresh sewage samples permitted bacterial growth and facilitated bioremediation of Zn, Cu and Mg through metal uptake and bioabsoption by Brevibacteria sp. This effectively reduced concentration of heavy metals, with treatment efficiency order Cu>Zn>Mg, and respective removal percentages of 77, 63 and 55. The optimum glucose concentration for effective bioremediation found as 2g/l for Zn and Cu, and 8g/l for Mg. Pb was resistant to bioremediation with Brevibacteria sp. Stale sewage produced inhibitory substances preventing adequate growth of bacterium with no bioremediation. Bioremediation with Brevibacteria sp. is found effective in removal of micro-units of Zn, Cu and Mg from domestic sewage. As a readily available low-cost agent, it is recommended for large- scale application on those metals while Pb should be further subjected to advanced treatments.

Keywords: Bioremediation, Biosorption, Bacteria, Sewage, Metal uptake, Absorbent

INTRODUCTION

One of consequences of industrialization and industrial production is the generation and release of toxic waste products including trace heavy metals like Cd, Pb, Mn, Cu, Zn, Mg, Cr, Fe, and Ni, which are polluting our environment (Baysal et al. 2013). Some conventional methods of heavy metals removal include ion-exchange, electro-winning, coagulation, cementation, reverse osmosis/electrodialysis, electro-coagulation, precipitation, and membrane separation (Kang et al. 2000; Sag and Kutsal, 2001; Wang and Tang, 2001; Ahalya et al. 2003; Wickramasinghe et al., 2004; Wan Ngah and Hanafiah (2008); Baysal et al. 2013). These methods are however characterized by disadvantages such as secondary pollution, high cost, high energy input, large quantities of chemical reagents, and poor treatment efficiency at low metal concentrations (Ahluwalia and Goyal, 2007; Gao et al. 2008). A proven alternative treatment approach, with capacity to overcome most of the identified flaws of the conventional methods, has been found in adsorption and biosorption (Bailey et al. 1999; Sag and Kutsal, 2001; Volesky, 2007; Turker, 2007; Wan et al. 2008; Sud et al. 2008; Demirbas, 2008). Natural adsorbents have gained popularity in recent research activities due to the fact that they are more readily available, require little processing, low-cost, usually byproduct of waste materials from waste industries and isolated organisms are cultivated or propagated for biosorption purposes using inexpensive materials (Sag and Kutsal, 2001; Sekhar et al. 2003; Baysal et al. 2013).

Active biosorption or bioaccumulation is regarded as the second part of the metal sequestering process

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by living biomass. It is capable of removing traces of heavy metals from dilute aqueous solution. Bioaccumulation is an intracellular metal accumulation process which involves metal binding on intracellular compounds, intracellular precipitation, methylation and other mechanisms. Biosorption is a passive immobilization of metals by biomass and presently one of the promising technologies in removal of toxic metals from aqueous solutions of municipal or industrial wastewater (Gadd, 1988; Brierley, 1990, Senthikumar et al. 2000). The mechanisms of sorption by cell surface are unrelated to cell metabolism, as they are based on physicochemical interactions taking place between the metal and functional groups of the cell wall. In the process, the first barrier to penetration of metals into micro-organisms is the cell wall or through an alternative process of metal uptake by bioabsorption (Kadukova and Vircikova, 2005; Mohan and Pittman, 2007). This provides some protection for the cytoplasmic membrane and therefore serves as a biosorbent. Bacteria are examples of biomass-derived sorbent for several metals (Thakur, 2012). In wastewater treatment there are 3 types of bacteria used to treat wastes in the treatment plants: aerobic, anaerobic and facultative all based on the presence or absence of dissolved oxygen for their respiratory activities. Aerobic ones are employed in aerated wastewater environments, anaerobic normally used in anaerobic digesters where they obtain oxygen for respiration from the substrate through fermentation process, and facultative bacteria are capable of switching their respiratory modes between the two above.

The central objective of this study is to determine the efficiency of *Brevibacterium* sp (MTCC 10313) in bioaccumulation of Zn, Cu, Mg and Pb in domestic sewage samples. It is a gram-positive aerobic bacterium that has been isolated from the coffee pulp by Nayak et al. 2012. The configuration and composition of *Brevibacterium* sp. with cross-lined cell walls, positive growth in lactose, fructose, citrate, and lysine are its potential metal binding features. Its temperature range for growth is another plus as a metal bioaccumulation property. At higher pH within which *brevibacterium* sp. grows, it has been observed that the adsorbent surfaces are generally of higher affinity thereby facilitating attraction/binding with the metal ions (Lohani et al. 2008). The study therefore focuses on bioremediation of the selected heavy metals with *Brevibacterium* sp., a low-cost adsorbent.

MATERIALS & METHODS

(a) Chemicals, reagents and culture media: The Dextrose anhydrous purified (*carbon source/glucose supplement*) and Tri-chloroacetic acid (*TCA*) were procured from E. Merck (India) Ltd., Mumbai. The nutrient broth, NaOH and HCl were all purchased from HiMedia Laboratories Pvt. Ltd., Mumbai.

(b) Sewage samples and the bioabsorbent: To facilitate the bioremediation study on very low concentrations of heavy metals (in micro-grams levels), samples were obtained from the inlet unit of the raw domestic sewage at the Wastewater Treatment Plant of NMAM Institute of Technology, NMAMIT, Nitte. The trace presence of these heavy metals in the wastewater may be attributed to leaching and wears from lead and metal sewer materials. Other likely cause may be wet-weather-flow, partially combined sewerage system encompassing erosion in the study area. Samples were taken for immediate laboratory experiments on fresh sewage study while those of stale sewage were conducted after 35-49 days of sampling. Industrial wastewater samples were taken from the influent of Lamina Foundry Treatment Plant, Nitte, Karnataka State, India to serve as the control. Fresh and stale samples from this were equally subjected to the bioremediation treatment. Sampling was in accordance with the Standards (APHA, 2005). A previously isolated and cultured *Brevibacterium* sp. as per the protocols of Holt, et al. 2000 was used as bioabsorbent. The culture was obtained from the Department of Biotechnology Engineering, NMAMIT, Nitte. Initial concentrations of heavy metals under study; Zn, Cu, Mg and Pb were determined in all the samples and the control with the use of Atomic Absorption Spectrometer (AAS) Avanta GM Model.

(c) Preparation of medium and flask culture experiment for growth curve: The minimal medium was prepared by dissolving nutrient broth ingredient in distilled water as 13g/l in a conical flask. Sampled sewage was collected in clean 2-litre plastic bottles with stoppers and was prepared by filtering through Dr. Watts' 9cm filter paper (CAT No.501). This was aimed at removing all suspended particles from the final samples for treatment. At room temperature, the pH in each of the sample's filtrate was adjusted with HCl or NaOH to between 6.5-6.8 for ensuring optimum metal binding condition. Supplemented culture was prepared by adding 1-8g/l of glucose into each of the 5 fresh samples in the side-arm flasks, at 1, 2, 4, 6 and 8g/l respectively.

The procedure was repeated for samples taken from the industrial wastewater but supplemented with only 1g/l in the 6^{th} flask, because the effluent is generated from a foundry it does not contain any nutrients or carbon source, therefore it was supplemented with minimum amount of carbon sourced. A blank sample was maintained in the 7^{th} side-arm flask to serve as

supplementation control during Optical Density (OD) measurements. These and nutrient broth solution were cotton plugged, wrapped, and sterilized by autoclaving at 121^{0}C for 15 minutes and cooled to room temperature. Then 250µl of the stock (*Brevibacteria* sp.) representing 1%, was aseptically inoculated in the Laminar Air Flow chamber into the sterilized broth and transferred into a rotary shaker incubator (ROTEK Rotary Make) at 120 rpm and maintained at a constant temperature of 37^{0}C. Then another 1000µl of the fresh (24hrs) grown sub-culture in nutrient broth, representing 1% was again aseptically inoculated into each of the supplemented and sterilized wastewater samples. The initial OD was taken at 600nm with the use of Spectronic 20 spectrophotometer at intervals of 2hrs from the time of inoculation (Aneja, 1996; Holt et al. 2000, Gokulakrishnan et al. 2005). The above procedures were adopted in both the control experimentation with the industrial wastewater sample and the main experimentation on the domestic sewage.

(d) Preparation of the samples and their analyses by AAS, SEM and EDAX: After the cultured samples have reached their stationary stage, 10ml of supernatant from each treated sample was taken and added to 12.5ml of 24% TCA solution as a denaturing agent to terminate microbial growth if any and precipitate all proteins. This was kept in the rotary incubator shaker at 100 rpm for 30 mins. The denatured culture was then subjected to centrifugation using REMI Cooling Microfuge; CENTRIFUGE CM-12; 405 at 5000 rpm for 15 mins. The procedure was repeated for all samples and the control. The separated supernatants were filtered and analyzed with AAS. The settled pellets were dissolved in distilled water to form a homogenous substance that was oven dried at 100^{0}C for 72 hrs and subjected to SEM and EDAX analysis for the determination of morphology and the constituents at the Metallurgy Department, National Institute of Technology Karnataka (NITK), Surathkal. In AAS analysis the supernatants of each treated samples were atomized and introduced to the flames. The flame temperature was varied by adjusting the fuel flow rate and the fuel itself. The high temperature of the flame atomizes the species being analyzed. A hollow cathode lamp is used to emit specific wavelengths that are characteristic absorption wavelengths for the metal being analyzed. The amount of light given off by this lamp into the sample is carefully measured (Hoffmann et al., 2005). This is achieved by using a hollow cathode made of the same metal as the one being analyzed. These atoms absorb the radiation and are excited into a higher electronic state. A photomultiplier tube is used to detect the amount of light passing through the flame. The difference in light is the one absorbed by the atoms being analyzed. Since the amount of light being absorbed is directly proportional to the concentration of the metal ions in the samples, a calibration curve of the metal can be prepared. This curve can be used to find the unknown concentration. In this study the focus was on Zn, Cu, Mg and Pb. Therefore the hollow cathode lamp containing each of the respective metals was used. A calibration curve was generated by the equipment after diluting 50ppm of the respective metal solution to produce other solutions of known concentration. The absorbance of these samples were measured at wavelengths (in nm) emitted by the hollow cathode lamps as instructed in the User's Guide of the AAS-GBC Scientific Equipment Pvt, Ltd. Avanta G. A Beer-Lambert's law plot is constructed as the calibration curve (Vinitha et al., 2009).

Specimens for Scanning Electron Microscopy/ Energy Dispersive X-Ray Analysis (SEM/EDAX) were prepared by carbon taping. They were stuck on the carbon tape plate and subjected to detail-obscuring conducive coating, gold coating using Auto-fine coater equipment JEOL, JEC-1600 to improve the conductivity of specimen since it is a non-conductive sample. The carbon-taped sample was transferred into JEOL fine-coater at pressure of 30Pa and allowed to be pressurized to a value <5Pa, creating a vacuum inside. After which certain rays were displayed, coating the specimen and the timing count-down was from 60 to 0 sec. Coated specimens were immediately transferred into the Specimen Stage of the SEM (Model: JEOL JSM-6380LA, Analytical Scanning Electron Microscope) after its cooling system has been topped with liquid nitrogen coolant. The surface morphology of the specimens were then examined by SEM and monitored on the attached Personal Computer (PC). At the same time EDAX of the specimens were performed and the elements present in the specimens identified with their concentration levels.

RESULTS & DISCUSSION

From the control experimentation, it is observed that there was no appreciable growth in the inoculated broth of industrial wastewater sample, labeled AF at1g/ l glucose supplementation. The OD measurements were less than 0.1 in most cases and therefore considered as insufficient growth. On the other hand, in the domestic sewage sample labeled BD after 26 hrs of incubation, there was good growth evidenced in measured OD values for 1g/l glucose supplement, whereas the same effluent and with much higher glucose concentration of 8g/l shows poor growth. This growth in 8g/l glucose supplemented domestic sewage is minimal and lacking agglomeration of particles probably because there is no nutritional deficiency and the bacteria is not stressed. On the other hand, with

the 1g/l supplement of glucose, the bacteria are perceived to have been under stress for nutrition, so the growth and agglomeration are more pronounced. Observing the trend in the levels of Zn and Cu after treatment with 2 and 4g/l glucose supplementation, it is evident that level of carbohydrate in the medium has some roles to play in bioremediation of these metals with *Brevibacterium* sp. (Table 1) The representation of various samples and their full descriptions are as shown on same Table.

For bioremediation of these 2 metals, the trend noted in 1 and 8g/l supplementation is almost similar, with values for Zn (0.41 and 0.42 µg/l) and for Cu (0.37 and 0.34 µg/l) respectively. For Mg, it is observed that even though there is bioremediation at different levels of sugar concentrations, maximum bioremediation was observed at 8g/l. The trend at 4g/l glucose supplementation there seems to be less bioremediation compared to at other sugar concentrations. At 4g/l the Mg bioaccumulation seems to have been inhibited. The bioremediation patterns of the metals with glucose supplementation at different concentration are shown in Fig. 1. It therefore follows that, *Brevibacteria* has proven to be an effective bioremediation agent for very low concentrations of trace metals in domestic sewage, such ranges as Zn (0.16 – 0.43µg/l); Cu (0.24 – 0.94 µg/l); and Mg (2.37 – 5.18 µg/l). This is a clear advantage of *Brevibacteria* over some commercial adsorbents. The range of values for Pb was however found to be between 0.01 and 0.78 in an irregular pattern. This could be due to the fact that Pb particles were not easily adsorbed by Brevibacteria. In addition to that, it has been reported that Pb at a certain concentration of 10^{-5} to $2x10^{-4}$ M produces about 50% inhibition of enzyme –SH and –COOH groups (Levina, 1972).

It is interesting to note that, when the stale/aged domestic sewage (of 49days) was subjected to bioremediation, there is no uniform pattern observed in bioremediation of the heavy metals (Table 2).

It correlates with the decreased growth kinetics of the microbe as compared with the fresh domestic sewage. This indicates that the stale sewage over the time produces certain inhibitors inherently for unknown reasons and these might have interfered with the growth of the bacteria and subsequently the bioremediation process has not taken place. This could be responsible for no bacterial growth observed and improper bioremediation in the stale domestic sewage. The haphazard trend of bioremediation result from the stale sewage is represented in Fig. 2.

The bacterial growth pattern observed in both fresh and stale samples are presented in Tables 3 and 4, while the trends with time are represented in Figs 3 and 4. It is evident from the tables and figures that the rate of growth in fresh sewage samples is 4 to 5 times higher than the rate of growth in the stale samples. Increased glucose supplementation in stale wastewater

Table 1. Summary of the AAS results of analyzed heavy metals contents in fresh domestic wastewater

Sample label	Full description	**Zn** (µg/l)	**Cu** (µg/l)	**Mg** (µg/l)	**Pb** (µg/l)
AFFr01Raw	Fresh raw foundry wastewater with 1g/l glucose supplement	0.55	1.28	2.65	0.07
BDFr01Raw	Fresh raw domestic wastewater with 1g/l glucose supplement	0.43	0.94	5.18	0.01
BDFr01In	Inoculated fresh domestic wastewater with 1 g/l glucose supplement	0.41	0.37	3.19	0.37
BDFr02In	Inoculated fresh domestic wastewater with 2 g/l glucose supplement	0.16	0.22	3.30	0.78
BDFr04In	Inoculated fresh domestic wastewater with 4 g/l glucose supplement	0.20	0.24	4.07	0.04
BDFr06In	Inoculated fresh domestic wastewater with 6 g/l glucose supplement	0.38	0.38	3.28	0.16
BDFr08In	Inoculated fresh domestic wastewater with 8 g/l glucose supplement	0.42	0.34	2.37	0.38

Table 2. Summary of the AAS results of analyzed heavy metals contents in stale domestic wastewater

Sample label	Full description	Zn *(µg/l)*	Cu *(µg/l)*	Mg *(µg/l)*	Pb *(µg/l)*
AFSt01Raw *(aged 49 days)*	Stale raw foundry wastewater	0.14	0.03	2.03	0.20
BDSt01Raw *(aged 49 days)*	Stale raw domestic wastewater	0.10	0.05	2.01	0.16
BDSt01In *(aged 49 days)*	Inoculated stale domestic wastewater with 1 g/l glucose supplement	0.45	0.03	2.93	1.12
BDSt02In *(aged 49 days)*	Inoculated stale domestic wastewater with 2 g/l glucose supplement	0.11	0	1.87	0.02
BDSt02C *(aged 49 days)*	Control stale domestic wastewater with 2 g/l glucose supplement	0.17	0.01	2.99	0.01
BDSt04In *(aged 49 days)*	Inoculated stale domestic wastewater with 4 g/l glucose supplement	0.19	0	3.39	0.19
BDSt06In *(aged 49 days)*	Inoculated stale domestic wastewater with 6 g/l glucose supplement	0.57	0	4.16	0.01
BDSt08In *(aged 43 days)*	Inoculated stale domestic wastewater with 8 g/l glucose supplement	0.99	0.41	2.77	0.03
BDSt08C *(aged 43 days)*	Control stale domestic wastewater with 8 g/l glucose supplement	0.64	0.31	2.13	0.21

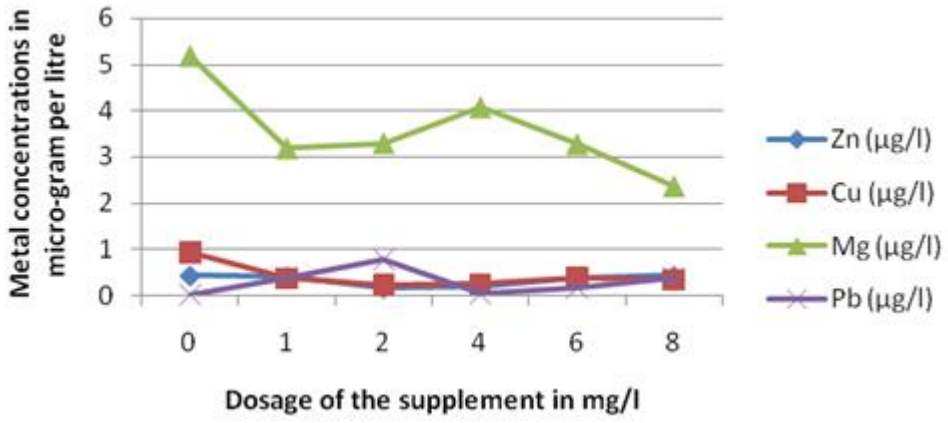

Fig. 1. Brevibacterium bioremediation trend observed in the fresh domestic sewage

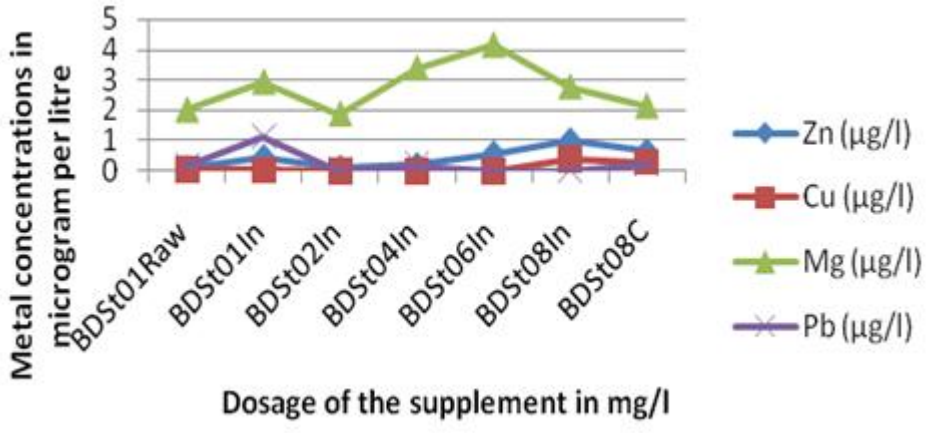

Fig. 2. Brevibaterium bioremediation trend observed in the stale domestic sewage

Table 3. Results of the bacterial growth monitoring/measurement experiments in fresh wastewater samples

	Samples and measured OD @600nm results					
Time/Hour	**BDFr01In**	**BDFr02In**	**BDFr04In**	**BDFr06In**	**BDFr08In**	**BDFr02C**
0th	0.19	0.23	0.24	0.24	0.24	0.22
2nd	0.30	0.30	0.27	0.28	0.23	0.26
4th	0.39	0.26	0.31	0.31	0.35	0.25
24th	0.52	0.71	0.58	0.57	0.56	0.24
26th	0.52	0.71	0.58	0.54	0.56	0.22
28th	0.52	0.72	0.58	0.55	0.53	0.23
48th	0.48	0.63	0.45	0.52	0.52	0.21
72nd	0.43	0.56	0.44	0.49	0.53	0.22
120th	0.43	0.56	0.44	0.54	0.60	0.22
144th	0.43	0.53	0.44	0.56	0.59	0.22
288th	0.43	0.53	0.44	0.56	0.59	0.22

Table 4. Results of the bacterial growth monitoring/measurement experiments in stale wastewater samples

	Samples and measured OD @600 nm results					
Time/Hour	**BDSt01In** *(aged 43days)*	**BDSt02In** *(aged 43days)*	**BDSt04In** *(aged 43days)*	**BDSt06In** *(aged 43days)*	**BDSt08In** *(aged 35 days)*	**BDFr02C** *(aged 43days)*
0th	0.04	0.02	0.01	0.04	0.05	0.04
2nd	0.12	0.09	0.08	0.10	0.13	0.03
4th	0.32	0.12	0.10	0.15	0.19	0.02
24th	0.37	0.14	0.10	0.14	0.43	0.03
26th	0.37	0.14	0.10	0.15	0.44	0.03
28th	0.37	0.15	0.10	0.14	0.46	0.03
48th	0.38	0.16	0.11	0.18	0.57	0.03
72nd	0.37	0.14	0.12	0.16	0.58	0.02
120th	0.37	0.14	0.12	0.16	0.59	0.03
144th	0.38	0.14	0.11	0.16	0.58	0.02
288th	0.38	0.14	0.11	0.16	0.58	0.02

samples did not facilitate the growth of microbes, whereas in the fresh sewage sample it did make difference and facilitated appreciable growth. One of the stale sewage samples of 35days old and supplemented with 8g/l glucose shows slightly higher growth as compared to others, which are 43days old samples. The matter of 8 days difference in the ageing of the sewage sample had a significant impact on growth of bacteria. This clearly shows that the more the ageing or staleness of the sewage sample, the stronger is its inhibitory action on the growth of the microbe. The presumption that inherent inhibitors are produced in the staling of sewage sample, which impedes the bacterial growth, is evident from Fig. 3, as compared to Fig. 4. The growth at 8g/l glucose supplementation in the stale sewage is about 60% of the growth found in 2g/l glucose supplemented fresh sewage sample. Similarly, the growth observed in 1g/l glucose supplemented aged sewage sample was about the same as in the control sample of the fresh sewage sample.

Percent bioremediation efficiencies of *Brevibacteria* on the heavy metals in both the fresh and stale sewage samples are represented on Tables 5 and 6. The trend in the fresh wastewater is graphically shown in Fig. 5. Of the studied metals, Cu was most efficiently bioremediated (80%) and response was positive at the range of 2-4g/l glucose supplementation, with the optimum concentration of glucose being 2g/l. In all the supplementation of glucose, Cu is found to have bioremediated beyond 60%. Zn was bioremediated at 2g/l supplementation of glucose up to 63%. Effective range of bioremediation of Zn was noticed to be between 2-4g/l glucose supplementation, where much more than half of its concentrations are removed. Highest bioremediation of Mg was observed at 8g/l glucose supplementation and with a value of nearly 55%. At lower glucose concentration the removal of Mg was less than 50%. Pb on the other hand, is observed to be totally resistant to the bioremediation by *Brevibacteria*. At any concentration of glucose supplementation it does not show bioremediation of Pb. This finding about Pb bioremediation corroborates

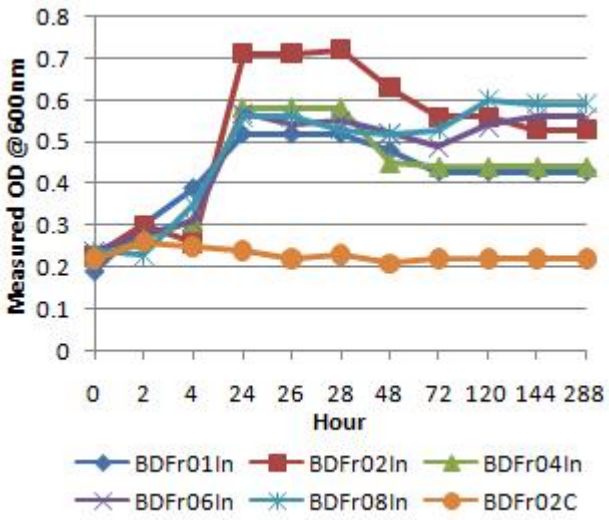

Fig. 3. Growth pattern of *brevibacterium* culture in the fresh sewage

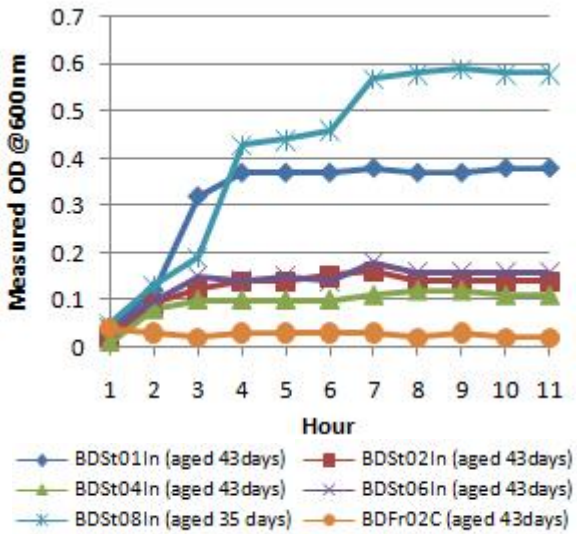

Fig. 4. Growth pattern of *brevibacterium* culture in the stale sewage

Table 5. Summary of the % removal of metals as compared with initial concentrations in fresh domestic wastewater

Sample label	Zn (µg/l)	% removal of Zn	Cu (µg/l)	% removal of Cu	Mg (µg/l)	% removal of Mg	Pb (µg/l)	% removal of Pb
BDFrRaw	0.43	-	0.94	-	5.18	-	0.01	-
BDFr01In	0.41	4.7	0.37	60.6	3.19	38.4	0.37	-3600.0
BDFr02In	0.16	62.8	0.22	76.6	3.30	36.3	0.78	-7700.0
BDFr04In	0.20	53.5	0.24	74.5	4.07	21.4	0.04	-300.0
BDFr06In	0.38	11.6	0.38	59.6	3.28	36.7	0.16	-1500.0
BDFr08In	0.42	2.3	0.34	63.8	2.37	54.2	0.38	-3700.0

Table 6. Summary of the % removal of metals as compared with initial concentrations in stale domestic wastewater

Sample label	Zn (µg/l)	% removal of Zn	Cu (µg/l)	% removal of Cu	Mg (µg/l)	% removal of Mg	Pb (µg/l)	% removal of Pb
BDStRaw(aged 49 days)	0.10	-	0.05	-	2.01	-	0.16	-
BDSt01In(aged 49 days)	0.45	-350.0	0.03	40.0	2.93	-45.8	1.12	-600.0
BDSt02In(aged 49 days)	0.11	-10.0	0	100.0	1.87	7.0	0.02	87.5
BDSt02C(aged 49 days)	0.17	-70.0	0.01	80.0	2.99	-48.8	0.01	93.8
BDSt04In(aged 49 days)	0.19	-90.0	0	100.0	3.39	-68.7	0.19	-18.8
BDSt06In(aged 49 days)	0.57	-470.0	0	100.0	4.16	-107.0	0.01	93.8
BDSt08In (aged 43 days)	0.99	-890.0	0.41	-720.0	2.77	-37.8	0.03	81.3
BDSt08C (aged 43 days)	0.64	-540.0	0.31	-520.0	2.13	-6.0	0.21	-31.3

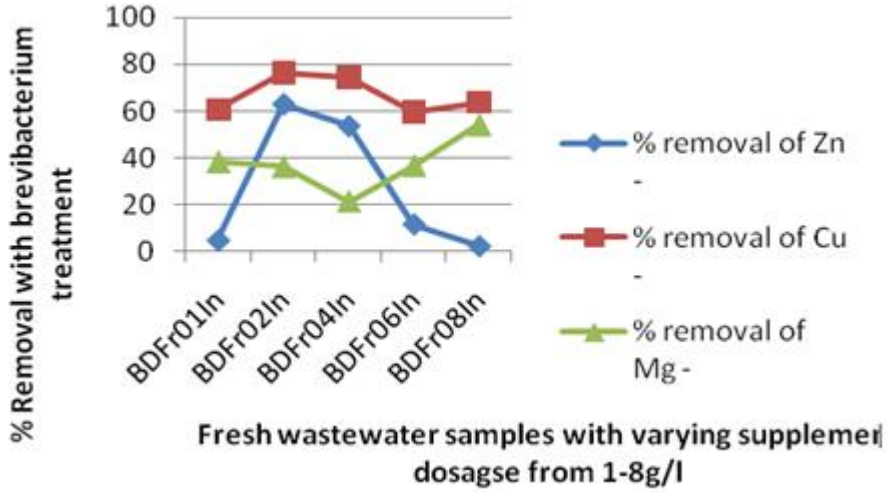

Fig. 5. Percentage bioremediation trend of Zn, Cu and Mg from fresh sewage water by *Brevibactrium*

a previous report by Thakur (2012) that gram-positive bacteria isolated from lead-contaminated soils are resistant to Pb. The effectiveness of *Brevibacteria* in the heavy metal bioremediation has therefore being found to be Cu, 77%; Zn, 63%; and Mg, 55%. The observations thus re-affirm the efficacy of biosorption methods in removal of heavy metals from wastewater and sludge as earlier reported (Goksungur, 2005; Ozturk, 2007; Azza, 2009). This bioremediation study is found to be negative on Pb.

Some outputs of the SEM analysis are shown in Figs 6 to 8 representing the magnification to 5, 000, 10,000 and 25,000 scales respectively. The outputs from EDAX are presented in Fig. 9. From the SEM outputs it becomes obvious that the there was metal uptake through bio-sorption with *Brevibacterium* sp. as there was no observed bound particles on the cell-wall surfaces of the bacteria. EDAX output shows the concentrations of the metals after bio-sorption. The identified elements apart from the heavy metals under study include Aluminum, Silicon and Iron. As shown by EDAX, the concentrations of studied metals in the residue also followed the reduction trend as measured by AAS, justifying the order: Cu > Zn > Mg, with Pb not being bio-remediated.

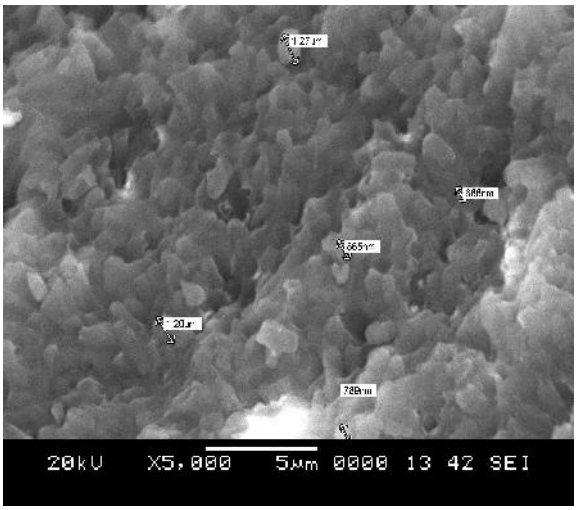

Fig. 6. Morphology of the bioremediation residue by SEM at X5,000 magnification

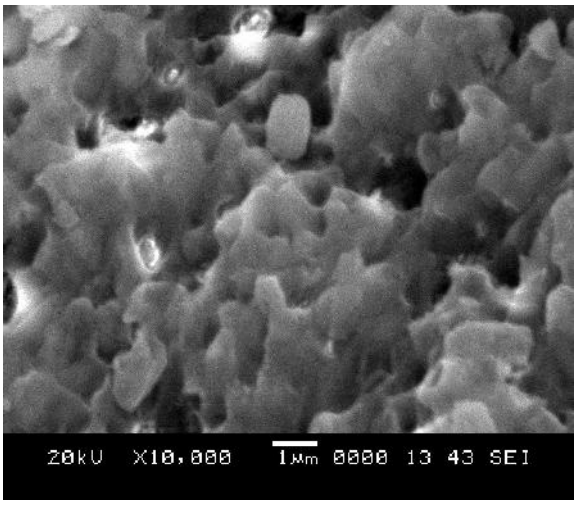

Fig. 7. Morphology of the bioremediation residue by SEM at X10,000 magnification

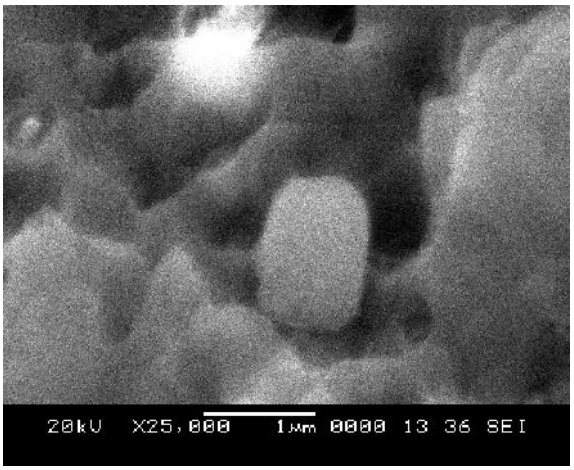

Fig. 8. Morphology of the bioremediation residue by SEM at X25,000 magnification

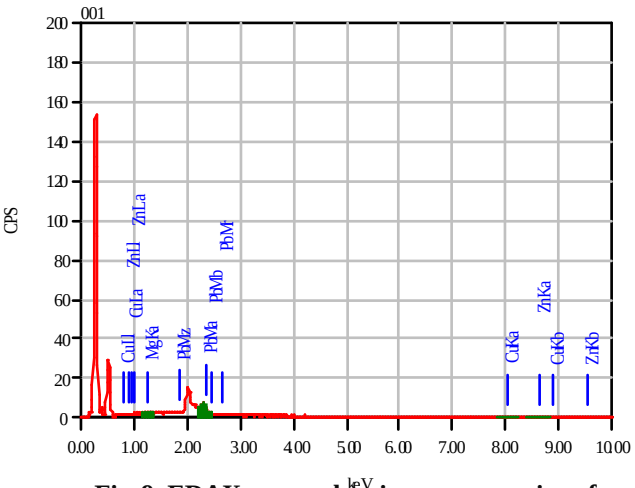

Fig. 9: EDAX output showing concentration of elements[* Object too big for pasting as inline graphic. | In-line.EMF *] in the residue after treatment

CONCLUSIONS

This study applied the previously isolated *Brevibacterium* sp. for the bioremediation of Zn, Cu, Mg and Pb in domestic sewage. Batch culture experiments were performed on both the fresh and the stale sewage samples after glucose supplementation of 1-8g/l. Fresh sewage samples permitted the growth of bacteria and facilitated bioremediation of Zn, Cu and Mg. Thereby, effectively remediation of microgram quantity of the heavy metals. The treatment efficiency is of the order Cu > Zn > Mg and with respective removal percentages of 77, 63 and 55. Pb was found to be resistant to the bioremediation with *brevibacteria* sp. as there was no reduction in the concentration after the treatment. The optimum glucose dosage for effective bioremediation was found to be 2g/l for Zn and Cu, and 8g/l for Mg. Stale sewage produced some unknown inhibitory substances that prevented the growth of the bacteria thereby resisting the bioremediation. The bioremediation with *brevibacteria* sp. isolated from coffee pulp has therefore been found effective in the remediation of Zn, Cu and Mg from domestic sewage. As a low-cost agent and being readily available, bioremediation by *brevibacterium* sp. is therefore recommended for large scale application on those metals while Pb should be further subjected to ion exchange, electro-winning, or membrane separation.

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