

## Research Article

# Bioremediation of Xenobiotic Compound and Heavy Metals by the Novel Marine Actinobacteria

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## ABSTRACT

Actinobacteria, one of the most important groups of microbes exhibit many interesting activities such as degradation and absorption of organic and metal substrates. The present study was designed to evaluate the degradation of xenobiotic compound (Carbaryl) and absorption of heavy metals (Cu and Zn) from the effluent by the marine actinobacteria named as *Streptomyces acrimycini* NGP (JX843532), *Streptomyces albogriseolus* NGP (JX843531) and *Streptomyces variabilis* NGP (JX43530), which were isolated from the marine sediments of south Indian coastal region, Tamilnadu, India. The rate of degradation of xenobiotic compound (Carbaryl) and heavy metals (Cu and Zn) by the isolates were determined by Gas Chromatography Mass Spectroscopy (GC - MS) and Atomic Absorption Spectrophotometer (AAS) respectively. The degradation of xenobiotic compound by the isolates were found to be 30.30, 35.90 and 36.20 per cent for 30 days of incubation. Similarly, the absorption of Cu and Zn by the respective isolates were 26.67, 57.35, 26.69 and 61.22, 62.33, 66.47 per cent up to 7 days of incubation.

**Keywords:** Actinobacteria, Bioremediation, Heavy metals, Xenobiotic compound.

## INTRODUCTION

Actinobacteria are the rich source of biotechnologically important enzyme and bioactive molecules. The industrial activities such as mining and metal plating could release the high amount of organic and heavy metals into the environment resulting in chronic pollution. The increased awareness of the role of micro organisms, leads to the treatment of waste water (Guest, 1987). Many researchers have been focused on microbial populations in the waste water treatment in order to enhance the eco - friendly approach (Sim et al., 2010). Many micro organisms have been involved in the treatment process. Among them, marine actinobacteria are more attracted towards the treatment. When compare the marine microorganisms with the terrestrial organisms, marine microorganisms have developed self protection mechanisms by the mean of evolution and competition for survival. This leads to the production of unique and complex chemical entities which are being unrevealed for various purposes (Sigrid et al., 2008; Hozzein et al., 2012).

Many studies showed a lot of environmental and health problems due to continuous and excessive use of xenobiotic compound (Cuthbertson and Murchie, 2010). The xenobiotic compound carbaryl (1-naphthyl methylcarbamate) is the third most-used insecticide in the world and it is toxic to

humans. It is classified as a likely human carcinogen by the United States Environmental Protection Agency (EPA). Many methods such as physico-chemical and biological methods have been employed in the degradation of xenobiotics. Physico-chemical methods are very expensive and create adverse effect in the environment. To overcome this problem, biological methods (microbes) have been practiced for degradation (Sridevi et al., 2011).

Another problem associated with health concern is, the discharge of heavy metals from electroplating industry. Some of the heavy metals can form compounds which are toxic at low concentration (Srisuwan and Thongchai, 2002). The overall waste water system is extremely variable, but usually high in heavy metals such as cadmium, chromium, lead, copper, zinc and nickel. Bioremediation is the effective method for the heavy metal absorption rather than other methods (Paknikar et al., 2003).

Atomic Absorption Spectroscopy (AAS), UV-visible detection and Gas Chromatography Mass Spectroscopy (GC-MS) are routinely used to determine transition of metal ions and highly conjugated organic compounds present in the sample (Hozzein et al., 2012). Therefore, the objective of the present study was to examine the ability of the degradation of xenobiotic compound and absorption of

heavy metals from the electroplating industry effluent.

## MATERIALS AND METHODS

### Primary screening of carbaryl degradation by actinobacteria

Degradative potential of actinobacteria against the xenobiotic compound-carbaryl was tested on Starch Casein Agar (SCA) plate supplemented with carbaryl compound at (20, 30, 40, 50 and 60 mg/l) different concentrations. The actinobacteria which showed potential growth were inoculated in starch casein broth containing carbaryl under shaking conditions (120 rpm, 30 days). The dry weight of the mycelium was noted and then degradation studies were analyzed by GC-MS (Modified method of Ningthoujam et al., 2012).

### Biodegradation of carbaryl

Culture filtrate of the medium containing carbaryl was extracted with dichloromethane. The extract was evaporated and the residue dissolved in acetone. The extracts were analyzed by GC-MS. It was performed in Electron Ionization (EI) mode (700 V) with a gas chromatograph equipped with MS detector. A HP-1701 capillary column (30 m X 0.25 mm X 0.25  $\mu$ m film thickness) was used with a temperature program of 80°C for one min; increased to 200°C at 8°C / min held for 2 min; finally the temperature was increased up to 260 °C at 15°C / min and held at 260 °C for 10 min. Nitrogen was used as the carrier gas at a constant flow of 1.0 ml/min. The samples were analyzed by split mode (1:20) at an injection temperature of 260 °C and an EI source temperature of 230 °C (Lin et al., 2011).

### Biosorption of heavy metals

#### Collection of effluent and detection of heavy metals

Effluent was collected from Nirmala electroplating industry, Coimbatore, Tamilnadu, India and the presence of heavy metals in the discharged effluent were analyzed by Atomic Absorption Spectrophotometer (AAS). Initial concentration of CU and Zn in the effluent was 31.8629 and 5.0775 ppm.

### Screening of Cu and Zn resistant marine actinobacteria

Primary qualitative screening test was carried out in starch casein agar (SCA) plates with different concentration of CuSO<sub>4</sub> and ZnSO<sub>4</sub> independently. The spore suspension (10<sup>-4</sup>) of the isolates were uniformly spread using

sterile cotton swab on a petridish containing some wells of 4 mm diameter. A total of five dilutions of the CuSO<sub>4</sub> and ZnSO<sub>4</sub> (10, 20, 30, 40 and 50 mg/l) concentrations were added to each of the wells. The petridishes were incubated for 24 h at 28°C. After incubation, the growth of the actinobacteria was observed. Inhibition of the bacterial growth was measured in mm (Modified method of Yadav et al., 2009).

### Efficiency of the actinobacterial strains in the removal of heavy metals

The biosorption of heavy metals were carried out in electroplating industrial effluent in starch casein broth medium. The medium was inoculated with actinobacterial suspension (10<sup>-4</sup>) which were showed potential activity in the preliminary screening and incubated at 30°C for 5 to 7 days in an orbital shaker. After 7 days of incubation, Cu and Zn removal were analyzed by AAS after digestion with concentrated HNO<sub>3</sub> (Hozzein et al., 2012).

## RESULTS AND DISCUSSION

### Biodegradation of carbaryl compound

Initial biodegradation studies on SCA medium containing carbaryl compound (20, 30, 40, 50 and 60 mg/l) indicated that, three novel marine actinobacterial strains have the potential to degrade carbaryl up to 50 mg/l concentration which were indicated by inhibition zone. An inhibition zone of 7.0, 5.0 and 4.0 mm diameter were produced by the isolates *S. acrimicini* NGP, *S. albogriseolus* NGP and *S. variabilis* NGP. The biodegrading capacities of these isolates were confirmed by shake flask studies. In this study, the degradation of carbaryl (50 mg/l) was carried out in starch casein broth (pH 7

.0). Biomass of the isolates *S. acrimicini* NGP, *S. albogriseolus* NGP and *S. variabilis* NGP were found to be 27.0, 29.0 and 31.0 mg. The GC-MS was carried out to analyze the degradation of carbaryl by three isolates. Results were expressed in table 1; figure 1, 2, 3 and 4. Finally, the degradation of the carbaryl was noted as 30.30, 35.90 and 36.20 per cent for the respective isolates. The strain *S. variabilis* NGP seem to be promising robust carbaryl degrader.

Isolation of microorganisms from unexploited environments may yield tremendous biotechnological applications that hopefully, will have greater impact (Hozzein and Goodfellow, 2007). Many actinomycetes can degrade different pollutants, including several pesticides. Benimeli et al. (2008) studied the bioremediation of pesticide lindane by *Streptomyces sp.M7* in soil samples and the

pesticide effects on maize plants were noted. Biomass of the *Streptomyces sp* was increased and concomitantly decreased residual lindane. Deschrijver and De Mot (1999) showed that the genera *Arthobacter*, *Clavibacter*, *Nocardia*, *Rhodococcus*, *Nocordioides* and *Streptomyces* involved in the degradation of pesticide. The metabolic pathways for pesticide degradation by actinomycetes have not been studied extensively. Based on the research of Siripong et al. (2009) the fungi isolates were tested for their ability to degrade polychlorinated hydrocarbons. It was observed up to 50% degradation was achieved when the fungi were cultured for 15 days. Similarly, Peace and Wunderlin (2004) reported the biodegradation of lindane by a bacterial consortium isolated from contaminated soil. These authors reported that *B. thiooxidans* and *S. parvicornis* degraded the compound after 3 days of aerobic incubation. The degradation of many xenobiotic compounds has been performed by mixed cultures, but most of the symbiotic mechanisms remain unclarified (Bul and Slater, 1982). Similarly, Lin et al. (2011) exploited the biodegradation of cypermethrin by actinomycetes HU-S-01 from waste water sludge. The strain completely degrades the compound within 96 h at the concentration of 50 mg/l and degradation products were identified using GC-MS. Although, the toxicity of 2, 4-Dichloro phenoxy acetic acid degradation was carried out by Bukowska (2006). Here, the soil microorganisms *Pseudomonas*, *Aspergillus* and *Streptomyces* utilized this compound as carbon and energy source. Based on the research of Debanandha et al. (2012) P-nitrophenol (PNP), a major nitro aromatic compound has been degraded up to 270 mg/l by actinomycetes from Hundung lime stone deposits in Manipur, India. It is highly toxic to soil micro flora and other non - target organisms.

### Biosorption of heavy metals

The isolates were checked for its activity to remove the heavy metals (Cu and Zn) with their respective salt solutions ( $\text{CuSO}_4$  and  $\text{ZnSO}_4$ ) at 10, 20, 30, 40 and 50 mg/l concentrations. An inhibition zone of 2 - 5 mm produced by the isolates on the agar surface was considered as resistant to the particular metal salts. The isolates *S. acrimicini* NGP, *S. albigriseolus* NGP and *S. variabilis* NGP produced an inhibition zone against  $\text{CuSO}_4$  and  $\text{ZnSO}_4$  were presented in figure 5 and 6. The maximum zone of inhibition (3.0 mm diameter) against  $\text{CuSO}_4$  was produced by *S. albigriseolus* NGP at the concentration of 10

mg/l. At the concentration of 30 mg/l  $\text{ZnSO}_4$  favoured the maximum zone of inhibition (2.0 mm diameter) by the isolate *S. variabilis* NGP. The efficient removal percentages of the heavy metals such as Cu and Zn by the isolates were confirmed by AAS and the mycelia growth was determined. The details were mentioned in table 2. The maximum removals of Cu and Zn from the effluent were achieved by *S. albigriseolus* NGP and *S. variabilis* NGP. The removal percentage was 57.35 and 66.47 respectively.

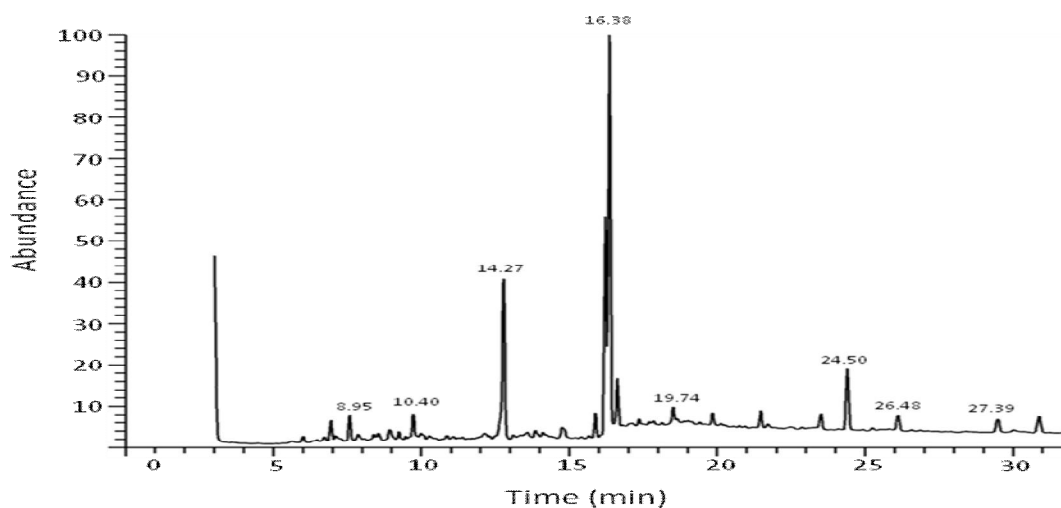
A total of thirty actinomycetes were isolated from the marine sediments of Martin's Island Bangladesh, which were screened for resistance towards heavy metal Cr (VI) on culture plates supplemented at concentrations ranging from 1-5 mM of Cr (VI). Among them, two were capable of showing complete reduction of 1 mM Cr (VI) within 24h (Jain et al., 2012). In addition, Hozzein et al. (2012) checked the ability of the actinomycetes in the removal of some heavy metals (Cu, Fe, Mn, Pb and Zn) from waste water. The results showed that most of the selected actinomycetes were reduced the concentrations of the tested heavy metals also reduced the values of biological oxygen demand (BOD), chemical oxygen demand (COD) and total suspended solids (TSS). Earlier, actinomycetes community in starch factory waste water, Saenna et al. (2011) indicated that 30 isolated actinomycete strains were tested for biological treatment and removal of heavy metals from the raw waste water. Also, the lead and cadmium biosorption capacity of *S. rimosus* and *S. clavuligerus* were reported in the batch mode (Selantia et al., 2004; Zouboulis et al., 2004). *Streptomyces sp* isolated from the marine sediment of Bay of Bengal showed multi-resistance against copper and mercury up to 480 mg/l (Yadav et al., 2009; Koushalshahi et al., 2012). Based on the research of Sabdon et al. (2012) twenty two coral bacteria were selected among sixty one, according to their resistance against Pb, Cr, Zn at 1 mM concentration using agar diffusion method. Although, multi metal resistance activity of *Streptomyces sp* VITDDK3 was reported by lakshmipathy et al., 2010; Saenna et al., 2011). While, Webster et al. (2001) reported that the total density and counts of microbial communities associated with the sponge *Rhopaloeides odorabile* was significantly reduced in response to  $\text{Cu}^{2+}$  concentration. In addition, strains of halophilic actinomycetes were isolated from the salt marsh environment of estuary, south east coastal of India by Senthil kumar et al. (2005) which showed

resistance towards chloride up to 100-150 mM. Das (2010) stated that, *S. phaeochromogens* HUT 6013 involved in the biosorption of gold. Selvam et al. (2013) studied the biosorption of chromium (VI) from

industrial effluent by *Saccharomyces cerevisiae*. According to that, immobilized form of the strain was found to be effective in the biosorption of chromium (VI).

**Table 1: GC-MS analysis of xenobiotic degradation by marine actinobacteria**

Marine actinobacteria	Incubation period (days)	Mycelial dry weight (mg)	Percent degradation (%)
Control	30	-	-
<i>S. acrimycini</i> NGP	30	27.0	30.30
<i>S. albogriseolus</i> NGP	30	29.0	35.90
<i>S. variabilis</i> NGP	30	31.0	36.20



Molecular formula :  $C_{12}H_{11}NO_2$   
Peak area : 558347.29

**Fig. 1: Biodegradation of carbaryl by control**

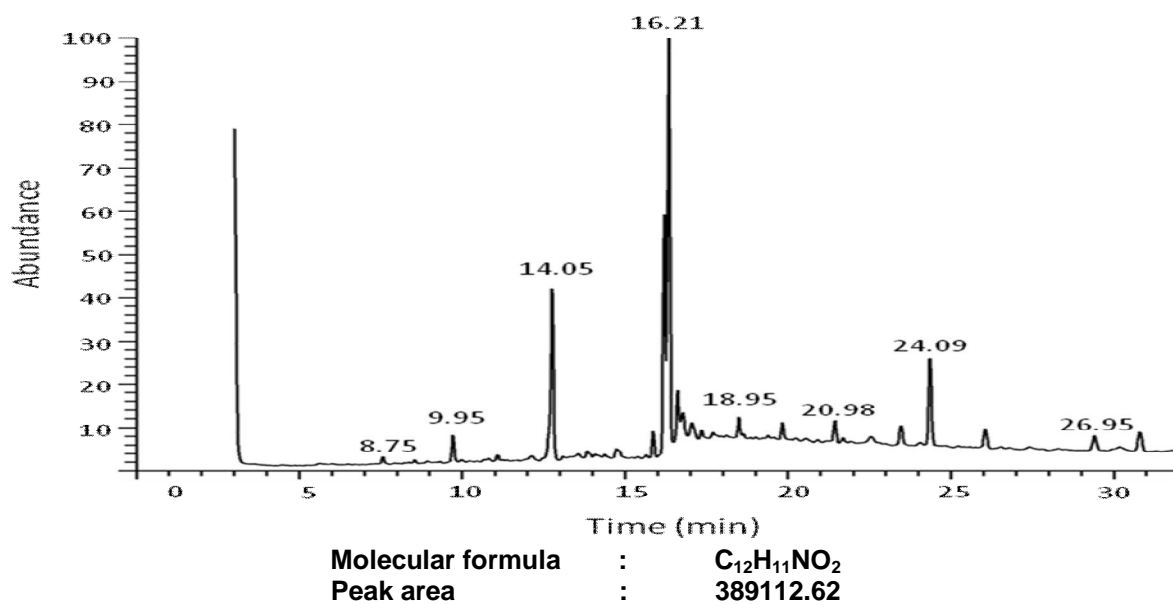


Fig. 2: Biodegradation of carbaryl by *S. acrimycini* NGP

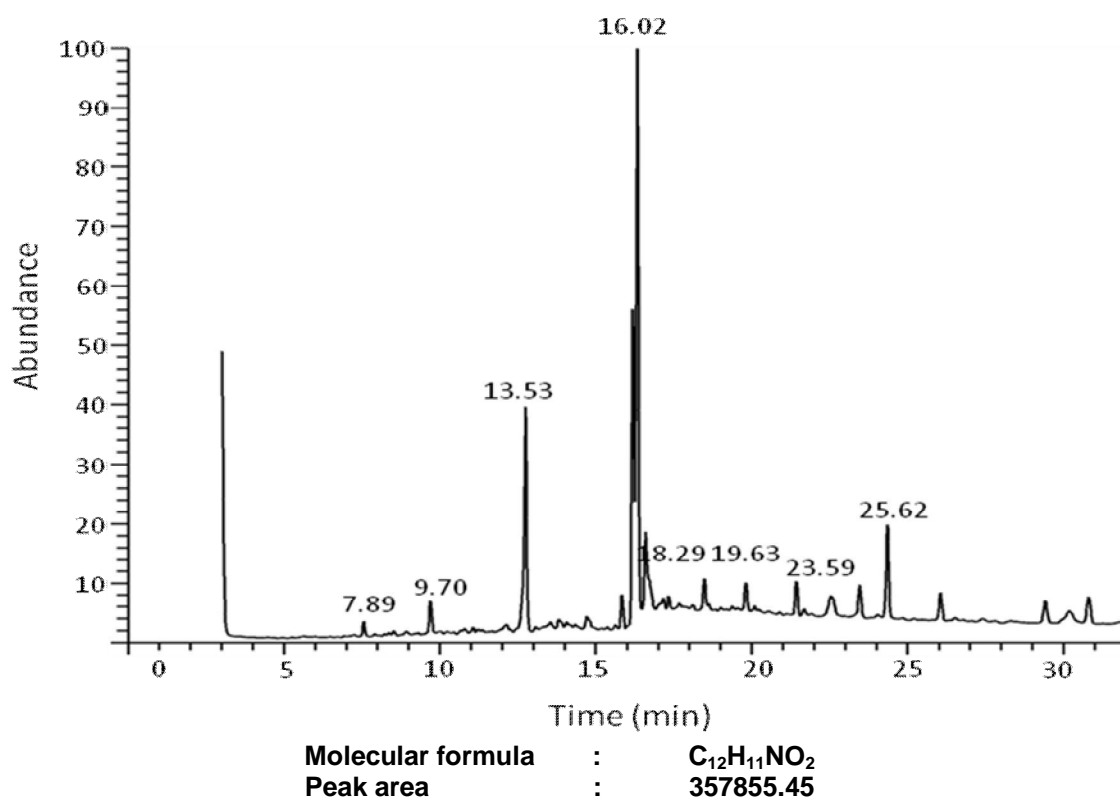


Fig. 3: Biodegradation of carbaryl by *S. albogriseolus* NGP

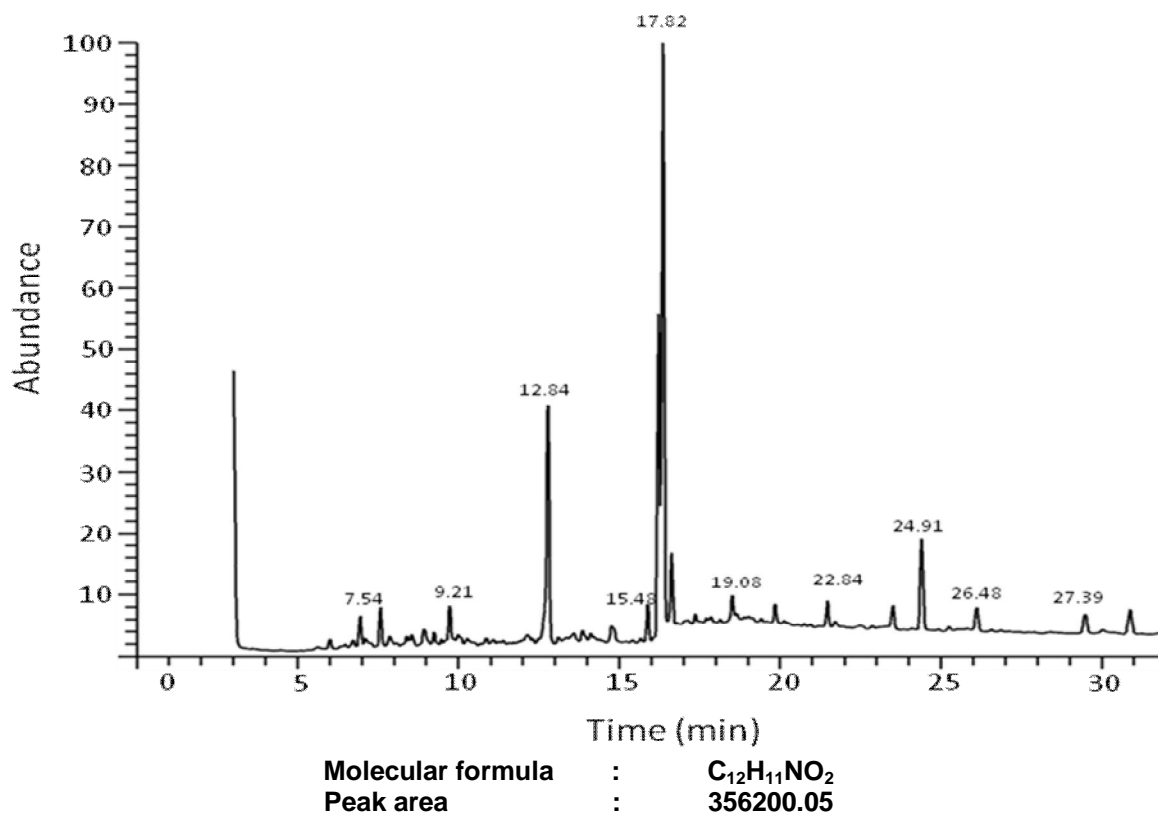


Fig. 4 Biodegradation of carbaryl by *S. variabilis* NGP

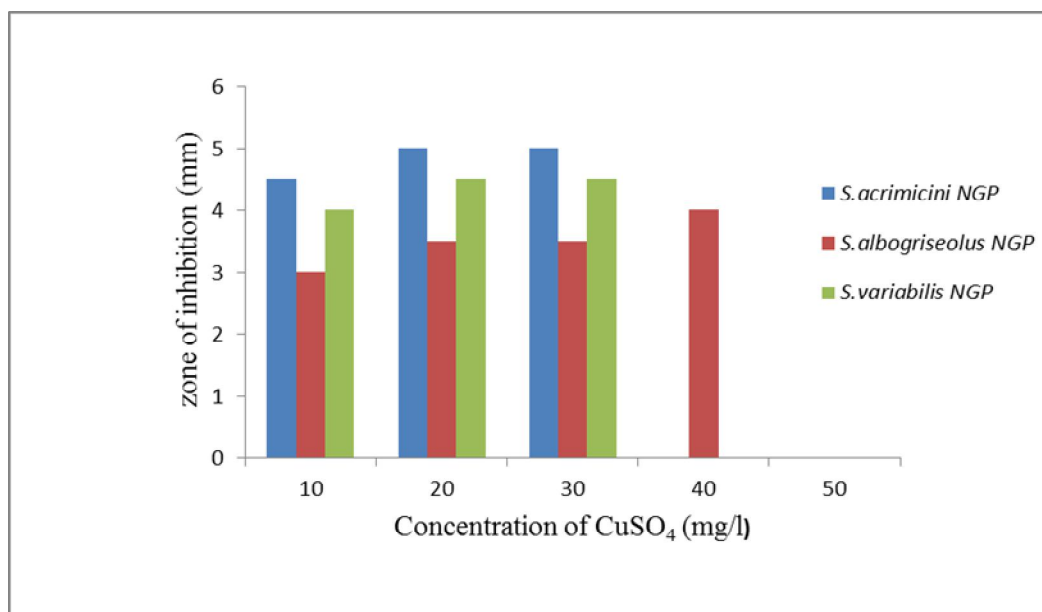


Fig. 5: Heavy metal resistance pattern exhibited by marine actinobacteria against  $CuSO_4$  salt solution



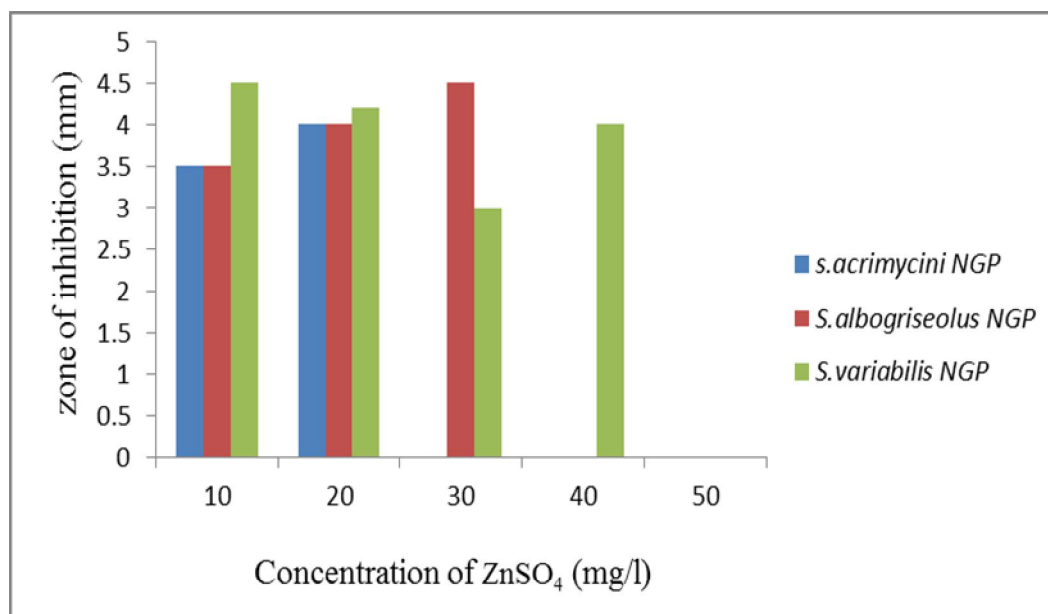


Fig. 6: Heavy metal resistance pattern exhibited by marine actinobacteria against ZnSO<sub>4</sub> salt solution

Table 2: Atomic Absorption Spectrophotometer (AAS) analysis of heavy metal absorption by marine actinobacteria

Marine actinobacteria	Mycelial dry weight (mg)	Concentration (ppm)		Absorption percentage (%)	
		Copper (Cu)	Zinc (Zn)	Copper (Cu)	Zinc (Zn)
Control	-	5.0775	31.8639	-	-
<i>S. acrimycini</i> NGP	32.0	3.7232	12.3561	26.67	61.22
<i>S. albogriseolus</i> NGP	27.0	2.1652	12.0017	57.35	62.33
<i>S. variabilis</i> NGP	25.0	3.7219	10.6812	26.69	66.47

## CONCLUSION

It could be concluded that the degradation of xenobiotic compound (Carbaryl) and absorption of heavy metals (Cu and Zn) from the effluent by the marine actinobacteria named as *Streptomyces acrimycini* NGP (JX843532), *Streptomyces albogriseolus* NGP (JX843531) and *Streptomyces variabilis* NGP (JX43530) were carried out. Based on the study, all the three isolates were efficiently involved in the degradation of xenobiotics compound and absorption of heavy metals from the effluent.

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