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Effects of silver, mercury,cobalt and nickel ions on *Sinorhizobium meliloti* bacteria

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SUMMARY

Sinorhizobium meliloti species of nitrogen fixation bacteria was isolated from *Medicago sativa* plant and exposed to different concentrations (5,10,20and 40 ppm) of Ag^{+1},Hg^{+1},Co^{+2} and Ni^{+2} ions as singly under the laboratory conditions .The growth rate of this bacterium was measured by using spectrophotometer at wave length 540 nm after different exposure periods (0,2,24,48 and 72 hrs.) to detect the toxic effect of these ions on the bacterium growth .Results showed that the inhibitory effect of these ions were in the following sequences $Ag^{+1} > Hg^{+1} > Co^{+2} > Ni^{+2}$ and the effect of these ions was depending on the ions type ,their charges ,ions concentration in the medium and the exposure periods.

Key word : *Sinorhizobium meliloti* , , Heavy metals, Growth rates

Introduction

Heavy metals discharged into the environment from various industries, agriculture and municipal sewages constitute one of the major causes of water and soil pollution (14).Among the microorganisms, bacteria , yeast and protozoa are generally the first category to be exposed to heavy metals present in the environment (11). Microorganisms require some metals like Cu^{+2}, Zn^{+2}, Co^{+2} and Ni^{+2} at low concentrations as essential micronutrients for vital cofactors for metalloproteinase and certain enzymes, however ,at higher concentration ,it has been reported that these metals form toxic complex compound in the cell that are too dangerous for any biological functions (9).There were several genera of essential bacteria found in soil such as *Sinorhizobium meliloti* , *Bradyrhizobium* , *Rhizobium* , *Azorhizobium* , *Allorhizobium* and *Mesorhizobium*, which are able to establish asymbiosis with leguminous plants. They elicit the formation of specialized organs, called nodules, on roots of their hosts,in which they reduce atmospheric nitrogen and make it available to the plant .Symbiotic nitrogen fixation is an important source of nitrogen, and the various legume crops and pasture species often fix as much as 200 to300 kg nitrogen per hectare(10).Very little information is available in the literature regarding the action of heavy metals against these bacteria , therefore, the aim of this study is to evaluate the toxic effect of Ag^{+1},Hg^{+1},Co^{+2} and Ni^{+2} ions on the growth of *Sinorhizobium meliloti* isolate under the laboratory conditions .

Materials and Methods

Bacterial suspension :-

Sinorhizobium meliloti isolate was isolated from the root nodules of *Medicago sativa* plant according to (13) method .This isolate was identification by (1).Pure isolate was grown on the nutrient agar media ,then ,the bacterial suspension adjusted to 10^6 cfu / ml according to Mc Farland method (4).

Growth medium :-

Nutrient broth medium was used after pH adjusted to 6.8 , sterilized by using autoclave at 121◦C for 20 min.

Heavy metals stock solutions :-

Stock solution (1000 ppm)of each metal was prepared by dissolved it's salt : $AgNO_3$, $Co(NO_3)_2.6H_2O$ and $Ni(NO_3)_2.6H_2O$ for Ag^{+1},Co^{+2}, and Ni^{+2} respectively in 1000 ml of deionized distilled water .Whereas, the mercury was prepared as metal (1g) dissolved in 1.5ml of con.HNO_3,The volume make to 1000 ml by deionized distilled water .Different concentrations (5,10,20, and 40 ppm) of each solution were prepared by using the dilution law and nutrient broth medium .

The effect of heavy metals test :-

The nutrient broth medium that supplemented with heavy metal ions (singly) was dispensed into sterilized tubes as 20 ml / tube in addition to the control tubes (medium free of heavy metal ions).The inoculums size (10^6 cfu/ ml) were added as 1ml / tube. These cultures were incubated at 28 ◦C .Then , the growth of bacteria was measured by using spectrophotometer as optical density at 540 nm. for different exposure periods (0,2,24,48 and 72 hrs).

Data analysis and figures :-

Minitab version 11 program was used for the analysis of variance test (ANOVA test) and reverse less significant differences (RLSD) was calculated to detect the differences among the means of the growth rates .Excel 2003 program was used to draw the figures.

Results

The analysis of variance (ANOVA test) showed significant differences ($p< 0.01$) among the studied ions effects on the growth rates of *Sinorhizobium meliloti* isolate and these inhibitory effects were in the following sequences $Ag^{+1} > Hg^{+1} > Co^{+2} > Ni^{+2}$ fig (1).The results showed that these inhibition effects were depending on the type of ions ,their charges ,their concentrations in the medium and the exposure periods, as described in the following :-

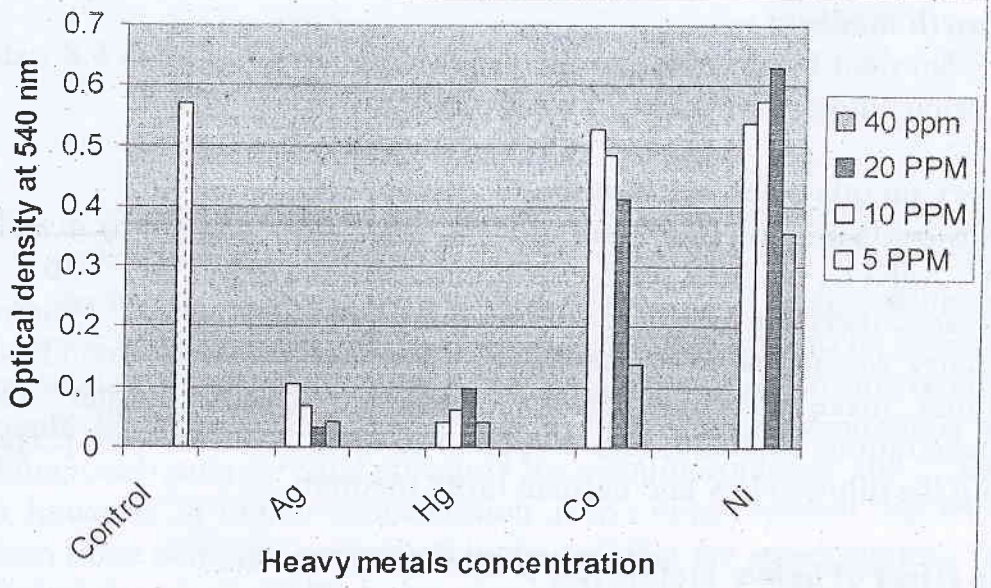
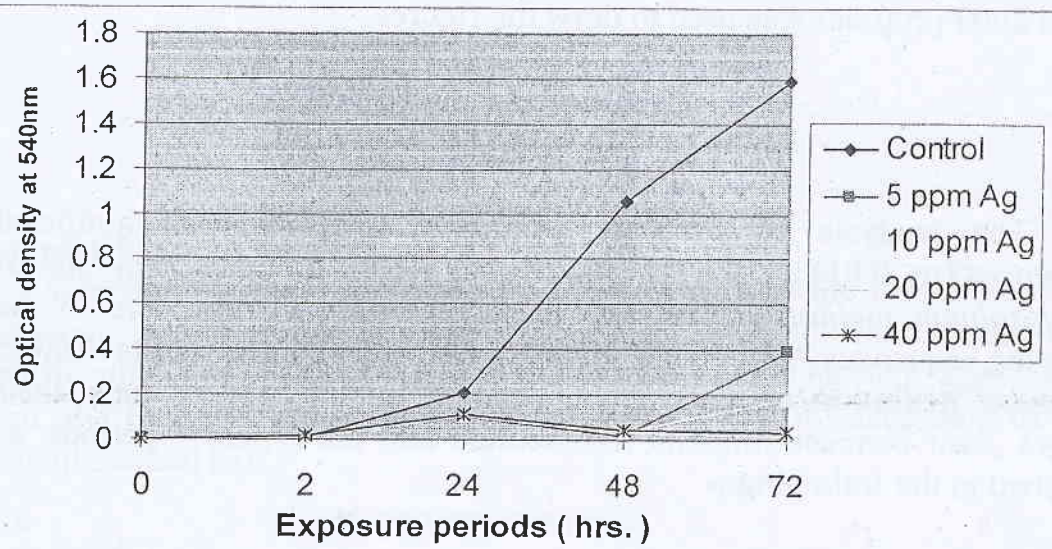


Fig (1) :- Effect of different concentrations of heavy metals on the growth of *Sinorhizobium meliloti*

1- The effects of silver ions (Ag^{+1}) :-

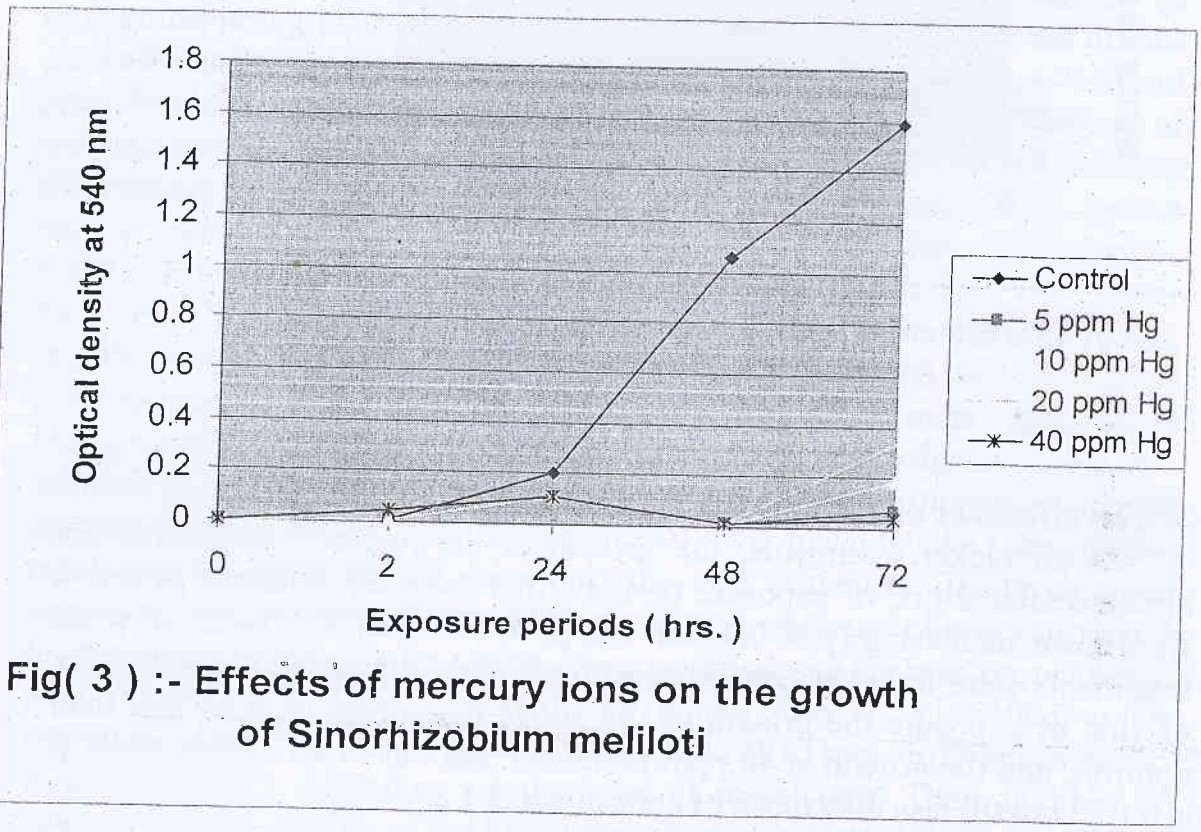
The growth rate of the present isolate was not affected after 2 hrs. of exposure periods at all silver ions treatments as compared with the control treatment .An elongation in the lag phase (48 hrs.) was appeared at 5 and 10 ppm treatments, Whereas no growth was appeared at 20 and 40 ppm . treatments along the exposure period (fig . 2) .



Fig(2) :- Effects of silver ions on the growth of *Sinorhizobium meliloti*

2- The effects of mercury ions (Hg^{+1}) :-

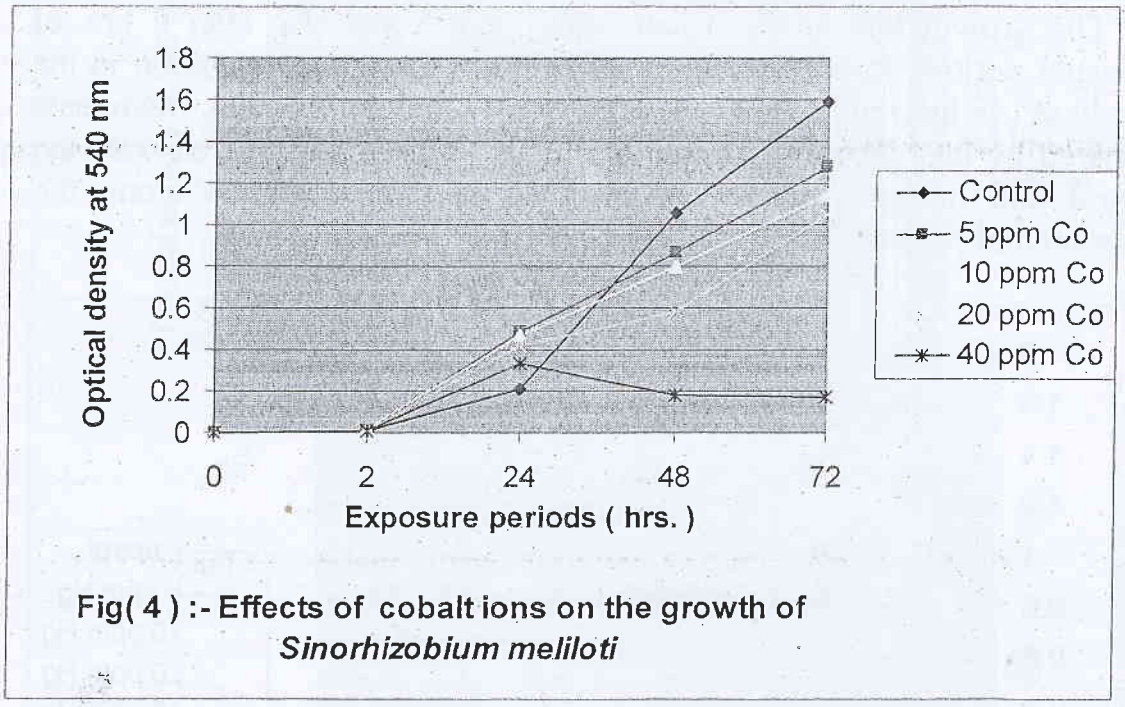
The growth rate of the isolate was not affected also after 2 hrs. of exposure periods for all treatments of mercury ions. An elongation in the lag phase (48 hrs.) of bacteria was appeared at 5,10,and 20 ppm. treatments followed by less growth appeared after this period as compared with the control and no growth was appeared at 40 ppm treatment along the experimental periods(fig .3) .



Fig(3) :- Effects of mercury ions on the growth of Sinorhizobium meliloti

3- The effects of cobalt ions (Co^{+2}) :-

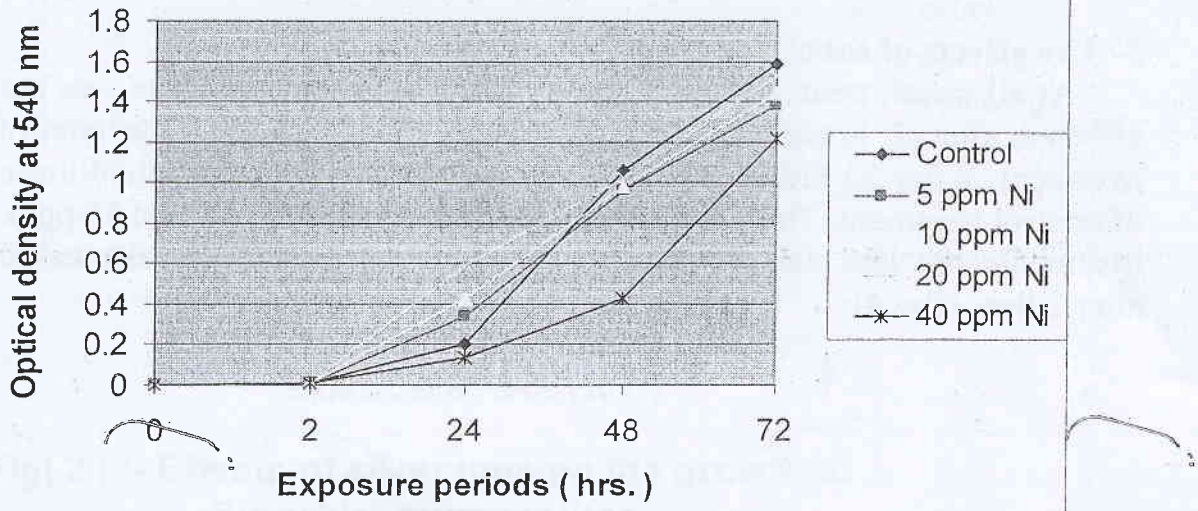
At all cobalt treatments ,the growth rate of the present isolate was not affected after 2 hrs.of exposure periods as compared with the control treatment .After 24 hrs.of exposure ,the growth became higher than those of control treatment. Then, the growth were continuous at 5,10 and 20 ppm. treatments but less than control. At 40 ppm. the growth was decreased after 24hrs. (fig .4) .



Fig(4) :- Effects of cobalt ions on the growth of *Sinorhizobium meliloti*

4- The effects of nickel ions (Ni^{+2}) :-

At all nickel treatments, the growth of the present isolate was not affected after 2 hrs. of exposure period .After 24hrs.of exposure period, a significant increasing ($p < 0.01$) in the growth rate at 5, 10 and 20 ppm. treatments were found as compared with the control treatment. Then , after 48 hrs. of exposure the growth of the above treatments became less than control , and the growth at 40 ppm treatment was increased significantly($p < 0.01$) but till less than those of control(fig .5)



Fig(5) :- Effects of nickel ions on the growth of *Sinorhizobium meliloti*

Discussion

The present study showed a significant decreasing ($p < 0.01$) in the growth rate of isolate that exposed to heavy metals ions especially silver and mercury ions .silver and mercury ions were classified as non-essential for the growth of organisms and have a toxic effect even at low concentration and their salts have got wide spread applications in controlling the microorganisms populations (7). Also results exhibited an elongation in lag phase after exposure to increasing concentrations of ions ,this observation appeared to be due to selection for ion-resistant phenotypes (9).Also microorganisms have acquired a variety of mechanisms for adaptation to the presence of toxic heavy metals .Among the various adaptation mechanism, metal sorption, mineralization, uptake and accumulation , extracellular precipitation, enzymatic oxidation or reduction to a less toxic form and efflux of heavy metals from the cell (8, 12 , 11). These mechanisms are sometime encoded in plasmid genes facilitating the transfer of toxic metal resistance from one cell to another (12). Crist *et al*(6) suggest that the biosorption of heavy metals consists of two phases: a fast phase (less than 4 seconds) is attributed to surface adsorption ,mainly based on ion exchange with the participation of the carboxyl groups of uronic acids and much slower metal uptake (2hrs.)phase represent the diffusion of ions into the cell structures .Bacteria make excellent biosorbents because of their high surface-to-volume ratios and high content of potentially active chemosorption site such as on teichonic acid in their cell wall (2).Nickel and cobalt ions are classified as an essential micronutrients at low concentration (9).These differences in the effects probably depending on the type of metal ions, Their number of charges and the affinity of the binding site for each metal (5).The inhibitory effects of ions (such as cobalt at 40 ppm treatment) became higher as soon as increasing these ions in the medium ,our result was in agreement with Brown and Lester (3) and it may be due to saturation of extracellular polymers binding sites occurred at the highest metal concentrations added of ions.

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تأثير ايونات الفضة والزئبق والكوبلت والنيكل على الجرثومة *Sinorhizobium meliloti*

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الخلاصة

تم عزل الجرثومة المثبتة للنتروجين *Sinorhizobium meliloti* من نبات الجت *Medicago sativa* L. وعرضت لتراكيز مختلفة (40,20,10,5 جزء بالمليون) من ايونات Ni^{+2},Co^{+2},Hg^{+1},Ag^{+1} (بصورة منفردة) تحت الظروف المختبرية .تم قياس معدل النمو باستخدام جهاز المطياف الضوئي وبطول موجي 540 نانومتر لهذه الجرثومة بعد فترات حضن مختلفة (72,48,24,2,0 ساعة) لتحديد التأثير السمي لتلك الأيونات على نمو الجرثومة.

أوضحت النتائج إن تأثير تلك الأيونات على نمو الجرثومة كانت بالتسلسل التالي :- $Ni^{+2} < Co^{+2} < Hg^{+1} < Ag^{+1}$ ، و إن تأثير تلك الأيونات اعتمد على نوع الأيون وشحنته ، تركيزه في الوسط الزرعي ،وفترة تعريض الجرثومة له .