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HEAVY METAL INDUCED RESISTANCE OF BACTERIA ISOLATED FROM TAMILNADU METAL INDUSTRY

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AUTHOR: **Joonu.J**

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[1]Dr.Joonu.J* & [2]Divya.P
[1] Asst.Prof, Department of Zoology, Bishop Heber College, Trichy-17
[2] Department of Zoology, Bishop Heber College, Trichy-17

CORRESPONDENCE AUTHOR
Joonu.J
Email: joonucharles@gmail.com

CONFLICTS OF INTEREST
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ABSTRACT
In the environment the heavy metals are generally more persistent and stable than organic contaminant such as pesticides and petroleum by products and are non-bio degradable . Elevated levels of heavy metals not only decrease soil microbial activity and crop production but also threaten the human health through the food chain .Microorganisms has the capability of biodegrading or detoxifying heavy metal present in contaminated soils and ground water. Bacteria have evolved uptake and efflux mechanisms to adapt in heavy metals contaminated environments and thus represent a potential source for bioremediation processes. In this study the heavy metals in the Tamil nadu metal industry effluent were analysed .The microbial degradation of heavy metals were observed .The bacteria from the effluent were isolated and characterized by different biochemical test like IMVIC, Catalase oxidase, staining methods etc . The metals like copper and nickel of different concentrations were prepared and the bacteria were induced to grow in presence of heavy metals .The growth of bacteria in 24 , 48, 72 hours were observed .The bacteria like *Bacillus subtillus , E.coli* showed maximum growth in the presence of nickel and copper.

INTRODUCTION:
Heavy metals from industrial processes are of special concern because they produce water for chronic poisining in aquatic animals .Environmental pollution due to chemicals including heavy metals is a problem that may have negative consequences on the biosphere .

In recent years , ground soil and other materials polluted with heavy metals have become a serious environmental problem throughout the world due to their use in many manufacturing processes , and up as waste in industrial effluent , through which heavy metals can enter water cycle , and then in food chain where they are concentrated and ultimately reaching the toxic levels. Bacteria tolerance to heavy metals has been reported in both gram positive and gram negative bacteria . It is generally believed that gram positive bacteria are less tolerant to heavy metals than gram negative bacteria .Some bacterial species like *Bacillus subtilus* may be resistant owing to their ability to sporulate .The mechanism of resistance include metal reduction or transformation to more volatile and less toxic forms . Bacteria like *E.coli* enzymatically reduce hg2+ to hg0 which is highly volatile and diffuses away from bacterial cell . Microbial communities in buried sediments may represent up to one-third of the earth's biomass (Whitman *et al*., 1998). The release of heavy metals into our environment is still large and causes an environmental pollution problem because of their unique characteristics ***(Soltan et al., 2008)***. Contamination of the aquatic environment by toxic metal ions is a serious pollution problems,

heavy metals may reach watercourses either naturally through a variety of geochemical processes or by direct discharge of municipal, agricultural and industrial wastewater ***(Semerjian, 2010; SrinivasaRao et al., 2010)***, to a lesser extent, from metals ***(Gerlach, 1981)*** and occupies the sixth position in the list of hazardous compounds (Nascimento and ***ChartoneSouza, 2003)***. At elevated concentrations, soluble metal compounds can be deleterious to human health as well as to aquatic and marine environments ***(Semerjian, 2010; SrinivasaRao et al., 2010***

2. MATERIALS AND METHODS

The effluent sample was collected from Tamilnadu metal industry at Pudukottai district, Tamilnadu, India. The sample was collected in a plastic sterile container and taken to the laboratory and maintained at 37°c for further studies The serially diluted colonies further characterized by inoculating the bacterial colonies in a nutrient agar medium. The colonies were subcultured by pour plate method

2.1 CHARACTERISATION OF COLONIES:

When microbes were grown in different media it shows differences in the macroscopic appearance of their growth. These difference called cultural characteristic and are used as the basis for separating microorganism into taxonomic groups

2.2 IDENTIFICATION OF BACTERIA PRESENT IN THE EFFLUENT:

The isolated bacterium was subjected to identification by staining and biochemical test .Gram staining procedure was carried out to identify the gram reaction of the organism. The biochemical test such as indole, methyl red, voges proskauver, citrate, urease test, catalase test, etc. were performed to identify the organism

2.3 GROWTH STUDIES OF HEAVY METAL INDUCED BACTERIA

Growth studies of *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae,* were carried out in nutrient broth medium supplemented with peptone and yeast extract, etc. 10µl of culture were inoculated into the nutrient broth medium supplemented with copper and nickel metal then it were analysed for growth studies (Nair et al.,(1992).

4.1 In broad range:

The three isolates were tested for metal tolerance using 3 different concentrations of two metals salts each. Copper chloride and Nickel ammonium sulfate salts were used in different concentrations like 50mM, 80mM, 100mM. In different concentrations the three isolates were inoculated in broth containing heavy metals and incubated for 24, 48 72 hours. Then OD were taken at different intervals of time in uv spectrophotometer. By using readings graph were plotted

4.2 In narrow range:

The three isolates were tested for metal tolerance using 3 different concentrations of two metals each. Copper chloride and nickel ammonium sulfate salts were used in different concentrations of 10mM, 15mM, 20mM. In different concentrations the three isolates were inoculated in broth containing heavy metals and incubated for 24, 48, 72 hours. Then O.D were taken at different intervals of time in UV spectrophotometer. By using readings graph were plotted.

RESULTS AND DISCUSSION:

This investigation highlights the presence of microbes in the metal industry effluent sample and shows the prevalent occurrence of metal tolerant microbial population in the TAMILNADU METAL INDUSTRY,MATHUR. Heavy metal pollution of soil and waste water is a significant environmental problem. Waste waters from the industries and sewage sludge applications have permanent toxic effects to human and the environment. The heavy metal and radionucleotide pollution from nuclear power plants, mining industries, electroplating industries and agricultural runoffs is a major cause of concern to public health, animals and ecosystems . (Kratochivil *et al.,* 1998). Persistent toxicants (heavy metals) in water and sediments affected by heavy metal pollution can have serious effects on the aquatic ecosystem and can make water unsuitable for human consumption (DPIW).

Biochemical test	Results
A. Morphological Properties: 1.Gram's staining 2.Spore staining 3.Motility	Gram positive rods Spore forming Motile
B. Cultural Properties: 1.Nutrient agar 2.Starch hydrolysis agar	Raised opaque, dull, white colonies White colour gas forming colonies
C. Biochemical Properties: 1.Indole test 2.Methyl red test 3.Voges Proskauer 4.Citrate test 5.Urease test 6.Catalase test 7.Oxidase test 8. Manitol test	- + + - - + - -

Table 1 : IDENTIFICATION OF BACTERIA : BACILLUS SUBTILIS

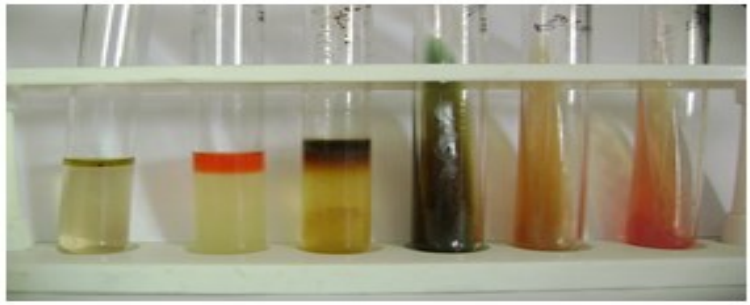


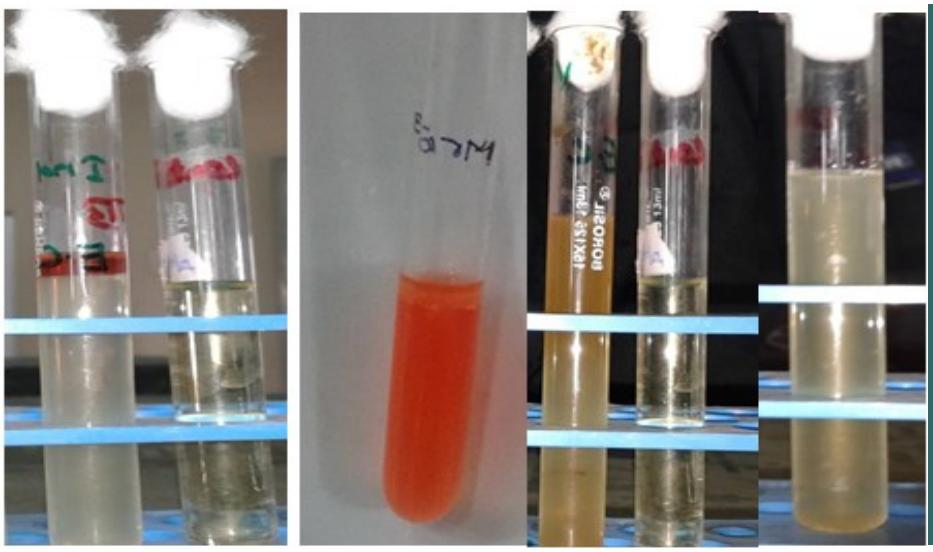
Fig 1: Identification tests of Bacillus subtilis

Nutrient agar EMB Agar Gram staining

Fig 2: Identification tests for E.coli

BIOCHEMICAL TEST	RESULTS
MORPHOLOGICAL PROPERTIES: 1. Gram's staining 2. motility	**Gram negative and non sporulating** **Motile**
CULTURAL PROPERTIES : 1. Nutrient agar 2. Eosin agar	Circular , convex , Mettalic sheen , translucent,
BIOCHEMICAL TEST : 1. Indole test 2. methyl red test 3. voges proskauer 4. Catalase test	 + + –

Table 2.1: Identification test results of E .coli



Bio Chemical Test Results- IMVIC test

IMVIC Test

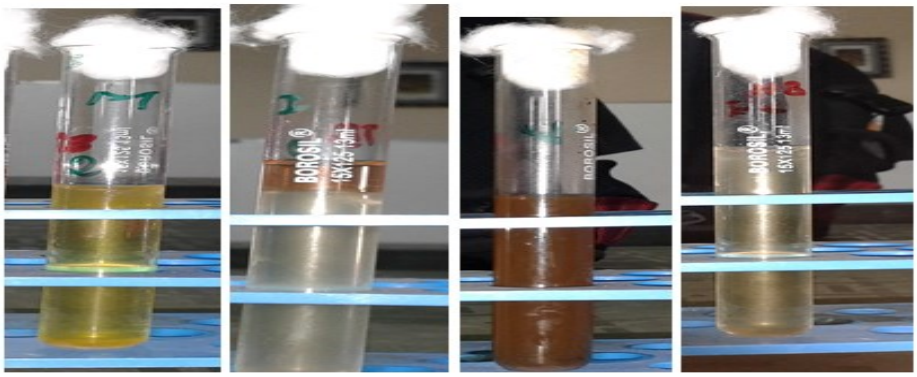


Figure 3. Identification tests of Klebsiella pneumoniae

BIOCHEMICAL TEST	RESULTS
MORPHOLOGICAL PROPERTIES: 1. Gram's staining 2. motility	Gram negative , non sporing encapsulated
CULTURAL PROPERTIES : 1. Nutrient agar 2. Eosin agar	Round, pink , slightly raised mucoid
BIOCHEMICAL TEST : 1. Indole test 2. methyl red test 3. voges proskauer 4. Catalase test	- - + +

Figure 3.1: Biochemical test results for Klebsiella pneumoniae

Broad range of copper and Nickel

Narrow range of copper and nickel

HEAVY METAL RESISTANCE

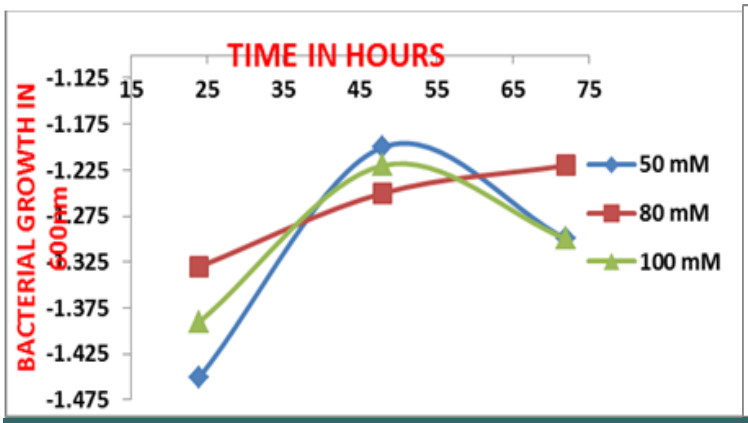


FIG 4.2.1.1 BACILLUS SUBTILIS

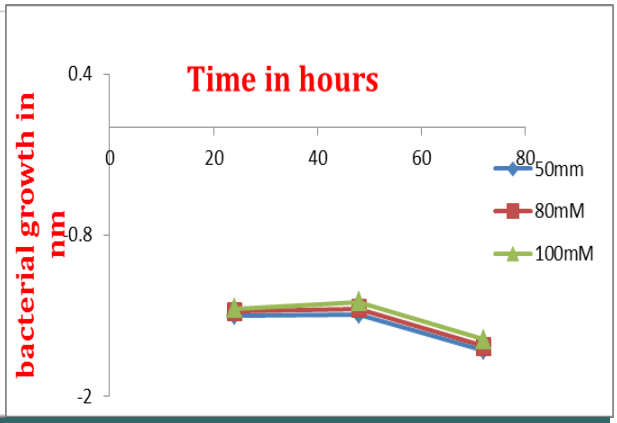


FIG 4.2.2 K.PNEUMONIAE

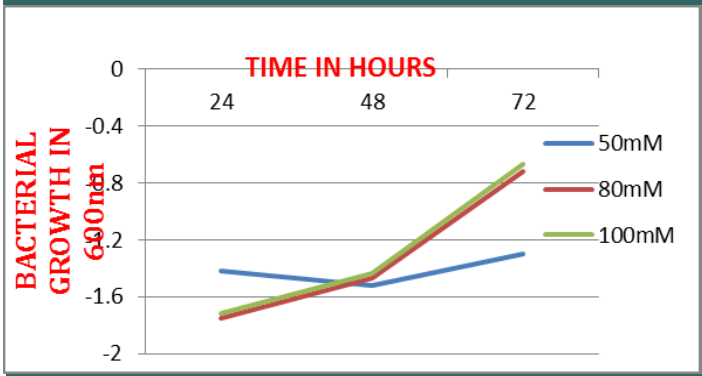


Fig.4.2.3 E.coli

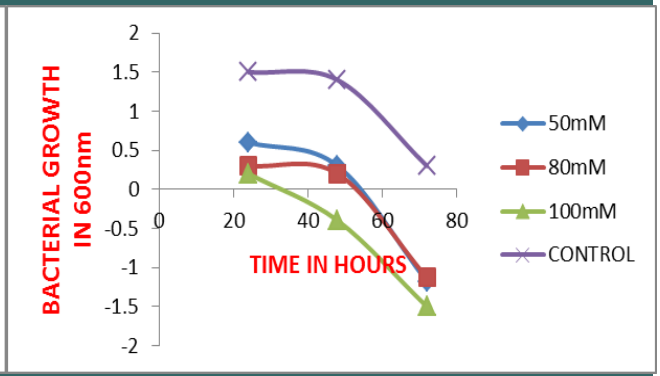


FIG 4.3.1 BACILLUS SUBTILIS

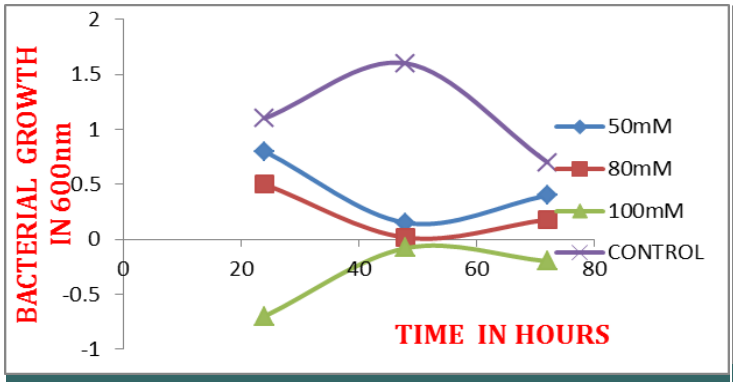


Fig.4.3.2 E.coli

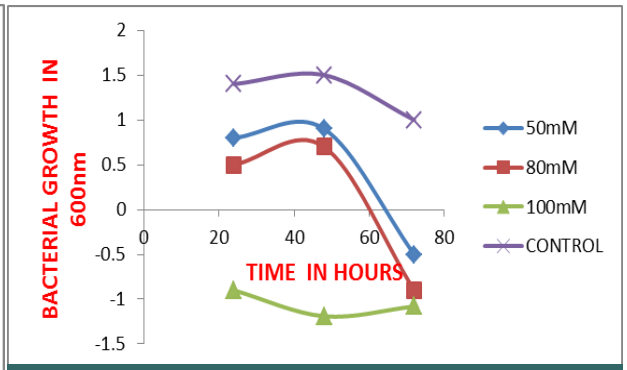


Fig. 4.3.3 Klebsiella pneumoniae

HEAVY METALS	CONCENTRATION	GROWTH OF BACTERIA
COPPER BROAD RANGE	100mM 80mM 50mM	Not Resistant Resistant Resistant
COPPER NARROW RANGE	20mM 15mM 10mM	Susceptible Susceptible susceptible
NICKEL BROAD RANGE	20mM 15mM 10mM	Resistant Resistant Resistant
NICKEL NARROW RANGE	20mM 15mM 10mM	Susceptible Susceptible susceptible

Table4.5 Resistance of bacteria to different heavy metals in different concentrations

Thus , from the above results it is clearly indicated that

- Bacteria can able to resist in heavy metals in narrow range than broad range of concentrations .
- Heavy metals like nickel is more toxic in heavy concentrations due to this bacterial growth is very less .Hence, Bacteria cannot tolerate their toxicity in broad range

But in narrow range bacteria like *Bacillus subtilus* was resistant.

- In copper, bacteria like *E.coli* , can able to tolerate even in higher concentrations .
- Hence , we can use these bacterial species to control the heavy metal toxicity in the environment.
- These bacteria can able to adsorb heavy metals at different concentratons. These bacteria can be widely used in bioremediation.

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SIFT DESK
Deerpark Dr, #75, Fullerton,CA,92831,United States.
E-mail:
helpdesk@siftdesk.org
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