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Heavy metal tolerance patterns of total heterotrophic bacteria isolated from the soils of Mahatma Gandhi University campus, Kottayam, Kerala

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Abstract

Industrialization of our society has led to an increased production and discharge of both xenobiotic and natural chemical substances. Many of these chemicals will end up in the soil. Pollution of soils with heavy metals is becoming one of the most severe ecological and human health hazards. Elevated levels of heavy metals decrease soil microbial activity and bacteria need to develop different mechanisms to confer resistances to these heavy metals. Bacteria develop heavy-metal resistance mostly for their survivals, especially a significant portion of the resistant phenomena was found in the environmental strains. Therefore, in the present work, we check the multiple metal tolerance patterns of bacterial strains isolated from the soils of MG University campus, Kottayam. A total of 46 bacterial strains were isolated from different locations of the campus and tested for their resistant to 5 common metals in use (lead, zinc, copper, cadmium and nickel) by agar dilution method. The results of the present work revealed that there was a spatial variation of bacterial metal resistance in the soils of MG University campus, this may be due to the difference in metal contamination in different sampling location. All of the isolates showed resistance to one or more heavy metals selected. Tolerance to lead was relatively high followed by zinc, nickel, copper and cadmium. About 33% of the isolates showed very high tolerance (>4000µg/ml) to lead. Tolerance to cadmium (65%) was rather low (<100 µg/ml). Resistance to zinc was in between 100µg/ml - 1000µg/ml and the majority of them shows resistance in between 200µg/ml - 500µg/ml. Nickel resistance was in between 100µg/ml - 1000µg/ml and a good number of them shows resistance in between 300µg/ml - 400µg/ml. Resistance to copper was in between <100µg/ml - 500µg/ml and most of them showed resistance in between 300µg/ml - 400µg/ml. From the results of this study, it was concluded that heavy metal-resistant bacteria are widely distributed in the soils of MG university campus and the tolerance of heavy metals varied among bacteria and between locations.

Key words: Soil; Heavy metals; Bacteria; Metal resistance.

Introduction

The value of life on earth is connected unquestionably to the overall quality of the environment (Anon, 1995). Pollution of the biosphere by heavy metals due to industrial, agricultural and domestic activities has formed a serious problem for the safe and rational utilization of soils (Srivastava *et al*., 2005). Industrial inputs and the agronomic application of fertilizers, pesticides and metal-contaminated sewage continue to contribute the metal accumulation in the soil (Herland *et al*., 2000). The pollution of the ecosystem by heavy metals is a real threat to the environment because metals cannot be naturally degraded like organic pollutants and persist in the ecosystem having accumulated in different parts of the food chain (Igwe *et al*., 2005). Metal toxicity may affect all forms of life including microorganisms, plants and animals, but the degree of toxicity varies for different organisms.

Heavy metals are often defined as a group of metals whose atomic density is greater than 5 g/cm. Some of the heavy metals are essential and, are required by the organisms as micronutrients, and they are known as 'trace elements, while some other heavy metals have no biological role and are harmful to the organisms even at very low concentration. Heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity. Heavy

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metals can damage the cell membranes, alter enzymes specificity, disrupt cellular functions and damage the structure of the DNA. Due to the selective pressure from the metal in the growth environment, microorganisms have evolved various mechanisms to resist the heavy metal stress.

At present, the tolerance of soil bacteria to heavy metals has been proposed as an indicator of the potential toxicity of heavy metals to other forms of biota (Hassen *et al*. 1998). Microbial survival in polluted environment depends on intrinsic biochemical and structural properties, physiological, and/or genetic adaptation including morphological, changes of cells, as well as environmental modifications of metal speciation (Wuertz & Mergeay, 1997). In the present work, an attempt was made to study the metal resistance patterns of total heterotrophic bacteria isolated from the soils of Mahatma Gandhi University campus, Kottayam, Kerala.

Materials and methods

Study area

For the present study, we selected Mahatma Gandhi University campus for soil sample collection. The Mahatma Gandhi University campus is situated at about 15km from Kottayam town, Kerala lies between 9º39'28" N latitude and 76º32'10" E longitudes. The campus is having an undulating topography with residual hillocks having laterite outcrops (Fig.1).

Collection of Sample

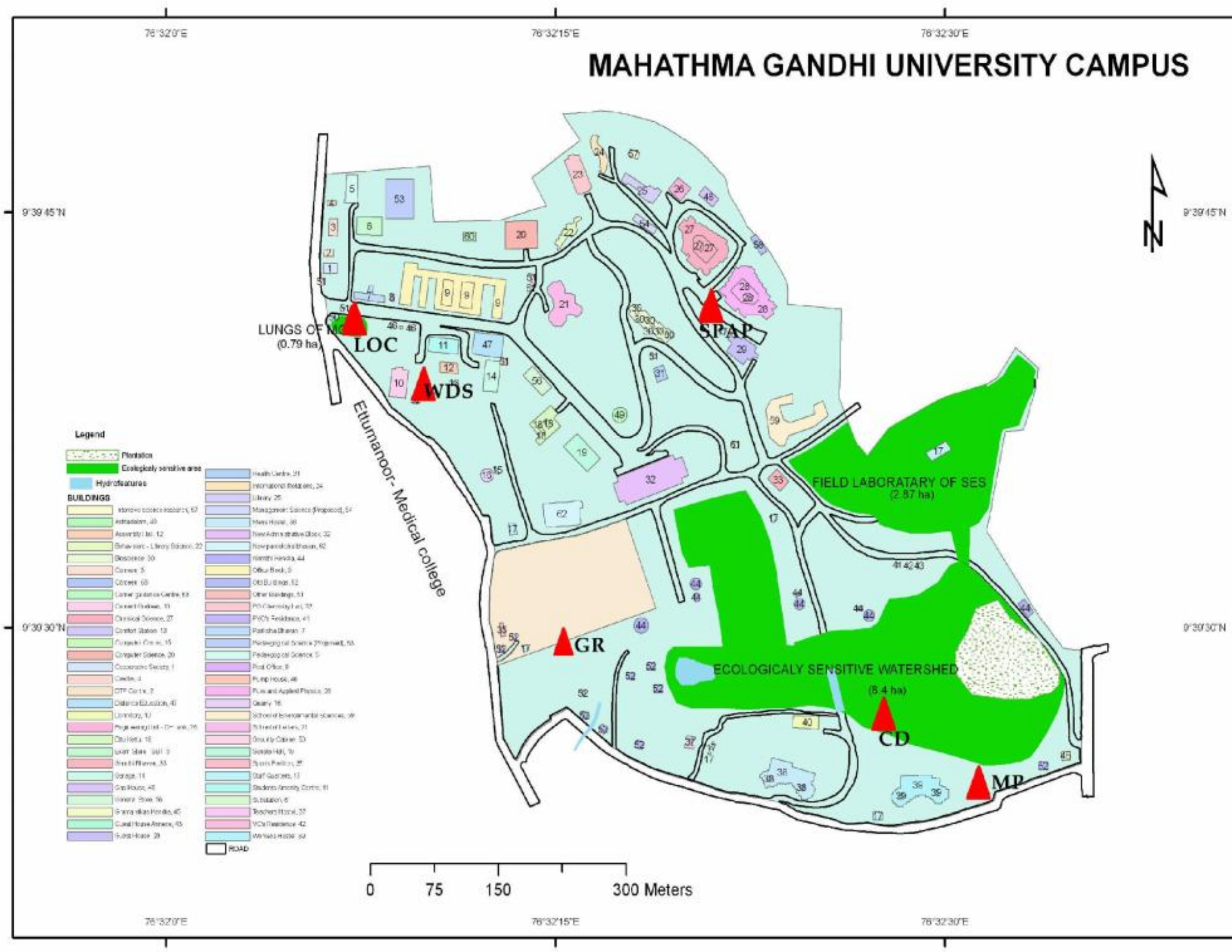

Fig.1. Mahatma Gandhi University Campus

The soil samples were collected from prefixed six sites of Mahatma Gandhi University campus. Samples were collected at a depth of 15 to 20cm from the surface after removing the top layer.

Table 1. Heavy metal resistance patterns of bacterial strains isolated from Manjium Plantation of MG University campus

Culture Name	Heavy metal concentration (µg/ml)				
	Zn	Ni	Cd	Cu	Pb
MP1	300-400	300-400	<100	300-400	>4000
MP 2	300-400	300-400	<100	400-500	>4000
MP 3	200-300	300-400	<100	300-400	>4000
MP 4	200-300	100-200	<100	200-300	1000-1500
MP 5	200-300	100-200	<100	100-200	1000-1500
MP 6	<100	100-200	<100	<100	>4000
MP 7	200-300	100-200	<100	<100	1500-2000
MP 8	200-300	300-400	<100	<100	1500-2000

Table 2. Heavy metal resistance patterns of bacterial strains isolated from Check Dam of MG University campus

Culture Name	Heavy metal concentration (µg/ml)				
	Zn	Ni	Cd	Cu	Pb
CD1	200-300	300-400	<100	<100	1500-2000
CD2	200-300	300-400	200-300	300-400	1500-2000
CD3	900-1000	300-400	<100	300-400	1500-2000
CD4	<100	300-400	<100	<100	<100
CD5	300-400	200-300	<100	<100	2000-3000
CD6	100-200	300-400	<100	100-200	>4000

For each of the sampling sites, sub-samples of soil were collected from different locations, pooled together and homogenized so as to obtain representative sample. Samples were collected using a spade that is thoroughly cleaned and disinfected between sampling to prevent cross-contamination.

Table 3. Heavy metal resistance patterns of bacterial strains isolated from Playground of MG University campus

Culture Name	Heavy metal concentration (µg/ml)				
	Zn	Ni	Cd	Cu	Pb
Gr1	<100	100-200	<100	<100	1000-1500
Gr2	400-500	300-400	<100	100-200	1000-1500
Gr3	900-1000	300-400	100-200	300-400	1000-1500
Gr4	<100	300-400	<100	<100	1000-1500
Gr5	<100	300-400	<100	100-200	1000-1500
Gr6	<100	300-400	<100	<100	2000-3000
Gr7	<100	300-400	<100	<100	1000-1500

Isolation and maintenance of bacteria

Isolation of bacteria, were carried by standard serial dilution plate technique. 10 g of soil was transferred to 90 ml sterile distilled water and agitated vigorously. Different aqueous dilutions, 10^{-1} to 10^{-7} of the suspensions were prepared and spread plated on Soil extract agar. Pure cultures of Bacterial colonies were prepared and persevered for metal tolerance studies.

Metal resistance testing

Heavy metals resistance of the isolates to lead, cadmium, copper, nickel and zinc were determined by agar dilution method (Lui *et al.,* 1983). Fresh overnight culture of the isolates grown in peptone water was aseptically inoculated in to nutrient agar plates, which were supplemented with increasing concentration of the afore-said metals individually (5µg/ml to 4 mg/ml). The plates were incubated at the room temperature and observed for bacterial growth. The lowest concentration of heavy metals at which no growth occurred when compared with the control plates was considered as the minimal inhibitory concentration (MIC). All metal salts were added to the medium after autoclaving and cooling to 45-50^{o}C from filter-sterilized stock solutions. The metal salts used for the study includes Lead nitrate ($Pb(NO_3)_2$), Zinc sulphate ($ZnSO_4.6H_2O$), Nickel sulphate ($NiSO_4.6H_2O$), Copper sulphate ($CuSO_4.5H_2O$) and Cadmium nitrate ($Cd(NO_3)_2.4H_2O$).

Results and discussion

A total of 46 bacterial isolates were isolated from different locations of the campus and tested for their resistant to five common metals in use (lead, zinc, copper, cadmium and nickel) by agar dilution method. The results of the present study revealed that most of the isolates showed resistance to one or more heavy metals selected. Resistance to lead was comparatively high followed by zinc, nickel, copper and cadmium.

About 33% of the isolates showed very high tolerance (>4000µg/ml) to lead. Tolerance to cadmium was rather low (<100 µg/ml). Resistance to zinc was in between 100µg/ml-1000µg/ml and most of the isolates

Table 4. Heavy metal resistance patterns of bacterial strains isolated from Waste dumping site of MG University campus

Culture Name	Heavy metal concentration (µg/ml)				
	Zn	Ni	Cd	Cu	Pb
WDS1 WDS2 WDS3 WDS4 WDS5 WDS6 WDS7 WDS8 WDS9	900- 1000 200- 300 500- 600 900- 1000 <100 900- 1000 <100 400- 500 300- 400	100- 200 300- 400 100- 200 300- 400 200- 300 300- 400 100- 200 100- 200 100- 200	100- 200 300- 400 100- 200 100- 200 100- 200 100- 200 100- 200 300- 400 200- 300	300- 400 100- 200 300- 400 300- 400 100- 200 300- 400 100- 200 300- 400 300- 400	>4000 500-1000 2000- 3000 >4000 2000- 3000 1500- 2000 2000- 3000 1500- 2000 >4000

shows resistance in between 200-500µg/ml. Resistance to Nickel was in between 100µg/ml-1000µg/ml and the majority of them shows resistance in between 300µg/ml-400µg/ml. Resistance to copper was in between 100µg/ml-500µg/ml and a good number of them showed resistance in between 300µg/ml- 400µg/ml. Heavy metal tolerance patterns of the bacterial isolates from the different locations of MG University campus are presented in Table1-6. The high levels of resistance and the widespread tolerance that was found among the isolates is probably attributed to the high metal contents in the soil (Abou-Shanab *et al.,* 2003).

All the isolates were tolerant to multiple metal ions. However, the patterns of tolerance among the 46 cultures varied may be due to the difference in the concentration of the different heavy metals in the soil.

Table 5. Heavy metal resistance patterns of bacterial strains isolated from lungs of Campus of MG University campus

Culture Name	Heavy metal concentration (µg/ml)				
	Zn	Ni	Cd	Cu	Pb
LOC1	<100	300-400	100-200	100-200	>4000
LOC2	900-1000	300-400	100-200	300-400	1500-2000
LOC3	500-600	300-400	>500	300-400	>2000
LOC4	900-1000	300-400	>500	200-300	>4000
LOC5	200-300	200-300	<100	<100	500-1000
LOC6	100-200	200-300	<100	100-200	500-1000
LOC7	900-1000	300-400	<100	300-400	>4000
LOC8	900-1000	300-400	100-200	200-300	>2000
LOC9	500-600	100-200	<100	<100	1000-1500
LOC10	900-1000	300-400	>500	300-400	>4000
LOC11	400-500	300-400	200-300	300-400	>4000

Conclusion

From the results of this study, it is concluded that heavy metal-resistant bacteria are widely distributed in the soils of MG university campus and the tolerance of heavy metals varied between bacteria even though they were isolated from the same soil.

Table 6. Heavy metal resistance patterns of bacterial strains isolated from SPAP site of MG University campus

Culture Name	Heavy metal concentration (µg/ml)				
	Zn	Ni	Cd	Cu	Pb
SPAP1	900-1000	300-400	100-200	300-400	>4000
SPAP2	400-500	900-1000	<100	100-200	>4000
SPAP3	900-1000	300-400	100-200	200-300	2000-3000
SPAP4	100-200	100-200	100-200	100-200	>2000
SPAP5	900-1000	300-400	100-200	400-500	>2000

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