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# ACTINOMYCETES: TOLERANCE AGAINST HEAVY METALS AND ANTIBIOTICS

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**Abstract:** Heavy metals can be both, essential as well as toxic for living beings. Micronutrients such as, Co, Fe, Mn have important role to play in living systems whereas, Pb Cd etc. pose harmful effects even at low concentrations. When these heavy metals get accumulated within the tissues of the organisms at various levels of the ecological chain, they cause decrease in the biomass and biological diversity by affecting the growth, morphology and activity of the organisms. Accumulation of heavy metals in soil also causes soil contamination, which can be overcome with the help of bioremediation. A large group of soil bacteria belonging to the *Actinomycetes* species are exposed to heavy metals in a variety of ways; although, they show resistance to heavy metals. The species of *actinomycetes* possess resistance for antibiotic synthesis as well. This makes the *actinomycetes* suitable agents for bioremediation. In this experiment, a total of 20 isolates from Shivpuri region of Madhya Pradesh were tested for the metal tolerance against selected heavy metals. After this, the most tolerant strains were tested to check their antibiotic susceptibility. Metal tolerance was tested by agar well diffusion method and tube dilution method. Out of the 20 isolates, Ash1, Ash 2, Ash 4,Ash 6, Ash 7, Ash 8, Ash 9, Ash 10, Ash 11, Ash 12, Ash 13, Ash 15 were resistant at 10 mM conc. of $CuSo_4$, but their growth was inhibited at higher concentrations of metal salts. Isolates Ash 10, Ash 11, Ash 12, Ash 13, Ash 19, Ash 20 were found to be resistant at 10mM conc. of ZnSO4, but they were also inhibited at higher concentrations. For different concentrations of Pb $(CH_3COO)_2$ most of the isolates showed same level of tolerance.

**Key Words:** Actinomycetes, Bioremediation, Heavy metal tolerance.

## INTRODUCTION

Metals play an important role in the vital processes of living beings. Heavy metals such as, Co, Cr, Ni, Fe, Zn & Mn etc are required by the living organisms in trace amounts and are known as micronutrients [1]. These metals are involved in various redox processes, enzymatic reactions and osmotic regulation [2, 3]. On the other hand, there are metals such as, Pb, Cd, Hg etc which have no biological role, instead are toxic and detrimental even at very low concentrations. However at higher concentrations, metals, both essential as well as non-essential, are toxic.

Heavy metals can accumulate within the tissues of organisms throughout the ecological chains at different levels and influence the growth, morphology and biochemical activity of the organisms resulting in decreased biomass and diversity [4]. Toxicity occurs through displacement of essential metal from their native binding site or as a result of alteration in conformational structure of the nucleic acids & proteins and interference with oxidative phosphorylation and osmotic balance [5, 1].

Both land as well as water is contaminated and both natural & anthropogenic sources account for this. Soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, coal combustion residues, spillage of petrochemicals, application of fertilizers, animal manures, sewage sludge, pesticides, waste water irrigation and atmospheric deposition [6, 7]

In this perspective many approaches have been used to assess the risk posed by the contaminating metals in the soil, water bodies etc. 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 [8, 9] Generally, gram-negative species appear to be more tolerant than gram-positive ones in soils that contain comparatively low levels of metal pollution [10].

Several studies have reported the role of microbes in bioremediation of heavy metals. Bioremediation is the process that uses microorganisms or their enzymes to return the environment, altered by contaminants, back to its original condition. Biosorpents like microbes can bind contaminants onto their cellular structures and have been used in environmental clean ups. It has been reported that there may be several potential microbial metal biosorbents. These include genera of *Bacillus, Pseudomonas, Streptomyces, Aspergillus, Rhizopus* and *Penicilium* [11].

Several toxicological studies have examined the heavy metal sensitivity of bacteria isolated from different habitats [12, 13, 14, 15]. The introduction of heavy metals in various forms in the environment can produce considerable modifications in the microbial

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communities and their activities [12, 14, 16, 17]. Their toxicity can be affected by abiotic factors such as pH, $E_h$, temperature, organic materials, or clay minerals, factors which also influence their speciation and bioavailability [18, 19, 20, 21]. Heavy metals generally exert an inhibitory action on microorganisms by blocking essential functional groups, displacing essential metal ions, or modifying the active conformations of biomolecules [12, 22, 23]. However, at relatively lower concentrations some metals (Co, Cu, Zn, Ni etc.) are essential for the growth of microorganisms, as they act as co-factors for metallo-proteins and enzymes [24, 12].

The mechanism of resistance to a metal may be of two types, either by accumulation of metal in the form of particular protein-metal association [25, 26], or by blockage at cell-wall level or membrane transport level [25, 27, 28]. Actinomycetes, comprises a large group of soil bacteria that are important in the recycling of carbon in polymeric macromolecules. They may be exposed to heavy metals in a variety of ways, especially when agricultural soils are subjected to treatment with sewage sludge [27]. The ways in which they interact with heavy metals are unknown. It has been reported that *actinomycetes* as a group are more tolerant to cadmium than other species. *Streptomyces* species that are able to detoxify $Hg^{2+}$ to volatile $Hg^0$ by means of a mercuric reductase enzyme have also been isolated [30, 31]. Therefore, there is a dramatic increase in the interest on studying the interactions of heavy metals with these microorganisms.

Traditionally, *actinomycetes* have been a rich source of biotechnological products like antibiotics, industrial enzymes and other bioactive molecules [32]. More than 70% of naturally occurring antibiotics have been isolated from *actinomycetes*. Naturally, these antibiotic producers also possess resistance for antimicrobial molecules they produce and these resistance mechanisms may be linked to antibiotic synthesis [33]. Several studies have indicated a correlation between multiple antibiotic resistance and heavy metal resistance [34, 35]. Heavy metal resistance together with metabolic diversity and specific growth characteristics of actinomycetes, such as, mycelium formation and relatively rapid colonization of selective substrates present them as suitable agents for bioremediation [36].

The genus *Streptomyces* comprises a large group of soil bacteria that are important in the recycling of carbon in polymeric macromolecules. They may be exposed to heavy metals in a variety of ways, especially when agricultural soils are subjected to treatment with sewage sludge [29]. The ways in which they interact with heavy metals are unknown.

Microbial resistance to heavy metals has been intensively studied over the past 35 years. However, most of the work has been directed towards biochemistry, genetics, and molecular biology of resistance mechanisms in laboratory cultures [37]. The most favored approach now is selecting organisms that can be used to develop tools to assess the metal levels in the environment. The objective of this study was to identify the bacteria previously isolated from uncontaminated soil to determine their tolerance towards lead, copper, and zinc and also to check the antibiotic susceptibility of the tolerant strains.

## MATERIALS AND METHODS

### *Sample collection*

Soil samples were collected from various places in Shivpuri, a district in Madhya Pradesh (India). The soil samples were collected from various locations: plant soil, stone mines, well, field, Harsi Dam and sewages.

### *Isolation of actinomycetes*

From these soil samples, microorganisms were isolated by serial dilution followed by spreading and re-streaking. Total 38 actinomycetes isolates were obtained which were morphologically and biochemically characterized [38]. Out of these, 20 isolates were selected and used for this experimental work.

### *Storage and maintenance of isolates*

For storage, purified isolates were inoculated into ISP1 media and incubated for 2-3 days and then stored at 4°C. For long term storage, glycerol stocks were prepared for all the isolated *actinomycetes*, by transferring them to 20% glycerol in ratio of 1:1. This preparation was then stored at -20°C in a deep freezing refrigerator.

### *Revival of previously isolated actinomycetes strains from their glycerol stocks*

For this experimental work, isolates starting from 1 to 20 were selected. These isolates were then revived from their glycerol stocks kept at -20°C in ISP1 media. For revival 10% of inoculum was inoculated in ISP 1 media and incubated overnight (24 hours) at 28°C.

### *Preparation of standard heavy metal salt solutions*

Heavy metal salt solution was prepared by following methods.

### *Preparation of PBS buffer (0.1 M, for 250 ml)*

Dissolve 2 g NaCl, 0.05 g KCl, 0.36 g $Na_2PO_4$ and 0.06 g $KH_2PO_4$ in 200 ml of distilled water and then make up the volume to 250 ml. Adjust the pH to 6.8.

***Preparation of metal solutions***

Weighed amounts of heavy metal salts : $CuSO_4.5H_2O$ (mol. wt. 249.68 g/mol), $ZnSO_4.7H_2O$ (mol. wt. 287.54 g/mol), $(CH_3COO)_2Pb.3H_2O$ (mol. wt. 379.33 g/mol); were dissolved in defined volume of PBS buffer to make solutions of the following concentrations; 10 mM, 50 mM, 100 mM, 250 mM and 500 mM. These solutions were sterilized by autoclaving at 121°C for 15 minutes, and then cooled down for further use.

***Methods for the assessment of metal toxicity***

Two methods were used to assess the metal tolerance of actinomycetes against the stress laid by the addition of heavy metals to its growth environment. Based on different growth behavior of bacteria on organic ligand supplemented and non-supplemented media [39, 40], both, semisolid agar media and agar-free (broth/liquid) media based methods were employed.

***Plate/Agar-well diffusion method***

7 days old loop full culture of isolates was taken for metal resistant study by Plate/Agar-well diffusion method. Starch Casein Agar plates were prepared and solidified. Using a sterile well borer (13mm width), a central well was made on the surface of Starch Casein Agar (SCA) plate. 500 µl of the standard metal salt solution (Cu, Zn, Pb) at different concentrations (10, 50, 100, 500 & 1000 mM) was poured into the well and was allowed to diffuse evenly across the well. On each plate six to eight strains were inoculated in radial streaks in duplicate such that two similar strains are opposite to each other.

All 20 strains were inoculated in the similar way by making agar plates diffused with different metal salts. These plates were incubated at 28°C for 7 days. The Zone of inhibition was measured (in mm) as the distance from the edge of the central well to the leading edge of the growing streak [9]. The percentage of metal tolerance by *actinomycetes* strains was calculated in terms of the ratio between length of the growth (in mm) and the length of the total inoculated streak. Hence, the greater the distance of growing colony from the edge of the well, the more was the inhibition exerted by that metal.

***Test Tube dilution method***

500 µl of the appropriate metal standard or salt solutions and 100 µl of the inoculum are taken and incubated at 28°C for 7 days (modified method) [41].

Positive control was also prepared by adding media, metal solution and inoculums, whereas negative control was prepared by adding media and metal solution only. The tubes were examined for turbidity. The tubes with turbidity were recorded as resistant and the ones without turbidity, as sensitive to that particular metal salt solution.

***Assessment of antibiotic susceptibility of the metal tolerant strains***

Metal resistant *actinomycetes* isolates Ash6, Ash8 (less metal stress tolerant) and Ash13, Ash15, Ash16 (more metal stress tolerant) were selected for antibiotic susceptibility test. The antibiotic susceptibility test was performed by disc diffusion method. Plates of Muller Hinton Agar media were prepared, sterilized and about 20 ml of MHA was poured on petriplates and was allowed to solidify. The 200 µl of selected strain cultures (5-7 days old) were spread on the surface of MHA plates; using sterile spreader. Antibiotic discs were prepared by cutting Whatman filter paper into 4mm circular discs and sterilized. Then these discs were aseptically dipped into antibiotic solutions (Gentamicin sulfate, Oxy-tetracycline, Ampicillin, Ceftriaxone sodium, kanamycin) with the help of a sterile forceps and then antibiotic discs were placed equidistantly on MHA plates. These plates were sealed properly and kept for incubation at 28°C for 2 days. Plates were observed for the zone of inhibition formed around the antibiotic discs used for the experiment.

## RESULTS

***Growth pattern on semisolid media***

Each isolate showed different tolerance level than the other, for different metals. Out of twenty, isolates Ash1, Ash 2, Ash 4, Ash 6, Ash 7, Ash 8, Ash 9, Ash 10, Ash 11, Ash 12, Ash 13, Ash 15 were resistant at 10 mM conc. of $CuSO_4$, but at other higher concentrations of metal salts their growth was inhibited. Isolate Ash 10, Ash 11, Ash 12, Ash 13, Ash 19, Ash 20 were resistant at 10mM conc. of $ZnSO_4$, but were inhibited at other higher concentrations. However most of the isolates showed same level of tolerance for different concentrations of Pb $(CH_3COO)_2$. On the basis of length of growing streak, tolerance of copper and zinc towards different *actinomycetes* isolates was calculated in terms of percentage of growth which was expressed in the form of graphs (figure XII & XIII).

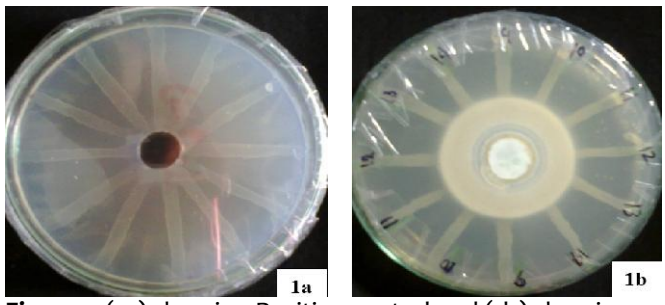



**Figure 1:** (1a) showing Positive control and (1b) showing growth patterns on SCA plate containing zinc sulfate at the concentration 500 mM.

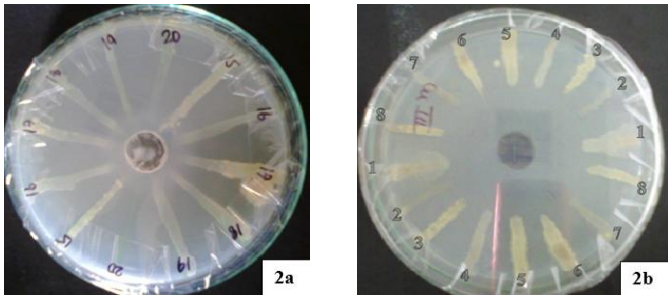



**Figure 2:** 2a showing growth patterns on SCA plate containing zinc sulfate at the concentration of 10mM, 2b showing growth patterns on SCA plate contained copper sulfate at the concentration of 100 mM.

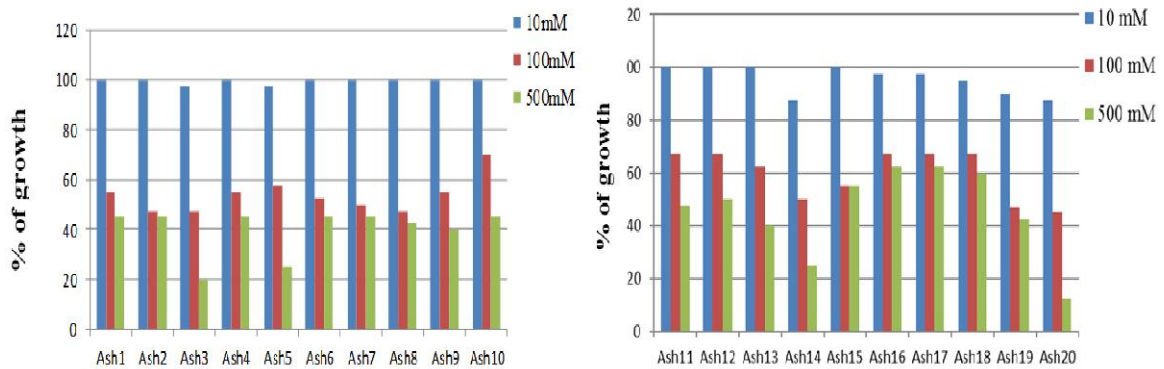
**Table 1:** Zone of inhibition (in mm) of isolates on SCA Plate.

| Actinomycetes Isolates | Zone of inhibition (in mm) on agar plates with different metal concentrations | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Conc. of copper sulfate (in mM) | | | | | Conc. of zinc sulfate (in mM) | | | | | Conc. of lead acetate (in mM) | | | | |
| | 10 | 50 | 100 | 250 | 500 | 10 | 50 | 100 | 250 | 500 | 10 | 50 | 100 | 250 | 500 |
| Ash1 | - | 8 | 18 | 21 | 22 | 1 | 12 | 16 | 18 | 20 | - | - | - | - | - |
| Ash2 | - | 7 | 21 | 22 | 22 | 4 | 11 | 14 | 18 | 20 | - | - | - | - | - |
| Ash3 | 1 | 13 | 21 | 30 | 32 | 7 | 14 | 24 | 25 | 27 | - | 15 | - | 35 | 20 |
| Ash4 | - | 7 | 18 | 20 | 22 | 3 | 11 | 16 | 20 | 22 | - | - | - | - | - |
| Ash5 | 2 | 15 | 17 | 28 | 30 | 23 | 23 | 23 | 24 | 25 | - | - | - | 33 | 25 |
| Ash6 | - | 6 | 19 | 20 | 22 | 3 | 13 | 19 | 23 | 24 | - | - | - | - | - |
| Ash7 | - | 7 | 20 | 22 | 22 | 9 | 14 | 17 | 20 | 23 | - | - | - | - | - |
| Ash8 | - | 8 | 21 | 23 | 23 | 3 | 12 | 22 | 22 | 22 | - | - | - | - | - |
| Ash9 | - | 18 | 18 | 24 | 24 | 4 | 18 | 20 | 17 | 21 | 3 | 5 | 7 | 5 | 9 |
| Ash10 | - | 15 | 12 | 21 | 22 | - | 16 | 18 | 17 | 18 | - | - | 7 | 9 | 13 |
| Ash11 | - | 18 | 19 | 21 | 21 | - | 17 | 19 | 15 | 20 | - | - | 9 | 10 | 14 |
| Ash12 | - | 8 | 13 | 16 | 20 | - | 17 | 19 | 16 | 20 | - | - | - | 7 | 9 |
| Ash13 | - | 5 | 15 | 20 | 24 | - | 16 | 12 | 13 | 18 | - | - | 9 | 11 | 15 |
| Ash14 | 5 | 18 | 20 | 26 | 30 | 10 | 11 | 15 | 17 | 17 | - | - | - | 6 | 11 |
| Ash15 | - | 13 | 13 | 18 | 18 | 1 | 15 | 18 | 18 | 21 | - | - | 5 | 5 | 5 |
| Ash16 | 1 | 14 | 13 | 13 | 15 | 3 | 11 | 13 | 19 | 19 | - | - | - | 10 | 11 |
| Ash17 | 1 | 14 | 13 | 13 | 15 | 2 | 10 | 13 | 19 | 20 | - | - | - | 5 | 6 |
| Ash18 | 2 | 12 | 13 | 14 | 16 | 1 | 11 | 15 | 19 | 21 | - | - | - | 5 | 5 |
| Ash19 | 4 | 14 | 17 | 18 | 20 | - | 15 | 16 | 21 | 22 | - | - | - | 10 | 10 |
| Ash20 | 5 | 16 | 22 | 30 | 30 | - | 15 | 16 | 30 | 30 | - | - | - | 10 | 10 |

**Calculation:** Percentage of bacterial tolerance/growth was calculated by using following formula:

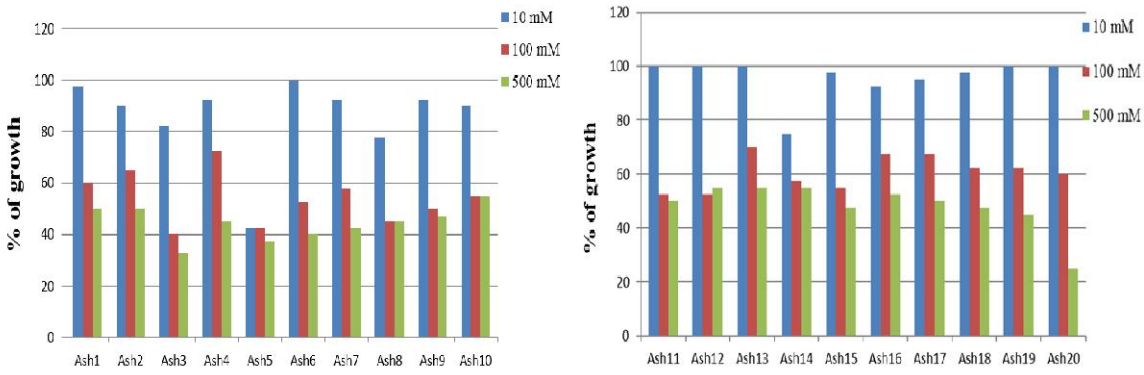
$$\% \text{ of growth} = \frac{\text{Complete inoculated length of streak - inhibited length}}{\text{Complete inoculated length}} \times 100$$

(Complete length of inoculated streak was = 40 mm)




**3a.** **3b.**

**Figure 3:** Represents the graph showing the calculated % of growth of isolates. Figure 3a & 3b showing toxicity of copper by actinomycetes isolates tested on agar media.




**4a.** **4b.**

**Figure 4:** 4a & 4b, showing tolerance of zinc by actinomycetes tested on agar media

***Growth of actinomycetes isolates in liquid media***

All 20 actinomycetes isolates grown in ISP1 broth containing varying concentrations of heavy metal salts were observed for turbidity, as shown in figure 14a to 14f. Table IV is showing the presence or absence of turbidity in the experimental tubes. Ash13, 15, 16 were most tolerant; Ash1, 2, 6, 8, 9, 10, 11, 12, 17, 18, 20, were moderately tolerant; and Ash3, 5, 7, 14, 20 were sensitive.

**Table 2:** Assessment of metal tolerance in liquid media

| Actinomycetes Isolates | Concentrations of zinc sulfate (in mM) | | | | | Concentrations of copper sulfate (in mM) | | | | | Concentrations of lead acetate (in mM) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | 50 | 100 | 250 | 500 | 10 | 50 | 100 | 250 | 500 | 10 | 50 | 100 | 250 | 500 |
| Ash1 | + | – | – | – | – | + | – | – | - | – | + | + | + | – | – |
| Ash2 | + | – | – | – | – | + | – | – | – | – | + | + | - | – | – |
| Ash3 | - | – | – | – | – | - | – | – | – | – | - | - | - | – | – |
| Ash4 | + | – | – | – | – | + | – | – | – | – | + | + | - | – | – |
| Ash5 | – | – | – | – | – | - | – | – | – | – | - | - | - | – | – |
| Ash6 | + | – | – | – | – | + | – | – | – | – | + | + | - | – | – |
| Ash7 | – | – | – | – | – | + | – | – | – | – | + | + | + | – | – |
| Ash8 | + | – | – | – | – | + | – | – | – | – | + | + | - | – | – |
| Ash9 | + | – | – | – | – | + | – | – | – | – | + | + | + | – | – |
| Ash10 | + | – | – | – | – | + | – | – | – | – | + | + | + | – | – |
| Ash11 | + | – | – | – | – | + | – | – | – | – | + | + | - | – | – |
| Ash12 | + | – | – | – | – | + | – | – | – | – | + | + | + | – | – |
| Ash13 | + | + | – | – | – | + | – | – | – | – | + | + | - | – | – |
| Ash14 | - | - | – | – | – | + | – | – | – | – | + | - | - | – | – |
| Ash15 | + | + | – | – | – | + | – | – | – | – | + | + | - | – | – |
| Ash16 | + | + | – | – | – | + | – | – | – | – | + | + | - | – | – |
| Ash17 | + | – | – | – | – | + | – | – | – | – | + | + | + | – | – |
| Ash18 | + | – | – | – | – | + | – | – | – | – | + | + | - | – | – |
| Ash19 | + | – | – | – | – | + | – | – | – | – | + | + | - | – | – |
| Ash20 | - | – | – | – | – | + | – | – | – | – | + | + | - | – | – |

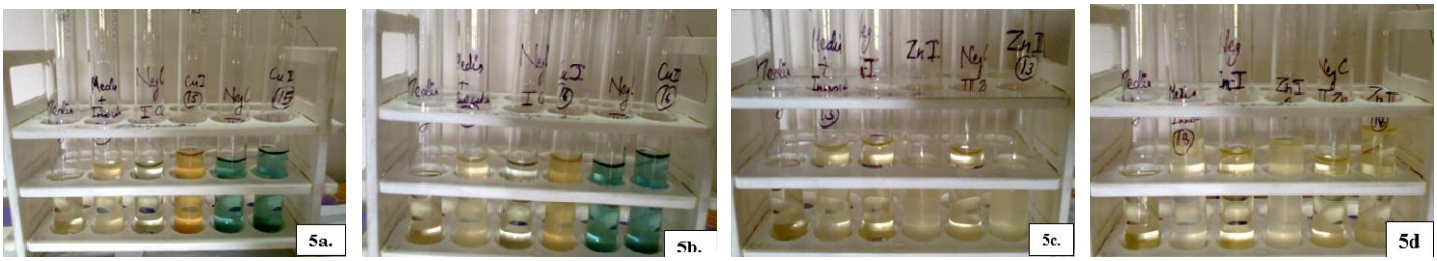



**Figure 5:** Actinomycetes isolates showing different levels of turbidity (growth) in liquid media supplemented with different concentrations heavy metals salts: Cu (14a &b), Zn (14a & b) and Pb (14e & f).

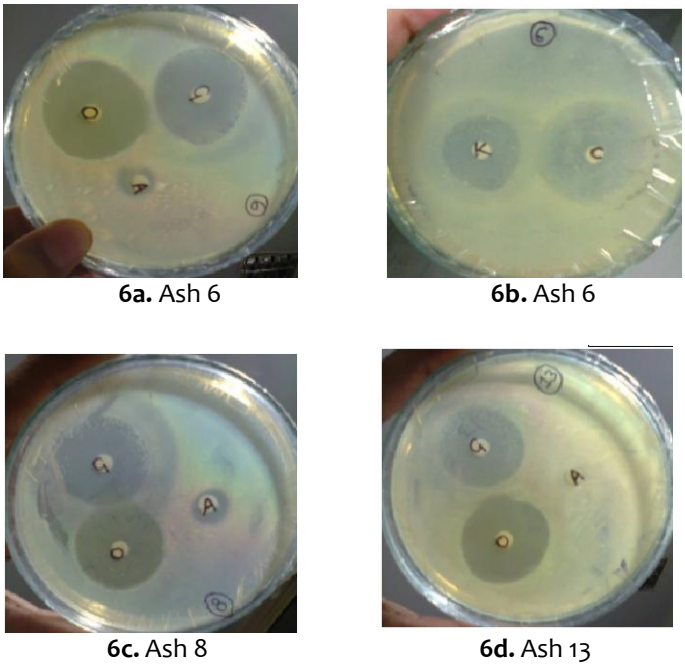

**Figure 5:** Actinomycetes isolates showing different levels of turbidity (growth) in liquid media supplemented with different concentrations heavy metals salts: Cu (14a &b), Zn (14a & b) and Pb (14e & f).

***Antibiotic susceptibility***

Five isolates (6, 8, 13, 15 &16) out of 20 were further tested for their sensitivity towards various antibiotics like gentamicin (G), oxy-tetracycline (O), ampicillin (A), kanamycin (K) and ceftriaxone (C) on MHA plates. The zone of inhibition was observed shown in table V. Greater zone of inhibition is observed for ceftriaxone and smaller or no zone for ampicillin, as shown in figure 15. According to the values of area of inhibition, antibiotic susceptibility shown by actinomycetes isolates, can be expressed in the following order: oxy-tetracycline> ceftriaxone> gentamicin> kanamycin>ampicillin.

**Table 3:** Antibiotic susceptibility of the metal tolerant actinomycetes isolates

| Actino-mycetes Isolates | Zone of inhibition (in mm) around antibiotic-disc | | | | |
|---|---|---|---|---|---|
| | Gentamicin | Oxy-tetracycline | Ampicillin | Kanamycin | Ceftriaxone |
| Ash6 | 31 | 33 | 10 | 23 | 33 |
| Ash8 | 28 | 29 | 11 | 26 | 37 |
| Ash13 | 27 | 28 | - | 26 | 42 |
| Ash15 | 30 | 26 | - | 28 | 36 |
| Ash16 | 28 | 33 | 8 | 27 | 35 |



**6a.** Ash 6 **6b.** Ash 6

**6c.** Ash 8 **6d.** Ash 13

**Figure 6:** Antibiotic susceptibility shown by zone of inhibition (ZOI)

## DISCUSSION

A total of 20 isolates were used in this experimental work which were previously isolated from Shivpuri region of Madhya Pradesh. These isolates were tested for the metal tolerance against selected heavy metals and the most tolerant strains were tested against five antibiotics to check their antibiotic susceptibility. Metal tolerance was tested by two methods: agar well diffusion method and tube dilution method. In agar-well diffusion method, all the isolates were grown on a semi-solid media (SCA) containing different concentrations (10mM, 50mM, 100mM, 250mM and 500mM) of heavy metal salts viz. copper sulfate, zinc sulfate and lead acetate. After an incubation period of 7 days, plates were observed for percentage of bacterial tolerance where,Ash1, Ash 2, Ash 4,Ash 6, Ash 7, Ash 8, Ash 9, Ash 10, Ash 11, Ash 12, Ash 13, Ash 15 were resistant at 10 mM conc. of $CuSo_4$, but at other higher concentrations of metal salts their growth was inhibited. Isolates Ash 10, Ash 11, Ash 12, Ash 13, Ash 19, Ash 20 were found to be resistant at 10mM conc. of ZnSO4, but were inhibited at other higher concentrations. However most of the isolates showed same level of tolerance for different concentrations of Pb $(CH_3COO)_2$.

In tube dilution method, all 20 isolates were grown on a liquid media (ISP1) supplemented with different concentrations of heavy metals and after 7-14 days of incubation, the tubes were observed for turbidity. The most turbid tube was considered as the one containing the most metal tolerant strain of actinomycetes. Ash13, 15, 16 were the most tolerant;

Ash1, 2, 6, 8, 9, 10, 11, 12, 17, 18, 20, were moderately tolerant and Ash3, 5, 7, 14, 20 were sensitive.

The most metal-tolerant (Ash 13, Ash 15 & Ash 16) and two of the moderately tolerant *actinomycetes* were selected to test their antibiotic susceptibility against five antibiotics. This was done by antibiotic disc diffusion method on MHA plates. After an incubation of two days, plates were observed for zone of inhibition. More the zone of inhibition; more is the susceptibility of a strain against that particular antibiotic. The sequence for the antibiotic susceptibility shown by actinomycetes isolates can be expressed in the following order: oxy-tetracycline> ceftriaxone> gentamicin> kanamycin>ampicillin.

Metal tolerance shown by actinomycetes may be due to the ability of these microorganisms to adhere to metal ions with their cell wall or accumulate these metals in intracellular fashion at higher concentrations. *Actinomycetes* are known to degrade the more resistant and indecomposable organic substance and produce a number of dark black to brown pigments which contribute to the color of soil humus.

It is just a preliminary study and further testing and proper justification of this result is requisite. Further, the evaluation of the toxicity level can be seen as a future prospective.

## REFERENCES


1. Bruins MR, Kapil S, Oehme FW, Microbial resistance to metals in the environment, Ecotoxicology and Environmental Safety, 2000,45,198-207.
2. Nies DH, Microbial heavy metal resistance, applied microbiology and biotechnology, 1999, 51, 730-750.
3. Hussein H, Farag S, Kandil K, Moawad H, Tolerance and uptake of heavy metals by pseudomonads, Process Biochemistry, 2005, 40, 955-961.
4. Roane TM, Pepper IL, Microbial responses to environmentally toxic cadmium, Microbial Ecology, 2000, 38, 358-364.
5. Poole RK, Gadd GM, Metals, Microbe Interactions, IRL Press, Oxford, 1989, 1-37.
6. Khan S, Cao Q, Zheng YM, Huang YZ, Zhu YH, Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China, Environmental Pollution, 2008, 152, 686–692.
7. Zhang MK, Liu ZY, Wang H, Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice, Communications in Soil Science and Plant Analysis, 2010, 41(7), 820–831.
8. Olson BH, Thronton I, The resistance patterns to metals of bacterial populations in contaminated land, Journal of Soil Science, 1982, 33, 271-277.
9. Hassen A, Saidi N, Cherif M, Boudabous A, Resistance of environmental bacteria to heavy metals, Bioresource Technology, 1998 ,64, 7-15.
10. Duxbury T, Toxicity of heavy metals to soil bacteria, FEMS Microbiol, Lett, 1981, 11, 217-220.
11. Vijayaraghavan K, Karamchandani P, Seigneur C, Balmori R, Chen SY, Plume-ingrid modeling of atmospheric mercury, J Geophys Res, 113.
12. Doelman P, Jansen E, Michels M, Van Til M, Effect of heavy metals in soil on microbial diversity and activity as shown as shown by the sensitivity resistance index, an ecologically relevant parameter, Boil Fertil Soil, 1994, 17, 177-184.
13. Duxbury T, Bicknell B, Metal tolerant bacterial populations from natural and metal-polluted soils, Soil boil Biochem, 1983, 15, 243-50.
14. Hiroki M, Effects of heavy metal contamination on soil microbial population, Soil Sci, Plant Nutr, 1992, 38, 141-47.
15. Jonas RB, Acute copper and cupric ion toxicity ion toxicity in an estuarine microbial community, Appl Environ Microbiol, 1989, 55, 43-49.
16. Guzzo A, Du Bow M, Bauda P, Identification and characterization of genetically programmed responses to toxic metal exposure in Escherichia coli, Metals and microorganisms, relationships and applications, FEMS Microbiol Rev, 1994, 14, 369-374.
17. Starzecka A , Bernarz T, Comparison of development and metabolic activity of algae and bacteria in soil under the influence of short-and long term contamination with metallurgic industrial dusts, Archive Hydrobiol, 1993, 98, 71-88.
18. Babich H, Stotzky G, Environmental factors that influence the toxicity of heavy metals and gaseous pollutants to microorganisms, Crit Rev Microbiol, 1980, 8, 99-145.
19. Gadd GM, Metals and microorganisms, A problem definition, FEMS Microbiology Letters 1992, 100, 197-204.
20. Sterritt RM, Lester JN, Interactions of heavy metals with bacteria, Sci Total Environ, 1980, 14, 5-17.
21. Stotzky G, and Babich H, Physico-chemical environmental factors affect the response of microorganisms to heavy metals, implications for the application of microbiology to mineral exploration, 1986, 5, 239-264.
22. Wood JM, Wang HK, Microbial resistance to heavy metals, Environ Sci Technol, 1983, 17, 582-90.

23. Li F, tan TC, Effect of heavy metal ions on the efficacy of a mixed bacilli BOD sensor, Biosens Bioelectron, 1994, 9, 315-324.

24. Eiland F, The effect of application of sewage sludge on microorganisms in soil, Tiddskrift Planteavl, 1981, 85, 39-46.

25. Ow D, Phytochelatin-mediated cadmium tolerance in *Schizosaccharomyces pombe, In Vitro* Cell, Develop Boil, 1993, 29, 213-19.

26. Rohit M, Sheela S, Uptake of zinc in *Pseudomonas* sp, Strain **UDG26**, Appl Environ Microbial, 1994, 60, 2367-70.

27. Wehrheim B, Wetterrn M, Comparative studies of heavy metal uptake of whole cells and different types of cells ills from *Chlorella fusca*, Biotechnol Tech, 1994, 8, 227-32.

28. Tomoika N, Uchiyama H, Yagi O, Cesium accumulation and growth characteristics of *Rhodococcus erythropolis* CS98 and *Rhodococcus* sp, Strain CS402, Appl Environ Microbiol 1994, 60, 2227-31.

29. Brookes PC, McGrath SP, Effects of heavy metal accumulation in field soils treated with sewage-sludge on soil microbial processes and soil fertility, FEMS Symp, 1986, 33, 327-343.

30. Silver S, Bacterial interactions with mineral cations andanions, good ions and bad, p, In P.

31. Summers AO, Bacterial resistance to toxic elements, Trends Biotechnology, 1985, 3, 122-125.

32. Lam KS, Discovery of novel metabolites from marine actinomycetes, Current Opinion in Microbiology, 2006, 9, 245–251.

33. Nodwell, JR, Novel links between antibiotic resistance and antibiotic production, J Bacteriol, 2007, 189, 3683-3685.

34. Bahig AE, Aly EA, Khaled AA, Amel KA, Isolation, characterization and application of bacterial population from agricultural soil at Sohag Province, Egypt, Malaysian J Microbiol, 2008, 4, 42-50.

35. Spain A, Implications of microbial heavy metal tolerance in the environment, Rev Undergrad Res, 2003, 2, 1-6.

36. Polti MA, Amoroso MJ, Abate CM, Chromate reductase activity in *Streptomyces* sp, MC1, J Gen Applied Microbiol, 2010, 56, 11-18.

37. Silver S, Phung LT, Bacterial plasmid-mediated heavy metal resistances, new surprises, Annu Rev Microbiol, 1996, 50, 753–789.

38. Chaudhary HS, Yadav J, Shrivastava AR, Singh S, Singh AK, Gopalan N, Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city of central India) J Adv Pharm Technol Res, 2013, 4(2), 118-23.

39. Gelmi M, Apotoli P, Cabibbo E, Porru S, Aessio L, Turano A, Resistance to cadmium salts and metal adsorption by different microbial species, Current Microbiol, 1994, 29, 335-341.

40. Sabry SA, Ghozlan HA, Abou-Zeid DM, Metal tolerance and antibiotic resisitance patterns of a bacterial population isolated from sea water, J Appl, Microbiol, 1997, 82, 245-252.

41. Kanopka A, Zakharova T, Quantification of bacterial lead resistance via activity assays, Journal of Microbiological Methods, 1999 37, 17–22.

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