altered DNA methylation (Shi *et al.* 2004), altered DNA repair (Hughes *et al.* 2002) mitochondrial damage (Liu *et al.* 2001), tumour promotion, proliferation of cell and co-carcinogenesis (Butt *et al.* 2011). The main reasons of high arsenic (As) contamination in any area are because of the alteration of the redox condition and geochemical properties of ground water. These alterations occur due to over- withdrawal of groundwater for drinking and irrigation, which leads to release of arsenic (As) from the minerals.

Contamination of arsenic in ground water is present in the form of ground water pollution which occurs due to high concentration of arsenic in the deeper level of ground water. Tube well supply near Ganga deltas cause serious arsenic poisoning to majority of population. In the year 2007, it was found that over 137 million people in more than 70 countries were affected by the poisoning of drinking water containing arsenic (Smedley *et al.* 2002).

Ganga River originating from the Himalayas is one of the natural source of arsenic (As) contamination in Ganga plains of Bihar. The arsenic (As) content in Ganga plains of Bihar is more than the permissible limit of 10 ppb.

In India, many states are affected by arsenic contamination in which some states like – West Bengal, Bihar, Assam, Jharkhand, Manipur, Chhattisgarh, Uttar Pradesh are reported highest arsenic concentration above the permissible limit.

In Bihar, the ground water arsenic (As) contamination was first found in Semariaojhapatti village of Shahpur block of Bhojpur district in the year 2002 (Chakraborti *et al.* 2003). Nowadays the groundwater contamination by arsenic (As) has spread to 16 more district of Bihar affecting more than 10 million people (Ghosh *et al.* 2007). In year 2012 Maner block of Patna district was reported to have a high health risk due to arsenic (As) contamination and the cancer risk in the arsenic (As) contaminated drinking ground water was as high as 192 (Singh *et al.* 2012). Other affected areas of Bihar include Bhojpur, Patna, Samastipur and Bhagalpur district. More than 500 µg/L of arsenic (As) were also detected in other regions of Bihar like Vaishali, Saran, Begusarai, Khagaria, Munger and Katihar district.

Arsenic resistant bacteria are very important in controlling the speciation and cycling of arsenic in nature (Inskeep *et al.* 2007). The main aim of this study is isolation of Arsenic resistant bacteria from different water sources and investigation of arsenate bioremediation efficiency by highly resistant isolates.

Materials and Methods :

Sample collection : Water sample of Ganga, Sewage and Tube well were collected from the arsenic contaminated region of Patna, Bihar. The sample was collected in previously sterilized bottles and was further used for the isolation of bacteria. The selected site for the Ganga sample was namely DIGHA GHAT (25.6383° N, 85.1005° E), for Sewage was namely KRISHNA GHAT (25.6221° N, 85.1684° E) and the sample of Tube well was from BUDDHA COLONY (25.6228° N, 85.1285° E). The samples were kept in sterilized plastic bottles at 4°C till further use.

Isolation of arsenic resistant bacteria : For the isolation of arsenic resistant bacteria from Ganga plane, 0.1ml of serially diluted water sample (upto 10^{-4} dilution) was spread on to Nutrient Agar (NA) (Aneja K.R. 2003) plates containing AsIII i.e. arsenite of different concentration i.e. 50µm, 100µm & 150µm respectively. Whereas for the isolation of arsenic resistant bacteria in sewage, 0.1ml of serially diluted water sample (upto 10^{-5} dilution) was spread on to NA media plate containing different concentration (50µm, 100µm & 150µm) of AsIII. For the isolation of arsenic resistant bacteria in tube well, 0.1ml of serially diluted water sample (upto 10^{-3} dilution) was spread onto NA media plate containing different

concentration (50µm, 100µm & 150µm) of AsIII. Plates were incubated at 37° C for 24 hrs (Arduino *et al.* 1991). The colonies which showed resistance to the AsIII and were morphologically different were picked up and isolated via purifying the colonies by streaking and growing them repeatedly on NA media and stored at 4° C.

Cultural and morphological characterisation : For Morphological characterisation, streak plate of fresh culture (after incubation at 37°C for 24) was observed and colony morphology was recorded. The morphological characteristics were observed in terms of their shape, elevation, margin, texture, colour and optical property. The cultural characteristics were identified using the Gram Staining technique (Salanitro *et al.* 1974) and the cellular structure was observed under light microscope at 100X magnification.

Biochemical analysis of the isolated strain: Different biochemical properties of the isolates such as enzyme production test (i.e. Indole, Catalase and Urease test), Nitrate reductase, Methyl Red, Voges-proskauer, Citrate, H_2S production test and utilization of different carbon sources were tested by following the standard methods (Pelczar *et al.* 1957).

Estimation of MIC Value : MIC i.e. Minimum Inhibitory Concentration of AsIII of all the isolates were evaluated by growing them on a higher concentration (Rahman *et al.* 2014, Liao *et al.* 2011) varying from 0.5 - 2mM of AsIII containing NA media plate. The plates were then incubated at 37° C with different concentration of AsIII for 24 hrs. and then growth was observed and absorbance was taken using spectrophotometer at 640nm.

Oxidation - Reduction Test : The ability of the isolates to reduce arsenite (AsIII) into arsenate (AsV) was evaluated by the Oxidation – Reduction test. Different isolates of Ganga, Sewage and Tube well water was inoculated in the nutrient broth media containing different concentrations of AsIII (0.5mM – 4mM) respectively. 2-3 drops of silver-nitrate solution was added in the nutrient broth media. After inoculation, the culture broth was kept at 37ºC for 72hrs and the colour change of the nutrient broth was observed.

Results and Discussion :

Isolation of arsenic tolerant bacterial strain: A total of five bacterial strains were isolated from the different water samples, they were placed in Nutrient Agar media with 750ppb – 7500ppb Sodium Arsenite solution. It was observed that MIC values of isolates-varied from 0.5mM**-** 4mM. Out of five bacterial strains only two strain of Ganga and tube well respectively showed highest tolerance to arsenite. These isolates were grown on increasing concentration of arsenite upto 1.5mM solution and then slowly declined and ultimately ceased growing at 2.5mM. It was found that bacterial strain that were isolated from sewage water didn't gave growth on increasing arsenite concentration and stopped its growth at 1.5 mM.

Cellular and colony morphology : The cellular morphology of isolates was observed under light microscope. The strain which were isolated from Ganga water are Gram positive and rod shaped while the bacterial strain isolated from tube well were Gram positive and cocci shaped and the isolates of sewage were Gram negative and rod shaped. The colony morphology of arsenic tolerant bacterial strain isolated from different water sources are as shown in (Table 1).

Table 1. Cultural characteristics of isolated strains

S. No	Sample	Dilution	Colour	Texture	Elevation	Margin	Shape
1.	Ganga	10^{-4}	Yellow	Creamy	Flat	Entire	Circular
2.	Ganga	10^{-4}	White	Creamy	Flat	Entire	Circular
3.	Sewage	10^{-5}	Orange	Smooth	Flat	Entire	Irregular
4.	Sewage	10^{-5}	Cream	Smooth	Flat	Entire	Circular
5.	Tube well	10^{-3}	White	Creamy	Flat	Entire	Circular

Biochemical analysis of isolated strain : The biochemical results of the bacterial strain were as follows for different water sources.

Table 2. Biochemical characteristics of isolated strains

Basic characteristics	Ganga water		Sewage water	Tube well water
	yellow	white	white	white
Gram Staining	+ve, Rods	+ve, Rods	-ve, Rods	+ve, cocci
Catalase	+ve	+ve	+ve	-ve
H_2S	-ve	+ve	-ve	-ve
Motility	+ve	-ve	+ve	-ve
Urease	-ve	-ve	-ve	-ve
Nitrate Reduction	+ve	+ve	+ve	+ve
Indole	-ve	-ve	-ve	-ve
MR (Methyl red)	-ve	+ve	-ve	+ve
VP (Vogesproskauer)	+ve	-ve	-ve	-ve
Citrate	+ve	-ve	+ve	-ve

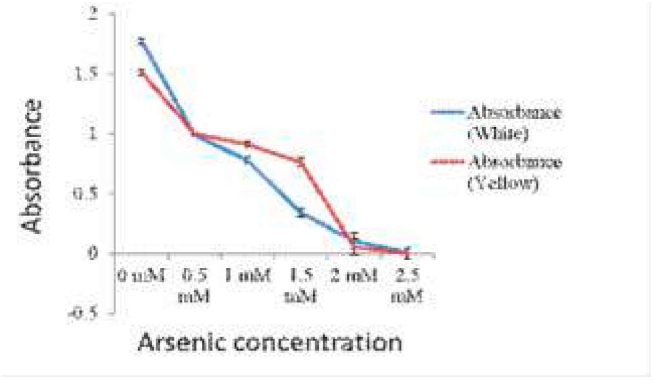
Different bacterial isolates shows different biochemical properties.

From the Ganga water the isolated bacterial strain was found to be Gram positive rods, Catalase positive, H_2S positive, Motility negative, urease negative, Nitrate Reduction positive, Indole negative, MR positive, VP negative and Citrate negative for white colony. For yellow colony Gram staining was positive rod shaped, Catalase positive, H2S negative, Motility positive, Urease negative, Nitrate Reduction positive, Indole negative, MR negative, VP positive and Citrate positive.

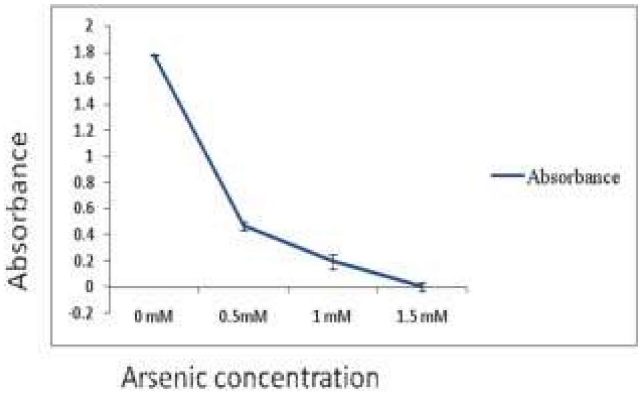
From the Sewage water the isolated bacterial strain were found to be Gram negative rods, Catalase positive, H2S negative, Motility positive, Urease negative, Nitrate Reduction positive, Indole negative, MR negative, VP negative & Citrate positive.

From tube well water the isolated strain was found to be Gram positive cocci, Catalase negative, H2S negative, Motility negative, Urease negative, Nitrate Reduction positive, Indole negative, MR positive, VP negative.

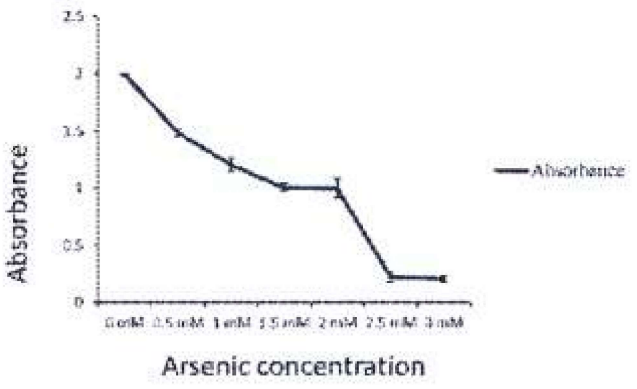
Evaluation of MIC of different bacterial isolates : The bacterial isolates were grown on increasing the arsenite concentration in Nutrient Agar media. The different concentration at which strains were grown are 0.5mM, 1mM, 1.5mM, 2mM, 2.5mM, 3mM, and 4mM (data not shown). Similarly, the experiment was performed in Nutrient broth media, where increasing concentration of arsenite was added in broth with isolates and absorbance were taken at 640nm (three replicates) for each isolates. The bacterial strain isolated from Ganga water were observed to grow up to 2mm while the isolates from tube well have highest tolerant value of 4mM, while isolates from sewage water have 1.5 mM tolerant value (Fig. 1.)



(a)



(b)

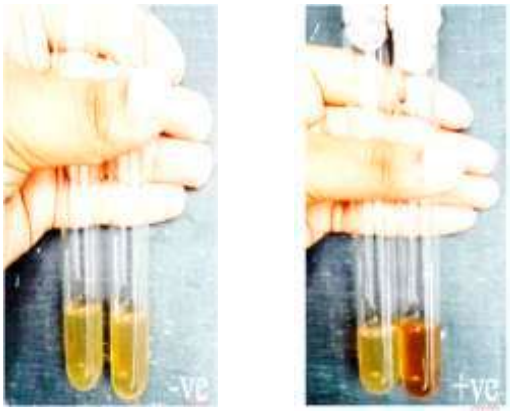


(c)

Fig. 1. Effect of different arsenite concentrations (0, 0.5, 1, 1.5, 2, 2.5, 3 and 4mM) on the cell growth of Ganga (a), sewage (b) & tube well (c) isolate in nutrient broth medium after incubation at 37°C

Oxidation – reduction test : Arsenite to arsenate oxidizing assay of the bacterial isolates was done for detection of bioremediation activity. After the addition of silver nitrate into nutrient broth containing the isolates and incubation of 72hrs, it was observed that broth turned brown slowly which confirmed the presence of silver arsenate in broth and it was found that when silver nitrate was mixed with old culture containing arsenate also turned brown confirming the presence of silver nitrate. The isolates which were isolated from Ganga and tube well water gave positive result by turning the broth colour to brown while sewage isolates gave negative result (Fig 2). Hence, it can be confirmed that both theisolates from Ganga and tube well oxidise arsenite to arsenate.

(a) (b)



(c) (d)

Fig. 2. Arsenite oxidation (a) arsenite broth without bacteria, (b) arsenite broth inoculated with isolates of tube well, (c) arsenite broth inoculated with isolates of Sewage & (d) arsenite broth inoculated with isolates of Ganga

From the earlier reports it has been confirmed that different water sources in Patna contain arsenic contemination (Chakraborty *et al.* 2003). All the samples of water contain certain arsenic resistant bacteria as arsenic compound are neutral to alkaline in nature, hence suitable for bacterial growth.

After performing the morphological and biochemical characterisation it was found that there are number of bacterial strain which can resist the toxicity of arsenic. These bacterial isolates from different water source have specific biochemical characteristics such as test for Urease, Catalase, MR, VP, Citrate, Indole, Nitrate Reduction, Motility, H_2S and also stains differently by Gram's stain.

From the Ganga water the isolated bacterial strain was found to be Gram positive rods, Catalase positive, H_2S positive, Motility negative, urease negative, Nitrate Reduction positive, Indole negative, MR positive, VP negative and Citrate negative for white colony. For yellow colony Gram staining was positive rod shaped, Catalase positive, H_2S negative, Motility positive, Urease negative, Nitrate Reduction positive, Indole negative, MR negative, VP positive and Citrate

positive. Thus on the basis of above characteristics we may conclude that the white coloured strain may be *Corynebacterium species* and yellow strain may be *Bacillus species*.

From the Sewage water the isolated bacterial strain were found to be Gram negative rods, Catalase positive, H_2S negative, Motility positive, Urease negative, Nitrate Reduction positive, Indole negative, MR negative, VP negative & Citrate positive. Thus on the basis of above characteristics we may conclude that the strain may be *Pseudomonas species*.

From tube well water the isolated strain was found to be Gram positive cocci, Catalase negative, H_2S negative, Motility negative, Urease negative, Nitrate Reduction positive, Indole negative, MR positive, VP negative. Thus on the basis of above characteristics we can conclude that the strain may be *Streptococcous species*.

From the Ganga water *Corynebacterium species* were found to resistant to arsenic while the *Streptococcous species* were isolated from tube well found to be resistant of arsenic. Both of these strains were the only strains which can tolerate upto 2mM and 4mM of arsenic concentration respectively. The isolates were grown on increasing concentration of arsenic and they were found to tolerate the toxic arsenate upto 4mM.

The isolated bacterial strains oxidised arsenite into arsenate by changing the colour of nutrient broth to brown after addition of silver nitrate. Once the bacterial strains converted the arsenite to arsenate they easily resist the effect as they were already resistant to higher concentration. Both the strains of Ganga water and tube well water respectively were found to oxidise arsenite to its less toxic form arsenate but they did not oxidise arsenate further.

Conclusion :

From the above study, we may conclude that the two strain of bacteria which were identified more similar to *Streptococcus species* & *Corynebacterium species* can tolerate arsenic concentration upto 4mM & 2mM of sodium arsenite concentration respectively.

And, it is also found that it can oxidise arsenite to arsenate which is its less toxic form by changing the nutrient broth colour to brown after the addition of silver nitrate but none of them can reduce arsenate. These two arsenic resistant bacteria can be further used for bioremediation of arsenic.

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References :

Aneja K.R. (2003). Experiments in microbiology, Plant pathology and biotechnology, (4th Edn), New Delhi: New age international (P) Ltd.

Arduino M.J., Bland L.A., Aguero S.M., Favero M.S. (1991). Effects of incubation time and temperature on microbiologic sampling procedures for hemodialysis fluids. *Journal of Clinical Microbiology.* 29(7):1462 – 1465 .

Butt A.S., Rehman A. (2011). Isolation of arsenite-oxidizing bacteria from industrial effluents and their potential use in wastewater treatment. *World J. Microbiol. Biotechnol.* 27(10):2435–2441.

Chakraborty D, Mukherjee SC, Pati S, Sengupta MK, Rahman MM, Chowdhury UK, Lodh D, Chanda CR, Chakraborty AK (2003). Arsenic groundwater contamination in Middle Ganga Plain, Bihar, India: a future Danger? *Environ Health Perspect.* 111(9):1194–1200.

Ghosh A.K., Shatrunjay K.S., Bose N., Sushant K.S., Singh A., Mishra R.C. (2007). Study of Arsenic Contamination in Ground Water of

Bihar (India) along the River Ganges: International Workshop on Arsenic Sourcing and Mobilization in Holocene Deltas. Department of Science and Technology, Government of India. pp. 83-87.

Hughes M.F. (2002). Arsenic toxicity and potential mechanisms of action. *Toxicol. Lett.* 133(1): 1-16.

Inskeep W.P., Macur R.E., Hamamura N., Warelow T.P., Ward S.A., Santini J.M. (2007). Detection, diversity and expression of aerobic bacterial arsenite oxidase genes. *Environ Microbiol.* 9(4):934.

Liao V.H.C., Chu Y.J., Su Y.C., Hsiao S.Y., Wei C.C., Liu C.W, (2011). Arsenite-oxidizing and arsenate-reducing bacteria associated with arsenic-rich groundwater in Taiwan. *Journal of Contaminant Hydrology.* 123(12): 20–29.

Liu, S.X., Athar, M., Lippai, I., Waldren, C., Hei, T.K., (2001). Induction of oxyradicals by arsenic: implication for mechanism of genotoxicity. *Proc. Natl. Acad. Sci.* U.S.A. 98(4):1643–1648.

Obinaju B.E. (2009). Mechanisms of arsenic toxicity and carcinogenesis. *Afr. J. Biochem. Res.* 3(5):232–237.

Pelczar M.J., Bard R.C., Burnett G.W., Conn H.J., Demoss R.D., Evans E.E., Weiss G.A., Jennison M.W., Mckee A.P., Riker A.J., Warren J, Weeks O.B., (1957). *Manual of Microbiological Methods,* Society of American Bacteriology, McGraw Hill Book Company, New York. 5th edition:315.

Rahman A., Nahar N., Nawani N.N., Jass J., Desale P., Kapadnis B.P., (2014). Isolation and characterization of a Lysinibacillus strain B1-CDA showing potential for bioremediation of arsenics from contaminated water. *J. Environ. Sci. Health.* 59(12):1349–1360.

Salanitro J.P., Fairchilds I.G. and Zgornicki Y.D. (1974). Isolation, Culture Charactristics, and Identification of Anaerobic Bacteria from the Chicken Cecum. *Applied Microboil.* 27(4):678 – 687.

Shi H., Shi X., Liu K.J. (2004). Oxidative mechanisms of arsenic toxicity and carcinogenesis. *Mol. Cell. Biochem.* 255(12): 67-78.

Singh S. K., Ghosh A. K. (2012). Health Risk Assessment due to Groundwater Arsenic Contamination: Children are at High Risk. *Human and Ecological Risk Assessment : An International Journal.* 18(4):751-766.

Smedley P.L., Kinniburgh D.G. (2002). A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochemistry,* 17(5):517–568.