



# IJEAST

INTERNATIONAL JOURNAL  
OF ENGINEERING APPLIED SCIENCE  
AND TECHNOLOGY



**VOLUME : 4    ISSUE : 07    Print / Issue Publication Date: 07-Jan-2020**



**ISSN : 2455-2143**



**DOI : 10.33564/IJEAST.2019.v04i07.021**

Indexed In



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# HEAVY METAL TOLERANCE OF INDIGENOUS MICROSymbionTS OF ARACHIS HYPOGAEA

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**ABSTRACT** - In this article, the heavy metal tolerance ability of the isolated indigenous micro symbiont partner of *Arachis hypogaea* was gauged on the basis of specific growth rate of the strains multiplied in YEMA amended with various concentrations of heavy metals. The success of a research can be determined through the knowledge of screening strategies exerted for intense and effectual selection of influential rhizobial strain for intensified field performance by exploiting the resistance properties of *Rhizobium* for identification of competent strains with phytoremediation properties.

**Keywords:** Rhizobium; PGPR; Bioremediation; Micro symbiont

## I. INTRODUCTION

Heavy metals discharged from industrial operations upon consequent accumulation in the environment cause drastic threat to the varied agroecosystems. Heavy metal ions at elevated levels are toxic and are excessively absorbed by roots, translocated to shoot and leads to destructive metabolism and reduced growth (Bingham *et al.*, 1986; Foy *et al.*, 1978). Giller *et al.*, 1998 proposed that the impact of heavy metals is dependent on duration of exposure, dose and type of metal used. The maximal yield in symbiotic legume *Rhizobium* relationship, is identified to be possible only if there are favorable condition for both. Many reports indicate striking influence on microbial composition due to abnormal levels of heavy metals accumulation (Paudyal *et al.*, 2007; Kruczak *et al.*, 2012; Heckman *et al.*, 1987; Broos *et al.*, 2005) and directly or indirectly affects the health of plants including legumes like green gram, peanut and chickpea leading to impaired chlorophyll synthesis, inactivated protein synthesis, severe reduction in crop yields and loss of soil fertility (Feng *et al.*, 2010; Bibi

& Hussain, 2005; Semane *et al.*, 2010; Moftah, 2000). Researchers have documented the effect of heavy metals on growth, genetic diversity, physiology and nodulation capacity of various strains of *R. leguminosarum* (Chaudhary *et al.*, 2004; Chaudri *et al.*, 2000; Shi *et al.*, 2002; Ahemad & Kibret, 2014; Hirsch *et al.*, 1993). Pereira *et al.*, 2006 stated that *Rhizobium* is a sensitive species which acts as a good indicator of soils contaminated with metals. Cevheri *et al.*, 2011 proved the hypothesis of suppressive action of heavy metals on rhizobial cells at higher concentrations when compared to low concentrations. Despite the negative impact of heavy metals on rhizobia, phytoremediation is possible in heavy metals accumulated soil (Ma *et al.*, 2011). Many reports support that the heavy metal resistance of rhizobial cells could be plasmid mediated or some mechanism may confer resistance (Lakzian *et al.*, 2002; Figueira *et al.*, 2005; Purchase & Miles, 2001). Native rhizobial isolate conferring resistance to heavy metals is of potential importance in symbiotic association for remediation of the affected area. To execute their beneficial PGP traits under stress conditions, rhizobia possess key tolerance mechanism or pathways against abiotic stresses, heavy metals and pesticides for sustainable agriculture. Indigenous and native microbes might be more effective and competitive in combatting against the environmental stresses.

## II. EXPERIMENT ON HEAVY METAL TOLERANCE STUDIES (STANIER *ET AL.*, 1985)

This research deals with exploration of heavy metal tolerance ability of native rhizobial isolates obtained from *Arachis hypogaea*. This specific growth study to the observed stress parameter could lead to the selection of efficient isolates for agricultural practices contributing to phytoremediation. A loop full of log phase culture of each strain was inoculated separately in flasks containing Yeast



Extract Mannitol broth amended with different metal concentrations of their corresponding salts Al( $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ), Fe( $\text{FeCl}_3$ ), Co( $\text{CoCl}_2$ ), Hg( $\text{HgCl}_2$ ) and Mo( $\text{Na}_2\text{Mo}_4 \cdot 2\text{H}_2\text{O}$ ). The inoculated flasks were then placed at  $28 \pm 1^\circ\text{C}$  on shaker at speed 150 rpm. To obtain growth rate, the optical density of each flask was measured after 3 days at wavelength 610 nm. Specific growth rate of rhizobial isolated strains were measured using the formula (Stanier *et al.*, 1985).

$$\text{Specific growth} = \frac{\text{Log } OD_1 - \text{Log } OD_0}{T_1 - T_0} \times 2.303$$

Where  $OD_1$  = Log value of O.D. of culture at time t

hour

Where  $OD_0$  = Log value of O.D. of culture at time

$t_0$ hour

$T_1 - T_0$  = Difference between time interval or duration of incubation.

### III. RESULT

#### Heavy Metal tolerance studies

The overall 45 *Rhizobium* isolates showed a level of tolerance to heavy metals such as Al, Fe, Co, Hg Mo as shown in figures 1 - 15 and majority of the isolates failed to exhibit luxuriant growth above  $25\mu\text{g}$  of all the heavy metals. Exceptionally, five (VRh2, VRh9, TRh11, KRh11, KRh7) isolates exhibited moderate to luxuriant growth at  $150\mu\text{g}$  of all the tested heavy metals, while rest of the strains showed scanty growth at concentration  $<150\mu\text{g}$  of the various metals and a reduced growth rate at concentrations  $\geq 150\mu\text{g}$  of heavy metals.

#### 3.1. Effect of heavy metals on Tiruvallur rhizobial isolates

Application of heavy metals was found to be inhibitory on the growth of majority of Tiruvallur isolates at concentrations above  $25\mu\text{g/ml}$ . The specific growth rate of the isolates decreased as the concentration of metals increased from  $25\mu\text{g/ml}$  to  $250\mu\text{g/ml}$  of the applied heavy metals (Figures 1, 2, 3, 4, & 5). However, *Rhizobium* TRh11 sustained its growth rate ranging from 0.35 - 0.4 at higher concentrations of heavy metals. Similar observations were recorded for other metals. Majority isolates exhibited a declined growth rate in the presence of Fe, Mo, Hg and Co as the exposure to the concentrations increased. Figure 1 indicates that few of the isolates tolerated aluminium concentration but failed to tolerate  $\geq 150\mu\text{g/ml}$  of Fe, Mo, Co and Hg (Figures 2 - 5). Fe and Co was noted to exhibit significant growth impact on the rhizobial isolates when compared to

other metals.

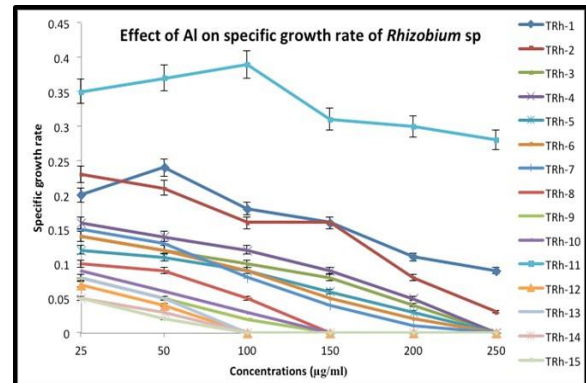


Figure 1: Effect of Aluminium on specific growth rate of *Rhizobium* isolates of Tiruvallur district rhizobial isolates (TRh 1-15)

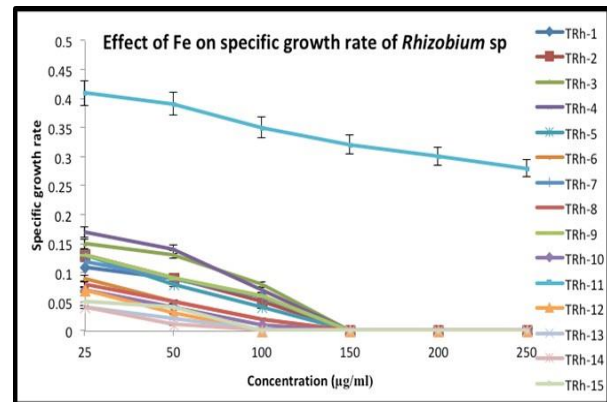


Figure 2: Effect of Ferrous on specific growth rate of *Rhizobium* isolates of Tiruvallur district rhizobial isolates (TRh 1-15)

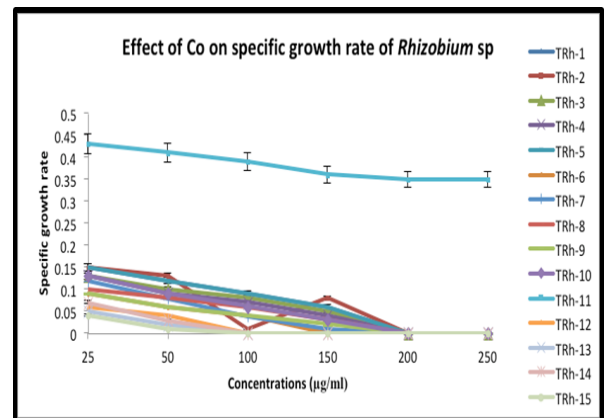


Figure 3: Effect of Cobalt on specific growth rate of *Rhizobium* isolates of Tiruvallur district rhizobial isolates (TRh 1-15).

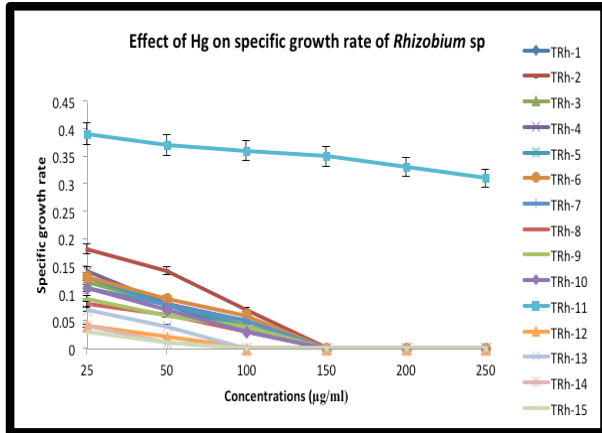


Figure 4: Effect of Mercury on specific growth rate of *Rhizobium* isolates of Tiruvallur district rhizobial isolates (TRh 1-15)

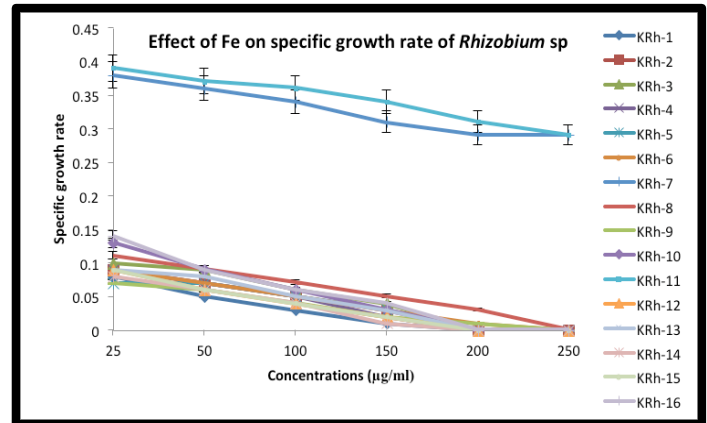


Figure 7: Effect of Ferrous on specific growth rate of *Rhizobium* isolates of Kanchipuram district rhizobial isolates (KRh 1-16)

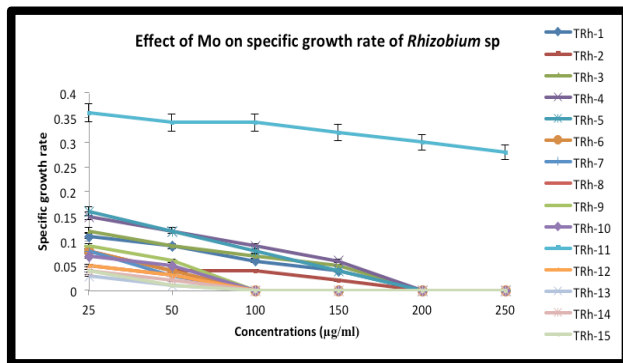


Figure 5: Effect of Molybdenum on specific growth rate of *Rhizobium* isolates of Tiruvallur district rhizobial isolates (TRh 1-15)

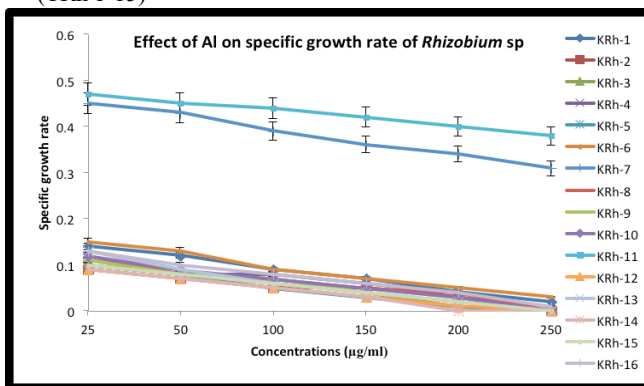


Figure 6: Effect of Aluminium on specific growth rate of *Rhizobium* isolates of Kanchipuram district rhizobial isolates (KRh 1-16)

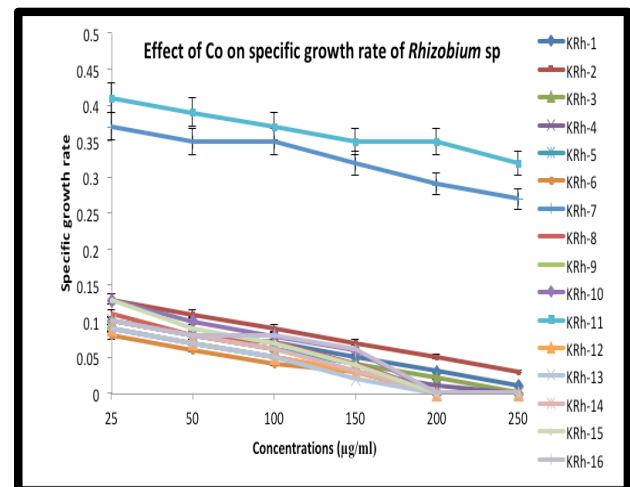


Figure 8: Effect of Cobalt on specific growth rate of *Rhizobium* isolates of Kanchipuram district rhizobial isolates (KRh 1-16)

### 3.2. Effect of heavy metals on Kanchipuram rhizobial isolates

Heavy metals were found beneficial up to 25 µg/ml concentration to culture of isolated strains of rhizobial bacteria (Figures 6, 7, 8, 9, & 10) isolated from Kanchipuram district of Tamil Nadu. Specific growth rate of most of the isolates in the presence of aluminium ranged from 0.1 - 0.2 in 25 µg/ml - 100 µg/ml respectively and declined with increasing concentrations of heavy metals. However, for KRh11 & KRh7, the growth rate increased from 0.4 to 0.5 in the amended concentrations of aluminum as presented in figure 6. Similarly, in case of Fe, the specific growth rate was  $\leq 0.15$  for many isolates (Figure 7).

High rate of specific growth was exhibited by KRh11 & KRh7. A declined growth rate of few isolates were observed in the presence of Cobalt, Mercury and



Molybdenum as the concentrations increased (Figures 8, 9 & 10). Of all the isolates, KRh11 & KRh7 reached 0.45 at above 200 µg/ml concentrations of heavy metals.

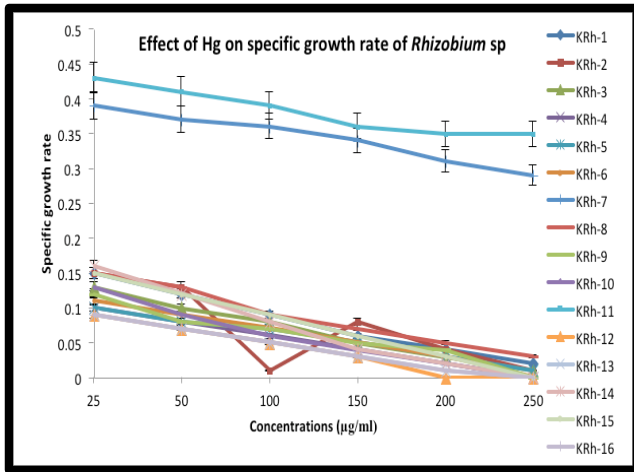


Figure 9: Effect of Mercury on specific growth rate of *Rhizobium* isolates of Kanchipuram district rhizobial isolates (KRh 1-16)

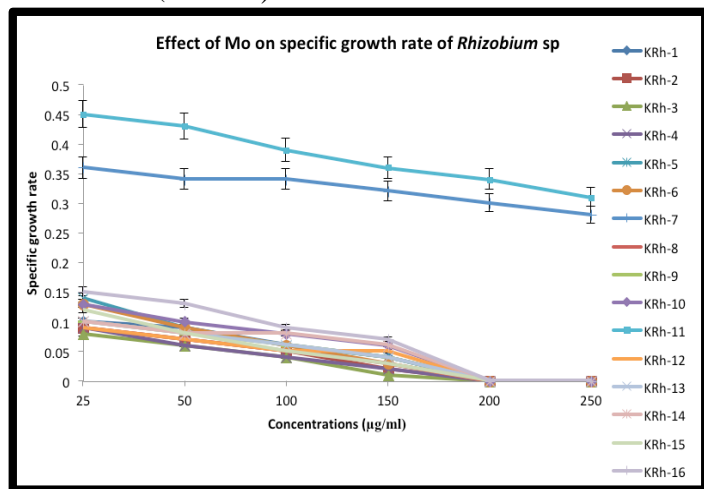


Figure 10: Effect of Molybdenum on specific growth rate of *Rhizobium* isolates of Kanchipuram district rhizobial isolates (KRh 1-16)

### 3.3. Effect of heavy metals on Vellore rhizobial isolates

The isolates of Vellore thrived in media amended with different heavy metals till a concentration of 150 µg/ml. Surprisingly, two isolates VRh2 & VRh9 reached a specific growth rate up to 0.45 at varied concentrations of the used metals in this study (Figures 11, 12, 13, 14 & 15). Beyond a certain limit, the heavy metals exhibited a negative effect on the growth rate of the isolates.

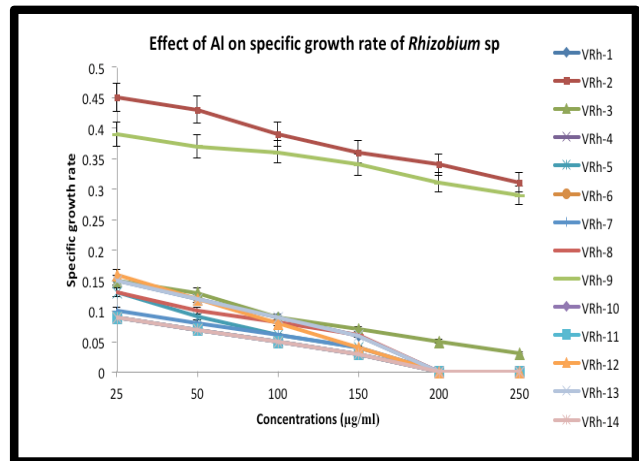


Figure 11: Effect of Aluminium on specific growth rate of *Rhizobium* isolates of Vellore district rhizobial isolates (VRh 1-14)

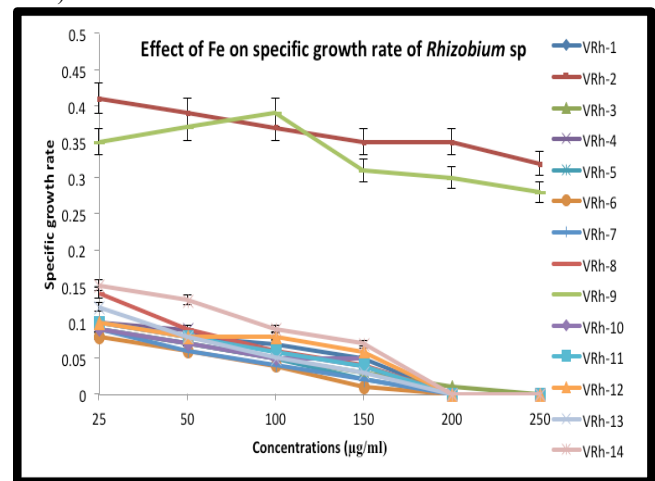


Figure 12: Effect of Ferrous on specific growth rate of *Rhizobium* isolates of Vellore district rhizobial isolates (VRh 1-14)

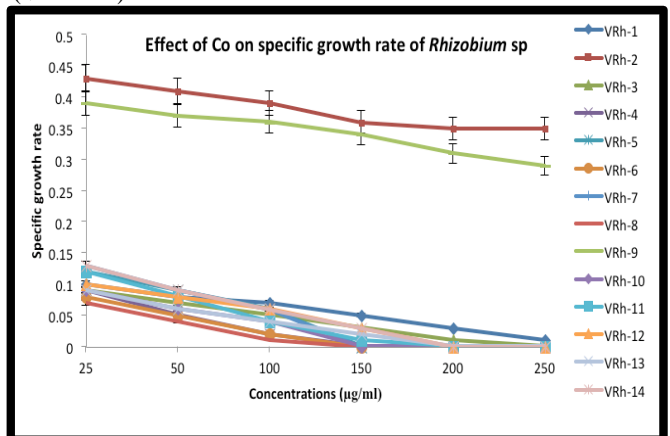


Figure 13: Effect of Cobalt on specific growth rate of *Rhizobium* isolates of Vellore district rhizobial isolates (VRh 1-14)

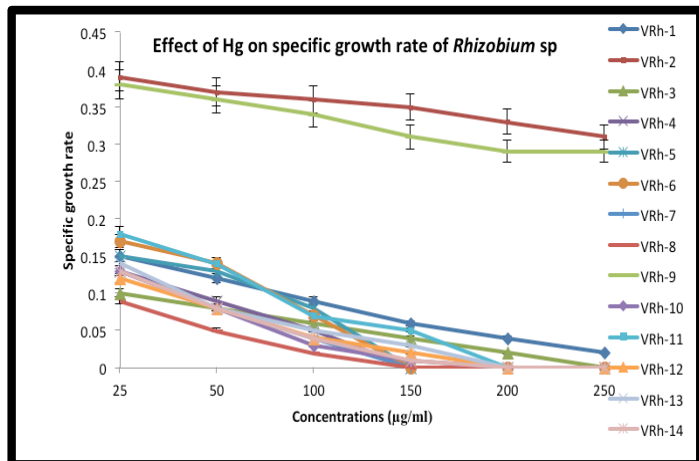


Figure 14: Effect of Mercury on specific growth rate of *Rhizobium* isolates of Vellore district rhizobial isolates (VRh 1-14)

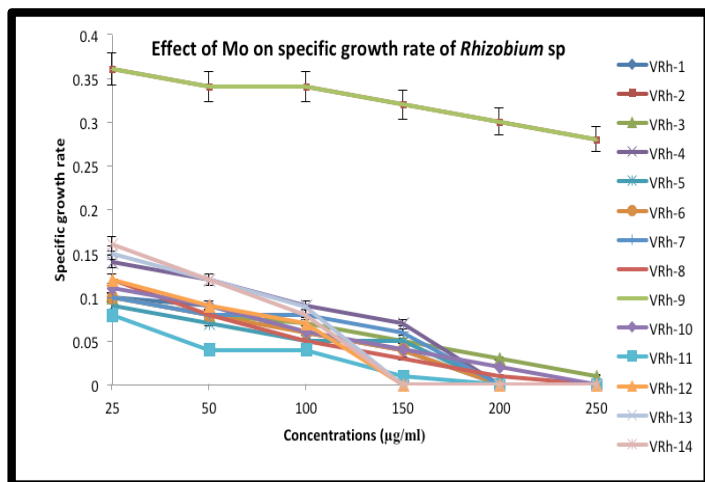


Figure 15: Effect of Molybdenum on specific growth rate of *Rhizobium* isolates of Vellore district isolates (VRh 1-14)

#### IV. DISCUSSION

Heavy metals are inhibitory to rhizosphere microbes and processes mediated by them. Nitrogen fixation ability is lost when the partners grow in metal enriched soil (Hernandez *et al.*, 2003). It is well known that iron is specially required for nitrogen fixing system as it is involved in the synthesis of major components like nitrogenase, leghemoglobin, ferredoxin, hydrogenase and cytochromes. Iron and molybdenum deficiency affect symbiotic partners and affects *Rhizobium* survival, formation of functional nodules which in turn reduces energy transfer to the bacteriodes. The inhibitive effects of Mo and Fe on nitrate reductase activities of soil microorganisms beyond a certain limit has been observed (Solaiman, 1999). Aluminium is found to be extremely toxic than

other metals. Copper is found to possess strong inhibitory effect on the rhizobial population (Arora *et al.*, 2010). Reduction in the bacterial count at high concentrations of heavy metals and variations in the nod genes exposed to metals have been documented previously (Stan *et al.*, 2011).

Present research investigated the effects of five different metals (Fe, Al, Mo, Co & Hg) at different concentrations on the specific growth rate of rhizobial isolates from each location *in vitro*. Figures 1, 2, 3, 4, & 5 indicate the specific growth rate analysis of Tiruvallur district rhizobial isolates when exposed to metals at different concentrations. At very low concentrations of heavy metals, the growth of the strains was partially inhibited. Mercury and Iron greatly affected the growth of isolates at higher concentrations as proved by the declined growth rate. Rhizobial isolate TRh11 exhibited better growth performance amongst the 15 isolates of Tiruvallur. Rane *et al.*, 2014 recognized that few metals possessed stimulatory effect on the growth of organisms at low concentration.

Figures 6, 7, 8, 9 & 10 implies that the survival rate of Kanchipuram rhizobial isolates was predominantly low at higher concentrations of metals. The isolates showed reduced/negative growth at  $\leq 150$   $\mu\text{g}$  levels of metals. Numerous metals have been reported previously to inhibit the growth, morphology and activities of various groups of microorganisms (Bondarenko *et al.*, 2010). Among the 16 isolates, KRh11 and KRh7 persisted the antagonistic effect of metals.

The Vellore isolates VRh2 and VRh9 (Figures 11, 12, 13, 14 & 15) have the ability to tolerate heavy metals such as Al, Fe, Co, Hg, Mo in all the studied concentrations. At higher concentrations, these metals exhibited detrimental effect on the other strains of Vellore. In a similar study, Abdel-Salam *et al.*, 2010 determined the effect of heavy metals on the strains of rhizobia which correlate with the current findings that as the concentration of metal increased, growth of the organisms ceased. Rhizobial response to different types of metals depends on the applied concentrations (El -Hilai, 2006).

Positive relationship between the amount of heavy metals and the tolerance levels of rhizobial isolates were obvious, since five isolates were in general tolerant to all metals in the evaluated results. Many workers have demonstrated the multiple heavy metal tolerance of rhizobia (Doleman *et al.*, 1994). Resistance mechanisms of microbes is supported by EPS and LPS of rhizobial strains which act as defense barriers against heavy metals. Principal mechanisms adopted by *Rhizobium* to tolerate heavy metals are accumulation of metal ions inside the cell,



bioreduction of the toxic metals, methylation, precipitation and chelation (Lima *et al.*, 2006). Several findings on tolerance of rhizobial strains to heavy metals have been reported by many researchers (Milicic *et al.*, 2006; Lakzian *et al.*, 2002; Kuperman & Carreiro, 1997). The toxic effects of the heavy metals can be reduced through phytoremediation process and promotion of growth of the plants through PGP activities with the help of rhizobia associated with the legumes which has a great biotechnological potential (Fagorzi *et al.*, 2018). Vidal *et al.*, 2009 evaluated heavy metal tolerance of *M. metallidurans*. Pajuelo *et al.*, 2011 suggested that the extrusion of heavy metals from the rhizobial cells could be attributed to resistance. Indigenous rhizobia adapting to local conditions of heavy-metal contamination are more likely to succeed over the long term compared to introducing strains which have less adaptation strategies.

## V. CONCLUSION

These indigenous rhizobia of *Arachis hypogaea* plant possess ecological benefit. Therefore, productive rhizobial candidates from such environment will definitely establish successful symbioses in extreme heavy metal rich environments useful for production of crop legumes.

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**ACKNOWLEDGEMENT:**

Nithyakalyani acknowledges her guide Dr. Kannan and Ms. J. Neenapriya for their support and guidance.

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