RESEARCH ARTICLE

Screening of Heavy Metal Resistant Bacteria from Nale Lake of Vasai Taluka of Maharashtra

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ABSTRACT

Due to rapid industrial progress and urbanization environmental pollution with toxic heavy metal has become one of the major concerns in today's world. We need advance chemical and mechanical technology for removal of heavy metals from polluted water, but this technology is quite expensive. Microbial technologies and inventions may provide an alternative to the current methods. Microorganisms and microbial products can be highly efficient bioaccumulators for heavy metals. In this study, preliminary investigation showed presence of few heavy metal resistant bacteria from a lake of Vasai Taluka situated in Nale village. Different isolates showed different degree of resistance to Arsenic, Lead and Cadmium proving that they can be used as tools for remediating the water contaminated with these toxic heavy metals. Further investigations need to be carried out to identify and characterize the isolates so as to establish their role as bioaccumulators.

Keywords : Heavy metals, Bioaccumulators, Vasai Taluka, Nale Lake.

INTRODUCTION

In recent years, due to urbanization and industrialization environmental pollution has increased by alarming rate. As a result more and more toxic heavy metals get discharged in the environment which is further responsible for population increase of heavy metal resistant bacteria.

Cadmium (Cd) is one of the most toxic pollutants for plants, animals and humans (Gupta and Gupta, 1998). It gets released into the environment by various human activities such as mining, smelting, incineration of plastics and batteries, burning of fossil fuels etc. (Tang *et al*., 2006). Lead (Pb) a major pollutant is found in soil, water and air and is highly toxic to living organisms (Low *et al*., 2000). Arsenic (As), which is also a toxic heavy metal, widely distributed in environment (Nriagu, 2002). Arsenic gets introduced in the environment from various natural sources such as weathered volcanic, marine sedimentary rocks, fossil fuels, mineral, water, air, living organisms and anthropogenic activities including

mining, agriculture, industrial activities etc. (Mandal and Suzuki, 2002).

Removal of heavy metal ions from wastewaters is essential due to their extreme toxicity towards aquatic life and humans. The uses of conventional technologies, such as ion exchange, chemical precipitation, reverse osmosis and evaporative recovery for this purpose is often inefficient and /or very expensive (Volesky, 1990). Microbes may provide efficient and economical alternative to current methods. The objective of current study is to isolate heavy metal resistant bacteria which show high degree of resistance towards these three toxic metals.

MATERIALS AND METHODS

Site Description

The Nale Lake is situated at Nale Village in Vasai Taluka. This is manmade lake existing since more than 100 years. There are agricultural lands on one side of lake and on the other side village. Nale Lake was one of the major sources of water around 65-70 years back. Currently this lake is used for agriculture and fishing as well as cleaning activity by the local populace.

Sample Collection

The water samples were collected from two sites in Nale Lake. The samples were from the surface (0-15 cm depth). Water samples for bacteriological studies were collected in sterile bottles and transported to laboratory. Water samples for heavy metal analysis were acidified with concentrated $HN0_3$ and stored at 4°C.

Water analysis

Water samples were treated with 0.6 ml concentrated HNO_3, 0.25 ml of 75% H_2SO_4 and 100 ml of water. Each sample was then evaporated and diluted to 23 ml (Grimalt, 1989). Water is then filtered through Whatman Filter Paper no. 42 and then it was analyzed with an atomic absorption spectrophotometer (Agilent) for Cadmium, Lead and Arsenic.

Bacteriological Examination and Enumeration

The total number of bacteria colonies was determined in the water samples using the Standard Plate Count method. For standard plate count Nutrient agar media was used. The population of the bacteria resistant to metals was found with the help of PHG II media with metals. The PHG II media contains peptone 4g, Glucose 2g, Yeast extract 1g and agar 15g per 1000 ml of water and its pH was adjusted to 7 (Sabry *et al*., 1997; Kermanshahi *et al*., 2007). For each metal, plate having 10 mg/l concentration of metal was prepared. Isolation was carried out by using spread plate technique and then the plates were kept in an incubator for 2 days at 30^0C. The population of the resistant bacteria was counted. In the later stage the pure cultures of resistant bacteria were obtained on slant tubes of Nutrient Agar containing Metals.

MIC determination

The MIC was done to find out the minimum concentration of metal, which inhibits the bacterial growth in the plate. Resistance to heavy metals was determined by an agar dilution method (Washington and Sutter, 1980). Plates containing 20ml of agar and different concentrations of metal were poured on the day of the experiments. Concentration range of metals was from 10 mg/L to 100 mg/L, with interval of 10 mg/l. The plates were incubated at 37°C for 2 days. Plates containing media with no added metal were inoculated in the same way to act as controls.

RESULTS AND DISCUSSION

Metal concentrations in the studied water samples

The characteristics of the tested water samples are summarized in Table 1. These values represent up to a 100-fold increase above those reported internationally and are even higher than data from polluted environments (Abosamra *et al*., 1989; Spivak, 1981; Makokha *et al*., 2008; Arbneshi *et al*., 2008).

Bacterial Resistance:

The relatively low count of aerobic bacteria is may be due to high concentration of heavy metals in the water. Cadmium shows the maximum inhibition compared to Lead and Arsenic for site 1 but for site 2 Arsenic shows the maximum inhibitory effect.

MIC for Bacterial Resistance:

The percentage of the isolates susceptible to various concentrations of the four heavy metal ions is shown in Table 3 and Table 4.

Table 1: Average Concentration of Metals in Water Samples

Sr. No.	Heavy Metal	Conc. (mg/l)		Permissible limit (mg/l)
		Site 1	Site 2	
1	Lead (Pb)	8	3	0.05
2	Cadmium (Cd)	5	2	0.01
3	Arsenic (As)	16	14	0.01

Table 2: Average Percentage of Bacteria resistant to Metals

Sr. No.	Heavy Metal	CFU/ml Site 1	Percentage Viability for Site 1	CFU/ml Site 2	Percentage Viability for Site 2
1	Lead (Pb)	228	24	156	18
2	Cadmium (Cd)	128	13	94	11
3	Arsenic (As)	152	16	86	10
4	Control	952	100	876	100

Table 3 : Percentage of isolates Resistant at the following concentrations for Site 1.

Metal	10 (mg/l)	20 (mg/l)	30 (mg/l)	40 (mg/l)	50 (mg/l)	60 (mg/l)	70 (mg/l)	80 (mg/l)	90 (mg/l)	100 (mg/l)
Lead (Pb)	31	22	18	9	0	0	0	0	0	0
Cadmium(Cd)	18	18	13	9	0	0	0	0	0	0
Arsenic (As)	9	4	0	0	0	0	0	0	0	0

Table 4: Percentage of isolates Resistant at the following concentrations for Site 2.

Metal	10 (mg/l)	20 (mg/l)	30 (mg/l)	40 (mg/l)	50 (mg/l)	60 (mg/l)	70 (mg/l)	80 (mg/l)	90 (mg/l)	100 (mg/l)
Lead (Pb)	31	18	9	0	0	0	0	0	0	0
Cadmium(Cd)	18	13	9	0	0	0	0	0	0	0
Arsenic (As)	4	0	0	0	0	0	0	0	0	0

Metal Concentration and Metal Resistance in Bacteria

According to statistical analysis (the t-test and P=0.05), for the resistance percentage of microbes to lead, there was a significant difference between site 1 and site 2. The average percentage of bacteria resistant to Lead (Pb) at site 1 is 24% and for site 2 is 18%. Similarly for Arsenic (As), there was a significant difference between site 1 and site 2. The average percentage of bacteria resistant to Arsenic (As) at site 1 is 16% and for site 2 is 10%. For the resistance percentage of microbes to Cadmium, there was no such great difference between site 1 and site 2. Site 1 shows 13% resistant bacterial population whereas site 2 shows 11% resistant bacteria.

This difference may be due to the prominent human activities near site 1. Site 1 is actually the part of the lake which is towards the village. The samples are collected during post monsoon season; there are the possibilities that waste water from the village has been mixed with the lake water. The village waste water may contains Grease, engine oil, Detergents, garbage etc.

One more reason for getting the resistant bacteria is the Heavy metal salt occurrence in this geographical region. Due to which the number of bacteria resistant to heavy metal must have increased. One more factor which we cannot neglect is the genetic makeup of the bacteria. Different factors such as Plasmids, stress proteins, membrane ion pumps, etc may also be responsible for this resistance (Beveridge and Murray, 1980, Traxler and Wood, 1990).

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