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RESEARCH ARTICLE

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STUDIES ON GROWTH PATTERN OF CITROBACTER SP. ISOLATED FROM RIVER GANGA IN DIFFERENT HEAVY METAL AND ITS ASSOCIATION WITH MULTI-DRUG RESISTIVITY

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Abstract

Citrobacter sp. AR2 was isolated and multi- heavy metal resistivity with different heavy metals like arsenic, lead, cobalt, zinc, manganese and strontium was conducted. The organism showed highest growth in Zinc followed by Strontium, Manganese , Copper , Lead , Chromium , Arsenic and Cobalt. The growth pattern study revealed that the organism favoured Strontium , Copper and Lead for growth whereas the media containing other heavy metals showed slow and declined growth rate . The organism also showed its multidrug resistance character when plated with Ampicillin (50µg/ml), Tetracycline (10µg/ml), Chloramphenicol (34µg/ml), Kanamycin (50µg/ml), Penicillin G (80µg/ml) and Streptomycin (50µg/ml). *Citobacter sp.* AR2 was also tested against several commercially available antibiotic discs from which it was concluded that the organism is highly pathogenic and can resist almost all the antibiotics tested against.

Introduction:-

"The level of fecal coliform are exceeding its range of acceptance in entire stretch of the Ganga (from Gangotri to the Diamond Harbour)" has been reported by the Central Pollution Controll Board (CPCB) in July 2013 . Along the bank of the Ganga there are several industries established such as – plastic manufacturing unit , mining and metallurgic industry , electroplating , fertilizer plant (Zouboulis et.al 2004) and many more are there that pollutes the river water . These are the common source from which the heavy metals discharges. Anthropogenic and natural sources are mainly the two major sources that release the heavy metals. Metal fishing industries and metal industries also discharge heavy metals containing effluents along with organic ions and inorganic anions (Edward Raja et al 2009) . The release of untreated waste water , surface run off , accidental pills and discharge of the effluents directly from the industries to the fresh water bodies effects the quality of the water which are used in the agricultural land through the irrigation system that effects the nature of the soil.
The density of the heavy metals is greater than 5gm/cm3 which contaminates the environment because of their toxic effects on the living organisms . Heavy metals are stable and persistent in nature (Rajkumar et.al 2008).They are unable to destroy or degraded. The heavy metals are

highly toxic such as – cadmium , copper , cobalt , lead , chromium etc . which shows adverse effect on human body as well as on its biological systems (Bailey et.al 1999). On the other hand the Ganga is habitat of several microbes which are initially got effected by these toxic heavy metals but gradually undergo numourous biologocial changes in their communities such as by effluxing the metal ions outside the cell etc. and also modified genetically to survive the toxic atmosphere of water bodies(Doelman et.al 1994, Hiroki 1994 , Staezecka and Bednatz 1993).
The present study focuses on the changes in growth pattern of *Citrobacter* sp. Previously isolated from the water sample of River Ganga.

Materials and methods:-

Determination of optimum growth condition:-

The optimum growth condition with reference to pH was determined. The test was performed with pH ranging from 4 to 11 and this was sequentially performed for 3 days in a continuation. The isolates were grown in LB broth at different temperatures (25 º C, 30 º C, 37 º C and 40 º C) to determine the optimum temperature for growth.

Determination of Growth pattern:-

Growth pattern was determined for the isolated organism. 24 hrs old culture was taken and was inoculated in LB broth containing different heavy metals in separate conicals. Inoculation in LB broth without heavy metal was kept as control. Readings were taken in triplicates at regular intervals for 3 days at 660 nm (UV-VIS Spectrophotometer 117, Systronics, India).

Multiple heavy metal tolerance:-

Nutrient agar incorporated with salt of different heavy metals to study the multi-heavy metal tolerance of the organism (Amanpreet k. sidhu et.al, 2015). The organism was subjected to an increasing concentration of chromium to determine the level of tolerance. The organism was tested against several other heavy metals such as arsenic, lead, cobalt, zinc, manganese and strontium. The initial concentration of 1mM was taken for each heavy metal and was increased until the organism showed no growth. The MIC for the heavy metal was noted when the culture failed to grow on the plate even after 10 days of incubation (Shakoori et.al, 1999).

Determination of multi-drug resistance:-

The isolated organism was also tested for multiple drug resistance (MDR) ability. The strains were pour plated on LB agar plates containing one or multiple antibiotics in different combinations and were incubated at 30o C for 24 hrs. The tests were performed in triplicates. The antibiotics used for determination of MDR ability were as follows:- Ampicillin (50µg/ml), Tetracycline (10µg/ml), Chloramphenicol (34µg/ml), Kanamycin (50µg/ml), Penicillin G (80µg/ml) and Streptomycin (50µg/ml). The organism was also subjected to antibiotic sensitivity test according to the Kirby Bauer disc diffusion method given by Bauer et.al. (Mackey and McCartney, 1996) against several commercially available antibiotic discs. After incubation the organisms were considered resistance or sensitive according to the diameter of the zone of inhibition around the isolates on the LB plates.

Results:-

Determination of optimum growth condition

The isolate showed a wide range of dependence for both temperature and pH. The optimum pH for growth for the organism was found to be pH 8 (Table 1) and the optimum temperature was found out to be 37^0 C (Table 2).

Table 1: shows the optimum temperature for the growth of the organism

	pH 4	**pH 5**	**pH 6**	**pH 7**	**pH 8**	**pH 9**	**pH 10**	**pH 11**
Citrobacter sp. AR2	+	+	+	+	++	+	+	-

-, Indicates no growth; +, Indicates growth; ++, Indicates optimum condition

Table 2: shows the optimum temperature for the growth of the organism

	25 º C	**30 º C**	**37 º C**	**40 º C**
Citrobacter sp.AR2	+	+	++	-

-, Indicates no growth; +, Indicates growth; ++, Indicates optimum condition

Determination of Growth pattern

After incubation the organism showed different growth rate when grown in media containing chromium as compared to media that does not contain chromium. With chromium the organism showed a slow start initially in comparison to the media that does not contain chromium (Figure 1).

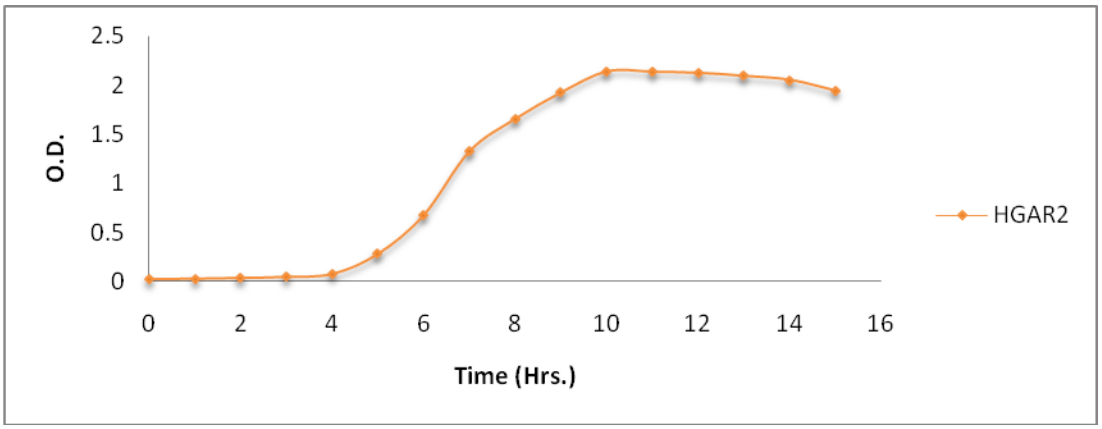

Figure 1: Growth curve of *Citrobacter sp* AR2

Chromium and other heavy metal tolerance

After incubation the organism showed growth in a varied range of heavy metal. The organism showed highest degree of tolerance to chromium up to 7.5 mM (Figure 2). The organism showed thick growth to many heavy metals such as arsenic, lead, zinc, manganese, cobalt and selenium at a lower concentration (Table 3) but as the concentration increased the growth decreased.

Table 3: Heavy metal tolerance by *Citrobacter sp*. AR2

	Concentration (in mM)														
	1	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**
Chromium	+++	++	++	++	+	+	-	-	-	-	-	-	-	-	-
Arsenic	+++	+++	++	++	+	-	-	-	-	-	-	-	-	-	-
Lead	+++	+++	+++	+++	++	++	++	+	-	-	-	-	-	-	-
Cobalt	+++	+++	++	-	-	-	-	-	-	-	-	-	-	-	-
Copper	+++	+++	+++	+++	+++	++	++	+	-	-	-	-	-	-	-
Zinc	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	-	-	-	-
Strontium	+++	+++	+++	+++	+++	+++	+++	++	++	+	-	-	-	-	-
Manganese	+++	+++	+++	+++	++	++	++	+	-	-	-	-	-	-	-

-, Indicates no growth; +, Indicates growth; ++, Indicates Moderate condition; +++, Indicates Thick Growth

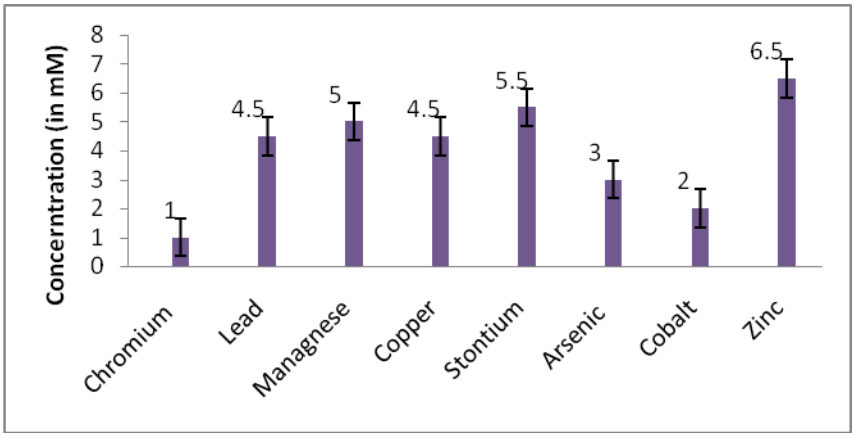

Figure 2: Heavy metal tolerance by *Citrobacter sp*. AR2

Determination of antibiotic resistance

Citrobacter sp. AR2 showed resistance to multiple antibiotics. When plated with 1X concentration of the following antibiotics: Ampicillin (50 µg/ml), Tetracycline (10 µg/ml), Chloramphenicol (34 µg/ml), Kanamycin (50 µg/ml), Penicillin G (80 µg/ml) and Streptomycin (50 µg/ml), the organism showed good growth thus can be conferred that the organism is multi-drug resistant.

Growth pattern in different heavy metal

After incubating the organism in different heavy metals the results showed that the growth rate is higher when the media contains heavy metals than when there is no heavy metal. The organism also showed a slowed growth when the media doesnot contain heavy metals (Figure 3, 4 5, 6, 7 and 8).

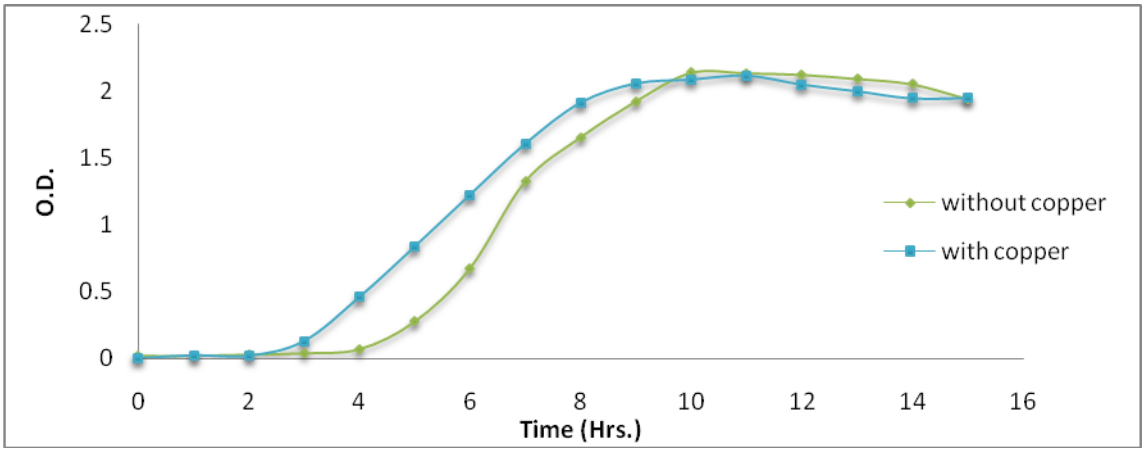

Figure 3: Showing Growth Curve with Copper

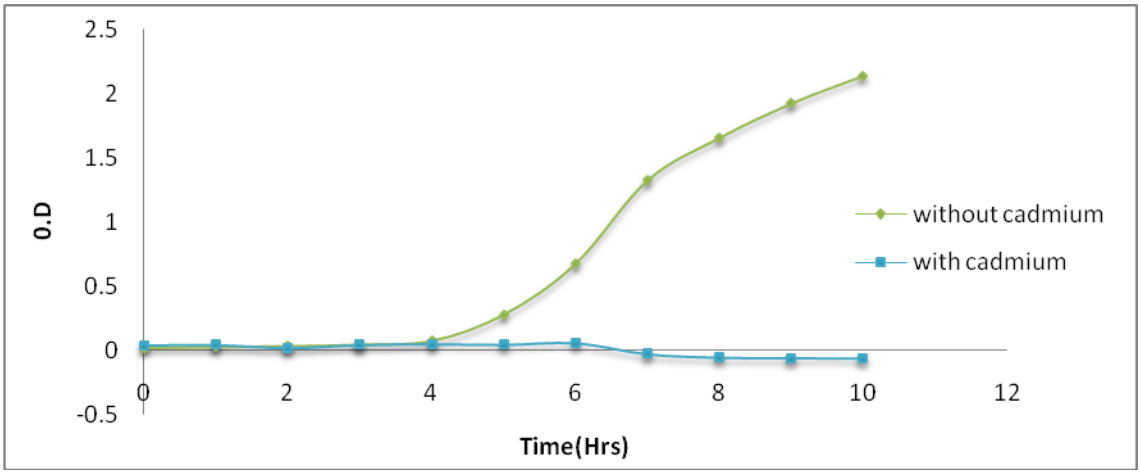

Figure 4: Showing Growth Curve with Cadmium

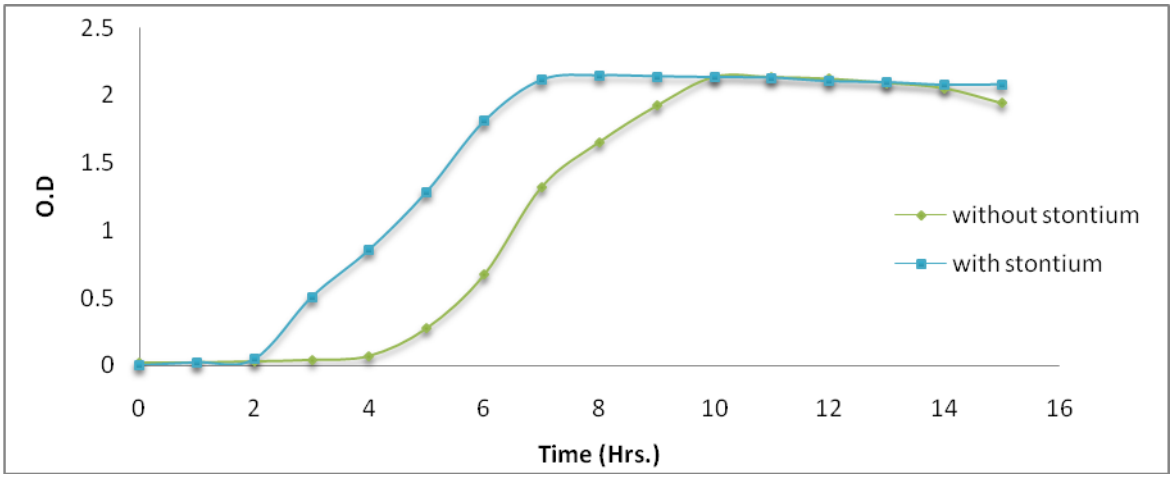

Figure 5: Showing Growth Cure with Strontium

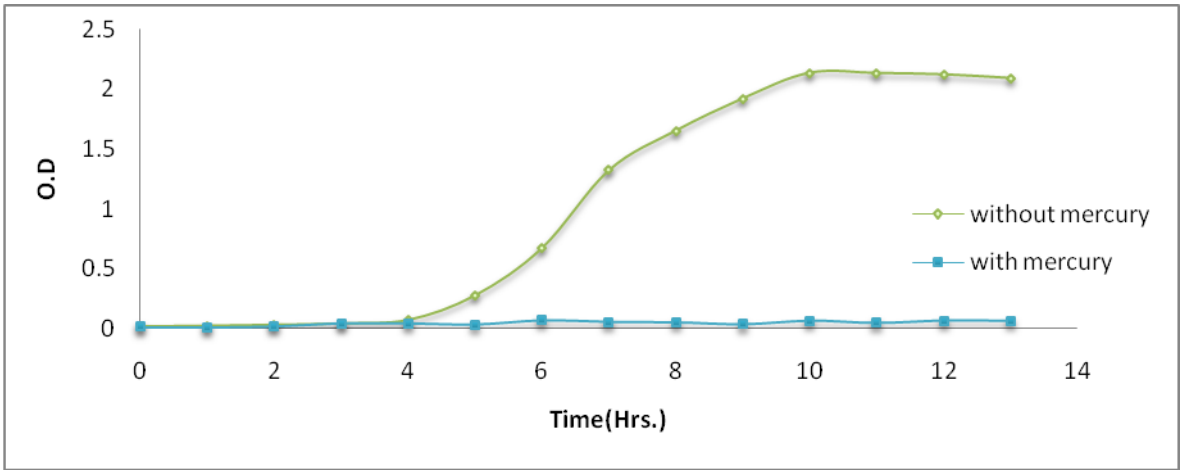

Figure 6: Showing Growth Curve with Mercury

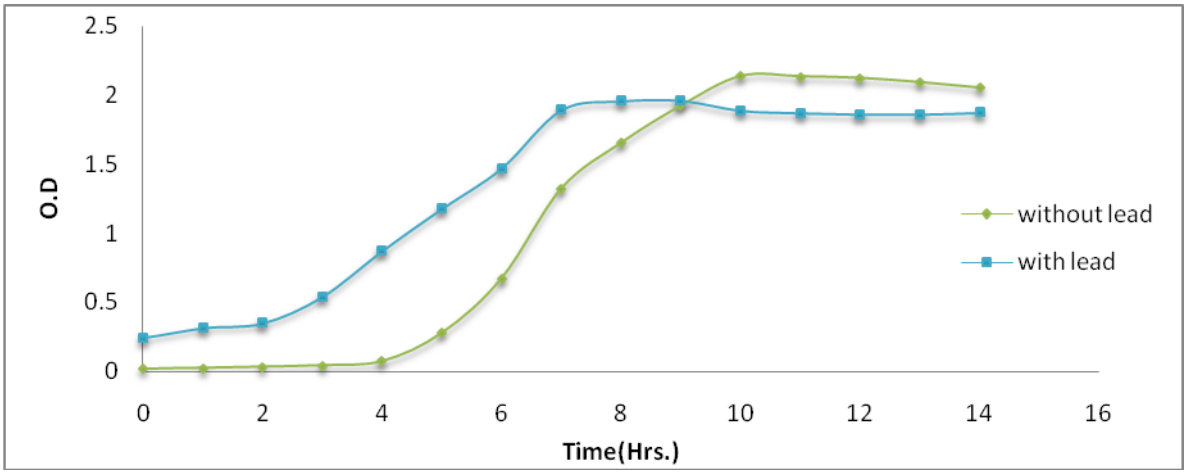

Figure 7: Showing Growth Curve with Lead

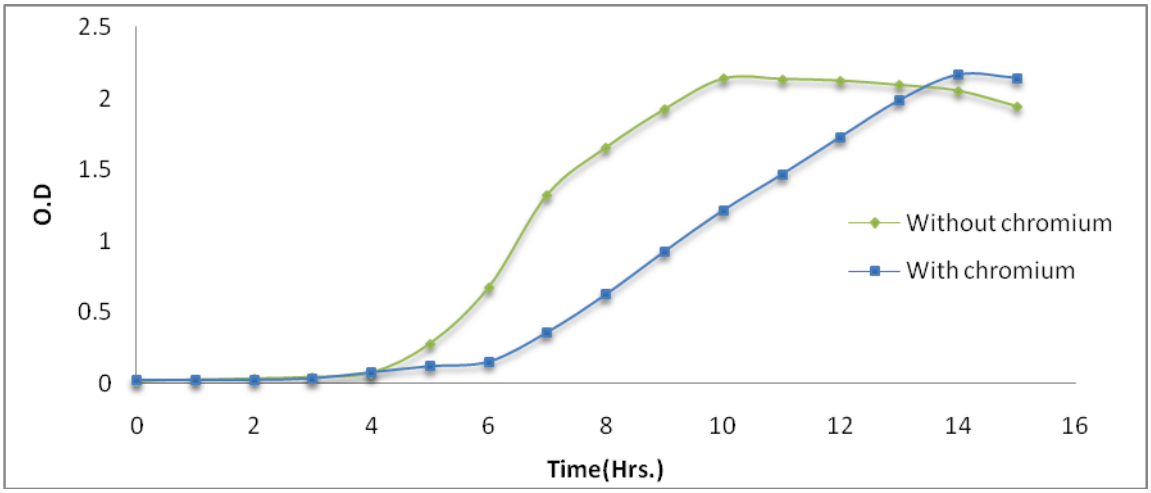

Figure 8: Showing Growth Curve with Chromium

Discussions:-

The organism showed growth in a varied range of heavy metal. The organism showed highest degree of tolerance to zinc (Figure 2) up to 6.5 mM. The organism showed thick growth to many heavy metals such as chromium, arsenic, lead, manganese and strontium at a lower concentration (Table 3) but as the concentration increased the growth decreased.

The growth pattern corresponding to the respective heavy metals were conducted that reveals that the organism possess a similar growth pattern (Figure 3, 4, 5, 6, 7 and 8). The organism does not show growth in cadmium and mercury (Figure 4 and 6) where as in other heavy metals the organism showed a show start and then an exponential growth curve was achieved by the organism (Figure 3, 5, 7 and 8). The organism showed best growth rate in copper followed by strontium, lead and chromium.

The organism *Citrobacter sp. AR2* was found to be efficient for remediating multiple heavy metals. This organism can be further studied for implementation of bioremediation of heavy metals used for this present study. The study also revealed that the present status of microbial load in the river Ganga needs an urgent attention since the study shows that the river is being polluted with huge amount of multiple drug resistant microorganisms. The presence of multiple heavy metal resistant microorganisms also confers that the river is polluted with increasing levels of heavy metals from the discharges of the effluents from the industries situated in its banks.

Thus finally it can be concluded from the results of this project is that the governing authority should give an urgent attention for clearing the pollution from the most sacred river of the Hindus such that it doesn't impose any health threat to the livelihood depending on it.

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