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“Growth kinetic of multi-metal resistant bacteria under metal stress”

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ABSTRACT

The use of multi-metal resistant bacteria as an alternative to heavy metal removal has become increasingly important in recent years. In this study, the potential growth of nine bacterial isolates on media with four different metals included copper, zinc, nickel, and chromium at 10, 50, and 100 mg/L, individually and quaternary were assessed. The data obtained were analysed to determine the effect of metals on the growth kinetics of the bacterial isolates in metal resistance. The growth kinetics included specific growth rate, doubling time and biomass production rate. Based on these parameters, the ability and selectivity of these isolates to grow under metal stress and produce biomass were determined. Tolerance parameters and performance index of all isolates at different metal concentrations were evaluated. The results showed that nine bacterial isolates were able to grow in culture medium integrated with individual and quaternary metals in all selected concentrations. They showed greater adaptability and resistance of metals with well and different rates. The highest biomass production was achieved with Ni followed by Zn were approximately more than half, while Cu, Cr and quaternary ranging approximately between one-third to half of the biomass produced without metals. *B megaterium*, *S ginsensidmutans* and *K rhizophila* the highest tolerance level and performance index with quaternary selected as multimetal tolerant bacteria. Based on the findings obtained, it is possible to conclude that isolates have potential applicability for further research related to bioremediation of metals and management of contaminated sites with vital methods.

Key words: specific growth rate, metal stress, kinetic parameters, multimetal resistant bacteria, Heavy metals, biomass production.

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الملخص

أصبح استخدام البكتيريا المقاومة للمعادن المتعددة كبديل لإزالة المعادن الثقيلة ذا أهمية متزايدة في السنوات الأخيرة. في هذه الدراسة، تم تقييم النمو المحتمل لتسع عزلات بكتيرية على وسط مع أربعة معادن مختلفة تشمل النحاس والزنك والنيكل والكروم عند 10 و 50 و 100 ملغم / لتر، بشكل فردي ورباعي. تم تحليل البيانات التي تم الحصول عليها لتحديد تأثير المعادن على حركية نمو العزلات البكتيرية في مقاومة المعادن. تضمنت حركيات النمو معدل نمو محدد، ووقت التضاعف ومعدل إنتاج الكتلة الحيوية. بناءً على هذه المعلمات، تم تحديد قدرة وانتقائية هذه العزلات على النمو تحت الضغط المعدني وإنتاج الكتلة الحيوية. معلمات التحمل ومؤشر الأداء لكل العزلات كانت قيمت . أظهرت النتائج أن تسع عزلات بكتيرية كانت قادرة على النمو في وسط مستنبت مدمج مع المعادن الفردية والرباعية في جميع التراكيز المختارة. أظهروا قدرة أكبر على التكيف ومقاومة المعادن بمعدلات جيدة ومختلفة. تم تحقيق أعلى إنتاج للكتلة الحيوية باستخدام النيكل متبوعًا بالزنك حيث كان أكثر من النصف تقريبًا، بينما تراوح النحاس والكروم والرباعي تقريبًا بين ثلث إلى نصف الكتلة الحيوية المنتجة بدون معادن. *B megaterium*, و *S ginsensidmutans* و *K rhizophila* العزلات الأكثر مستويات تحمل ومؤشر أداء مع معادن الرباعية. بناءً على النتائج التي تم الحصول عليها، من الممكن أن نستنتج أن العزلات لها إمكانية تطبيق محتملة لمزيد من البحث المتعلق بالمعالجة الحيوية للمعادن وإدارة المواقع الملوثة بالطرق الحيوية.

الكلمات المفتاحية: معدل النمو المحدد، الإجهاد المعدني، البارامترات الحركية، البكتيريا المقاومة متعددة المعادن، المعادن الثقيلة، إنتاج الكتلة الحيوية.

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Introduction

Microbial communities have acquired adaptations to unfavourable growth conditions, in order to survive in their natural environments. These changes are usually associated with stress conditions that cells may be exposed to it, such as metallic stress or expose to high concentrations of heavy metals which known as one of stressors (Matarredona *et al*., 2020). This stress exerts a selective pressure on microbial community leading to the emergence of resistant strains (Hao *et al*., 2020). Resistant metals bacteria are candidate to play a large role for metal removal applications. This is due to their rapid multiplication and growth rates beside the evolutionary effects of dealing with the toxicity of heavy metals have redressed their adaptation mechanisms with stressful conditions (Mathivanan *et al*., 2021).

The application of bacterial cells to the remediation of toxic metals has become reliably, this is as a result of a number of advantages such as their versatile survival in harsh environments and considered some of them extremophile microorganisms due to the rapid occurrence of mutations and evolutionary adaptations (Yin *et al*., 2019). Other advantages as previously assured which include high specific growth rate; doubling time and larger surface area to volume ratio make them as superior agents of bioremediation than other microbial counterparts (Mathivanan *et al*., 2018). Heavy metal tolerant bacteria have the ability to reduce the harmful effects of heavy metals (Chalini and Sharu, 2018). This is a result of the ability of many bacteria to increase the efficiency of genetic elements through gene transfer and the availability of active resistance genes, allowing them to survive in severe conditions (Zahir *et al*., 2021).

Microbiological studies and techniques have attempted to broaden the understanding of heavy metal resistant microorganisms to accompany the effort to implement successful bioremediation of metal contaminants (Verasoundarapandian *et al*., 2019). Growth rate is an important characteristic of living cells and is particularly valuable for determining the activity of microbial groups that grow at exponential rates (Zakaria *et al*., 2020). It was assumed that bacterial growth would be concomitant and proportional to the contaminants which can use by microbial cells that is because the metallic contaminants can be transferred by living cells through reactions that occur as part of their metabolic processes (Tarekegn *et al*., 2020). Studies of the ability of microorganisms to use chemical pollutants as an energy source through their own metabolic processes throughout the microbiological process were confirmed (Tarekegn *et al*., 2020; Kapahi and Sachdeva, 2019).

Growth kinetics is an auto-stimulating reaction which indicates that the rate of growth is directly proportional to the concentration of cells. The common methods for measuring the mass and number of cell are direct methods include dry weight, turbidity (optical density) and plate counts (Sakthiselvan *et al*., 2019). Normally bacterial growth measurements have been performed using the viable counts method. However, estimation of microbial growth parameters from growth functions fixation to optical density measurements has become a more common application, with the advantages of being fast and low cost compared to a viable count method. Optical density measurements are widely used to characterize bacterial growth in various fields of microbiology, and also allow accurate comparison of model-derived growth parameter estimates (Powell *et al*., 2020). Through the relationship between the amount of light absorbed and the number of cells in the culture medium, optical density measurements provide an estimate of microbial density (Mauerhofer *et al*., 2019).

Batch growth kinetics of microbial cells follows a growth curve which start with lag phase which is adapt stage of cells to a new environment. Following it the log phase in which the cell mass and cell number increases exponentially, the log phase considered as a balanced growth phase because the average cell composition remains constant as the characteristics of the bacterial culture (protein and DNA) increase at the same rate (Barbera *et al*., 2019). Then stationary phase through which the depletion of nutrients occur and balance of cells result in growth rate equals the death rate which generally leads to a sigmoid shape. The last stage is death phase through it appear accumulation of toxic products and increase the numbers of dead cells (Sakthiselvan *et al*., 2019; Powell *et al*., 2020).

Kinetic studies allow the determination of the time required for pollutant to decay to a target concentration and support the design of a bioremediation kinetics model (Zahri *et al*., 2021). In addition, yicrobial growth kinetics explains the relationship between the specific growth rate of a microbe (μ) and the substrate concentration (S) using mathematical models, thus is an important tool in the field of biotechnology (Sakthiselvan *et al*., 2019). It may be attributed some substrates reinforce higher levels

of bacterial growth and more biomass production than other. In bioremediation kinetics, substrates are utilized may directly proportional to the amount of microbial cells, and concentration of substrate (Zakaria *et al*., 2020). However, in the metals bioaccumulation process, high concentrations of heavy metals may interact with microbial cells which would result in prolong lag time and reduce growth rate. Therefore, microbial growth kinetics are affected by heavy metals (Mersin and Açıkel, 2021).

Researchers have developed mathematical models for bacterial growth and decay as well as using substrate and electron acceptors to predict and evaluate bioremediation efficacy. Various models of microbial growth kinetics have been established, which generate several important kinetic parameters for analysis. Moreover, kinetic studies provide a strategy for solving real application problems on an industrial scale, including the bioremediation process (Zahri *et al*., 2021). The study of specific growth rate may be more significant due to that the increase in biomass during bacterial growth is a reflection of the increase in cell numbers and the ability of cells to use or metabolize the substrate. The maximum specific growth rate (μmax) is one of the most important parameters to be determined. It is evaluated by growing microbial cells in batch systems and then studying experimental data obtained during the exponential phase of growth. However, it was found that the (μmax) value determined in this way is affected by experimental conditions, which are not constant during growth as biomass, substrates and product concentrations alter exponentially (Barbera *et al*., 2019). The main active part of the cell growth curve in which cells multiply rapidly is the exponential (log) phase. This stage can determine the kinetic parameters, thus, this growth period is the best stage because all the components of the cell grow at an equal rate, enzymes are available and they are at their peak of activity (Sakthiselvan *et al*., 2019).

It was summarized that metal stress caused by heavy metals and its relationship to microbial activities, may be useful for studying microbial activity as specific growth rate and produced biomass under metal stress to determine the effect of metals on the biology of microbes and response extent.

This study aims to determine growth kinetics, biomass concentration and the indexes of tolerance and performance of nine bacterial species, isolated from electroplating effluents, in a nutrient medium supplemented with different concentrations of single and quaternary metals during the exponential phase to quantitatively determine the ability of bacteria to grow and produce biomass. This can be done using logarithmic equations, as well growth kinetics and tolerance study contribute in highlighting the levels of inhibition and the interaction between metals and bacterial isolates. Thus, may be valuable study to optimize the growth and biomass yield of bacteria at stressful conditions. Other potential uses of mathematical models can assist to improve the yield of biomass quantities, reduction of toxic ions by optimizing of the growth kinetics. In addition, the study of produced biomass may be a part of a whole treatment for the production of more and new biomass applicable in the bioremediation of metals.

Research questions

- How the unfavorable growth conditions affected the microbial communities?
- What is the role of resistant metal bacteria in the metal removal application?
- How become the bacterial cells application reliable?

Research hypotheses

First hypothesis: The bacterial isolates possess strong adaptive capabilities and grew in presence metals but the growth reduced relatively with increasing metals concentrations.

Second hypothesis: the metal tolerance level of isolates and determination suitable isolates to metals remediation are carried out using the index of tolerance and performance.

Research aim:

To prove that in case of the high concentrations of metals the isolates were able to produce new cells despite their lower rates compared to cells that were far from the metal stress. As the level of biomass yield ranging approximately between one-third and one half of the maximum biomass produced with isolates without metals.

Research terminology:

Specific growth rate: The rate at which the biomass of a cell population increases per unit of biomass concentration is known as the specific growth rate period (Zhu, 2018).

Heavy Metal stress: Heavy metal stress has emerged as a severe issue in many worldwide terrestrial ecosystems. Heavy metal stress inactivates or denatures heavy critical enzymes and other proteins and obstructs substitution reactions of necessary metal ions from biomolecules, which negatively impacts soil and crop yield today due to increased industrialization (Shahid et al., 2015).

Kinetic parameters: Most frequently, kinetic statistics like the Michaelis-Menten constant (KM) and maximum velocity are described in the chapter titled "Kinetic parameters" (Vmax). This subsection may additionally include additional information (such as the substrate that exhibits the best catalytic efficiency, etc.) (Wang, 2020).

Multimetal resistant bacteria: are bacterial resistance mechanisms that can be used to 'clean-up' heavy metal pollutants from water (Nanda, 2019).

Biomass production: Energy created or generated by living or formerly living creatures is known as biomass energy. Plants like the aforementioned corn and soy are the most prevalent types of biomass that are used for energy. These creatures' energy can be used to generate electricity or be burned to produce heat (Antar, 2021).

Literature review

Microbial stress response to heavy metals in the environment

Both humans and the environment's resident bacteria are at risk from heavy metal contamination. When anthropogenic heavy metal contamination rises above a particular threshold, it becomes harmful. Metals' non-degradable qualities add to their toxicity. Some microorganisms evolved defence systems to adapt to the environment and survive. Microbes that adapt to these harsh environments undergo genetic as well as physiological modifications. Ion selective ATPase pumps improve heavy metal efflux transport to lessen toxicity in microorganisms. Application of heavy metal resistant bacteria in bioremediation is aided by the formation of aggregations, biofilm, and EPS. The creation of modified microorganisms for application in bioremediation has been explored through genetic modification. The last ten years of research have improved our understanding of the processes and signalling pathways needed to cause heavy metal stress in microorganisms. However, a deeper comprehension of these mechanisms and signalling pathways is essential to dealing with the worrisome rise in heavy metal contamination (Prabhakaran, 2016).

Study of Heavy Metal Uptake and Analysis of Plant Growth Promotion Potential of Multiple Heavy Metal-Resistant Bacteria

Pollution brought on by heavy metals is a significant environmental issue. The goal of this study was to identify heavy metal-resistant and plant growth-promoting native bacteria from arable land that could be used to provide environmentally friendly solutions to bioremediation and sustainable agriculture problems. All twenty of the heavy metal-resistant bacterial isolates identified in this investigation underwent extensive screening and characterisation. Three powerful isolates were chosen from this group, and their heavy metal resistance and absorption potentiality were further investigated. The three isolates showed high tolerance to Ni, Zn, Fe, Cd, and other metals (500 g/mL) according to the minimum inhibitory concentration determination. The isolates' growth kinetics in the presence of different heavy metals showed differences between regular and metal-induced growth. The isolates' capacity for pigmentation and tolerance of pH were both examined. A study using inductively coupled plasma-mass spectrometry showed that the isolates absorbed the most Cd during the exponential phase of growth. One of the isolates showed the ability to promote plant growth, which was discovered utilizing several in vitro qualitative screening assays. The isolates were identified as Pseudomonas aeruginosa strains by 16S rRNA molecular analysis. This was the first

investigation into the heavy metal-resistant and pro-plant growth bacteria in this area. The development of efficient bioremediation and sustainable agriculture strategies may be facilitated by further research into such native bacteria that are multi-metal resistant (Saha, 2022).

Evidence of Resistance of Heavy Metals from Isolated Bacteria

The goal of this study is to find connections between the levels of heavy metals in water and the bacteria of bacterial resistance. Watercourse samples in one of Mexico's most significant mining regions were taken. The resistance of 71 isolated bacteria to Cr, Zn, Cu, Ag, Hg, and Co was investigated. A Multiple Metal Resistant index was computed, and the Minimum Inhibitory Concentration range was identified. Then, kinetic parameters for 11 isolated bacteria were estimated. According to the results, the examined bacteria behaved differently when heavy metals were included in the media: without an effect, growth was inhibited; and significantly, growth was inhibited. Last but not least, a Performance Index was suggested to choose suitable bacteria for heavy metals removal; five bacteria were chosen. Pseudomonas koreensis was chosen as a standout among them because it can promote growth even in the presence of all the metals examined, making it a promising candidate for a future biosorption system (Escamilla, 2021).

Material and methods

The study was done using isolated bacteria from electroplating effluents. The effect of heavy metal ions on the specific growth rate, doubling time and biomasses yield during the exponential phase was investigated in a batch system.

Bacteria isolation and cultivation

Bacteria were isolated from electroplating industrial effluents and was identified and deposited in the GenBank database under accession numbers. All were heterotrophic, but they contained gram-positive and negative bacteria, also they were spore-forming, non-spore forming bacteria. The dominant bacterial isolates were belong to the following genera and species: *Microbacterium paraoxydans* (NR_025648.1), *Streptomyces werraensis* (NR_112390), *Microbacterium arabinogalactanolyticum* (NR_0449321), *Staphylococcus haemolyticus* (NR_036956.1), *Bacillus paramycoides* (NR_1577341), *Bacillus megaterium* (NR_117473.1), *Sphingobacterium ginsenosidimutans* (NR_117473.1), *Kocuria rhizophila*(NR_026452.1) and *Sphingobacterium detergens*(NR_116238). For further screening of multi metal resistant bacteria, the isolates were grown on nutrient agar plates (Oxoid, Lab-Lemco Powder) contain (10 - 50mg/L) of Cu, Zn, Ni, Cr individual and quaternary, and incubated at 37º C for 24h (Pandit *et al*.,2013), then maintained on nutrient agar slants at 4°C for further use (Silva *et al* ., 2012).

Preparation of metal stock solution

The stock solutions of chromium (Cr), copper (Cu), zinc (Zn) and nickel (Ni) were prepared in deionized water and sterilized by membrane filtration (0.22 µm) and stored at 4°C. The salts used were potassium dichromate ($K_2Cr_2O_7$), copper sulphate ($CuSO_4.5H_2O$), zinc sulphate ($ZnSO_46H_2O$), and chloride nickel ($Nicl_2.6H_2O$). All working concentrations were obtained by diluting the stock solution (1000 mg/l) with deionized water. The solutions were then left for 30mins until complete dissolution occurred and sterilization was followed by membrane filtration (Odokuma and Akponah, 2010). The solutions were checked for their concentration using atomic absorption spectrophotometer (Shaaban *et al.*, 2015).

Calculations of optical density (O.D) and colony forming unit (CFU/mL)

The biomass calculation was performed in order to estimate kinetic parameters through exponential phase by combining the results of the optical density method and total plate count method (Avirasdya *et al*., 2022). Bacterial isolates nine were incubated overnight in nutrient broth containing 10, 50 and 100 mg/L at 37 °C for 24 h. Aliquot of 1mL was drawn after 24h from each culture to evaluate O.D Then, the cultures were centrifuged at 3000 g for 10 min, the precipitated cells were dissolved with sterile distilled water, then serial dilutions with different O.D values (1/2, 1/4, 1/8, 1/16 and 1/32) were

performed with each culture, so that the absorbance was in the linear range of 0–0.6. A zero-point calibration was performed with blank nutrient broth. Optical density (O.D) of dilutions measured using Spectrophotometer at a wavelength of 600 nm (Thermo Spectronic Genesis 20, Spectrophotometer).Thereafter, Suspensions of OD=0.6 from serial dilutions were selected to inoculate sterilize nutrient media. Aliquot 1ml of each suspension was spread on nutrient agar plates containing 10, 50, and 100 mg/L copper, zinc, nickel chromium metals and quaternary. The suspensions spread across the entire surface of the plates and every spread plate was made in triplicate. Colonies were counted via colony counter after incubation at 37 °C for 24h and growth was expressed as colony forming units per millilitre (CFU/mL) (Hou *et al*., 2020; Monballiu *et al*., 2015). Simultaneously, growth of the isolates on nutrient broth and nutrient agar with no metal supplementation was done. The bacterial growth on metal supplemented media were compared by plotting the optical density at 600 nm to spread plate method and the correlation is determined between them according to (Chien *et al*., 2013).

Estimation of Growth Kinetics of bacterial isolates during Exponential phase

Exponential or Logarithmic phase is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the existing bacterial population. Simple equations can be used to describe the results of a batch culture growth experiment especially during the exponential phase, the growth of the bacterial isolates can be modelled using specific parameters which can be applied (Solís *et al*.,2015). In order to assess the effectof metal ions on the growth of bacterial isolates, kinetic parameters were estimated as follows:

The first parameter; specific growth rate (μ, h^{-1}) = 1/X (dx/dt)

The optical density data was carried out to calculate the specific growth rate μ (h^{-1}),

$$OD_t = OD_0 e^{\mu \Delta t} \quad (1)$$

$$ln \frac{OD_t}{OD_0} = \mu \times \Delta t \quad (2)$$

Where t is time (hours), constant μ is termed the specific growth rate or often simply growth rate, (unit: $d^{-1}, h^{-1} or\ min^{-1}$). The optical density (OD), is proportional (~) to the cell density, where (**OD_0** = optical density at time 0, $\boldsymbol{OD_t}$ optical density at time t (Zakaria *et al.*, 2020) .

The second parameter; generation or doubling time (t_d =h) during the exponential growth phase and the time of lag phase which is short period immediately after inoculation can be used to assess this parameter (Friedrich Widdel, 2007). It is expressed by the following equation:

$$\frac{ln2}{t_d} = \frac{0.693}{t_d} = \mu \quad (3)$$

$$t_d \ or \ G = \frac{0.693}{\mu} \quad (4)$$

The third parameters**;** biomass yield

In both batch and continuous culture systems the rate of growth of bacterial cells or the rate of biomass production (r_X) [in units of biomass ($time^{-1}$) can be also defined by the following relationship:

$$\boldsymbol{r_{x_1}} = \mu \times (x) \quad (\mathbf{5})$$

Where ; $\boldsymbol{r_{x_1}}$ = biomass concentration or the biomass production rate, μ : specific growth rate and X: concentration of microbes (Smeaton and Cappellen, 2018).

Determination Tolerance parameters and Performance Index

In order to determine the tolerance limits of isolated bacteria used the following parameters as indicators of multiple metal resistant including the index of tolerance and performance and multi metal resistance (Escamilla-Rodríguez *et al*., 2021). The tolerance index (TI) which represents the relative growth rate of the bacteria in metal solution to control solution, was calculated as follows:

$$TI = \mu_{ms} / \mu_c \quad (6)$$

The Tolerance index (TI) represents the relative growth rate of the bacteria, where μ_{ms} is the growth rate in metal-containing solution and µc is the growth rate in a control. TI utilize to quantify metal tolerance in the isolates, thus the higher the TI value indicate the greater the tolerance.

A Performance Index (PI) this indicator was used to select the most suitable isolate that could tolerate and remove multimetals from the contamination site.

Multiple Metal Resistance (MMR) was estimated based on adapted method to multiple metal resistance determination. The PI was calculated by combining the MMR, the TI, and the t_{dm} as expressed in the Equation:

$$PI = MMR \times 1/T_{dm} \times TI_{\ quaternary} \quad (7)$$

Where, Multiple Metal Resistance (MMR) evaluation was determined as follows Equation:

$$MMR = a/b \quad (8)$$

In equation: (a) represent the number of metals tolerated by the isolates to, and (b) the total number of metals the isolates were tested agains

⇑

Statistical analysis

Microsoft Excel 2013 was used to plot growth graphs. The significance of individual and quaternary metals on bacterial viability by CFU/ OD determination were analysed statistically using ANOVA and Tukey Honest Significant Difference(SPSS version 25). *P* value was calculated to determine the significant results. Results showing that *P* value less than 0.05 were considered as significant ($P < 0.05$).

Results and discussion

Bacterial growth in optical density (O.D) and colony forming unit (CFU/mL)

The growth of isolates under heavy metal stress was performed in liquid and solid cultures under the influence of tested metals at three concentrations 10, 50 and100 mg/L. The results were compared with a blank free metal nutrient-rich growth medium. Measuring of the optical density (O.D= 600) was used to determine growth kinetic for its fast, inexpensive and does not cause any harm to the tested bacteria. As for of the resulting biomass through growth was determined using the spread plate (Sutton, 2011). Growth was measured during 24 h the incubation time and data for cells logarithms obtained from the spread plates method and cell turbidity (O.D= 600) of optical density method were plotted in addition to the control as shown by graphs (Figure 1- 6). The graphs demonstrates the correlation between cells count based on spread plating and the measurement of the optical density, the isolates exhibits a linear gradient up to an optical density of about 0.5, high densities of the isolates diluted to get cells count with each metal through the spread plate then the logarithms of the cells were calculated and compared cells count obtained as logarithms to the optical density to nutrient broths. The graphs showed the positive linear relationship and the direct proportionality between the two measurements, CFU/ml and OD600. The effect of metals at concentrations 10, 50 and100 mg/L on the bacterial growth of bacterial isolates investigated using the spread plate and optical density tests. The figures showed the correlation between cell count based on spread plating and the measurement of the optical density, the isolates

exhibited higher densities with zn and ni compared to the others. The correlations a linear gradient up to an optical density of about 0.5 with zinc and nickel whereas was less than 0.5 with Cu, Cr and quaternary. The lower optical density values obtained in the presence of all metals show that the growth of bacteria is less compared to the state where no metal is present. Optical density measurements and the spread plates were performed together. Nutrient agar media supplemented with metals and free were used for counting colonies. The results of these tests showed that the CFU number did not differ significantly for cultures with and without metals despite the metals concentrations were gradually raised. These results are consistent with the results of (Monballiu *et al*., 2015). This may confirm the role of bacterial resistance to metals. It has been reported that production of a matrix of external polymeric substances (EPS) by bacteria minimize the bioavailability and activity of the ionizable metal forms, such as Zn, Ni and Cu (Flemming and Wingender, 2010). Moreover, it was showed that specific proteins produced during (EPS) capable of binding to metal ions can be formed that enhance bacterial tolerance to heavy metals.

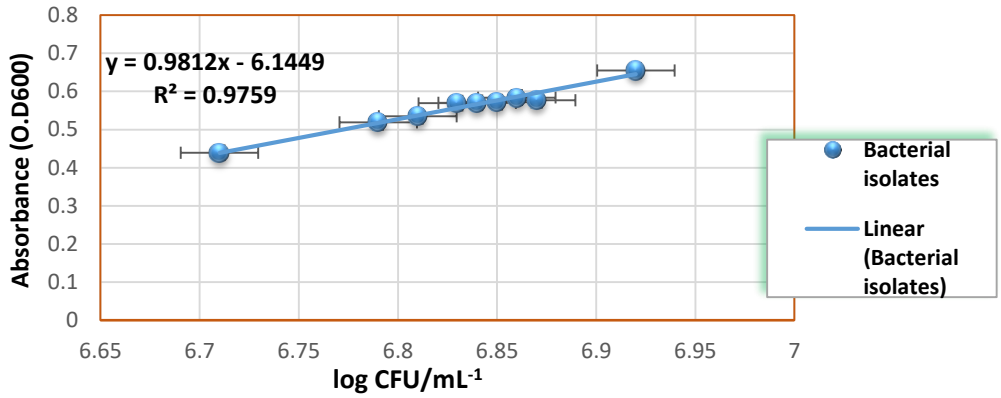


Figure 1- The correlation between viable cell count and the optical density for isolates in the blank

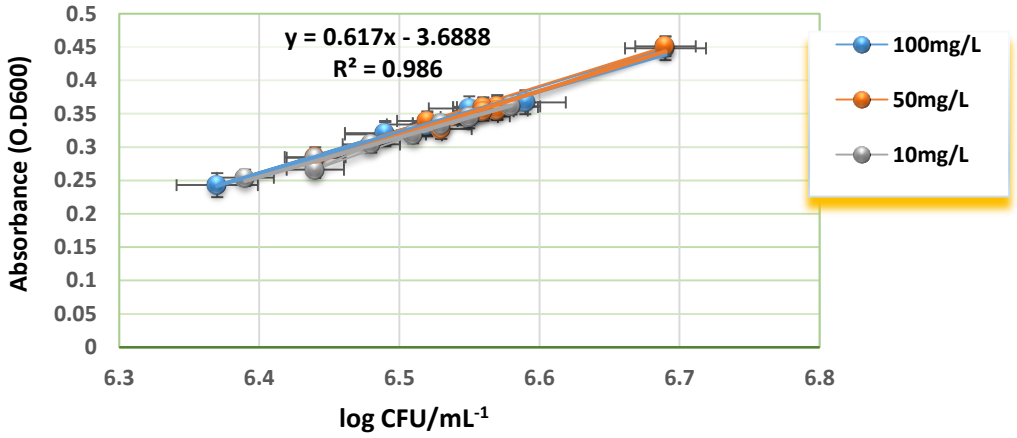


Figure 2- The correlation between viable cell count and the optical density for isolates in the presence Copper

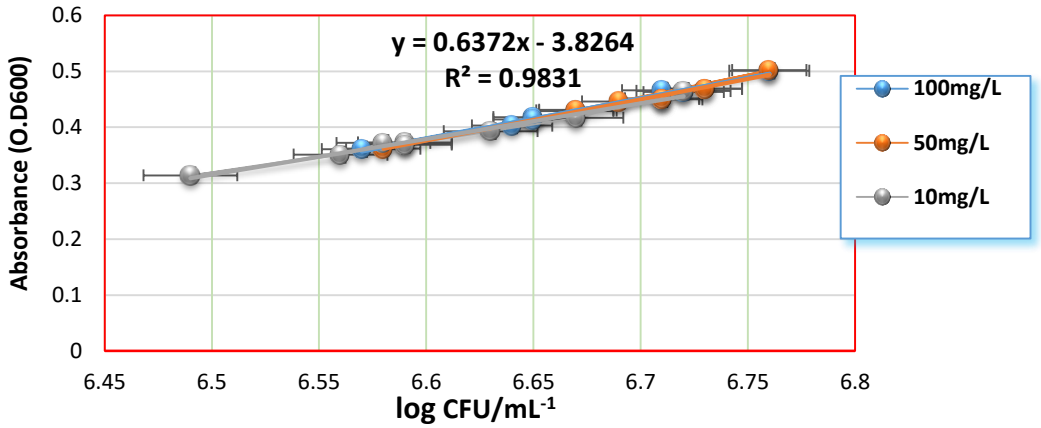


Figure 3- The correlation between viable cell count and the optical density for isolates in the presence Zinc

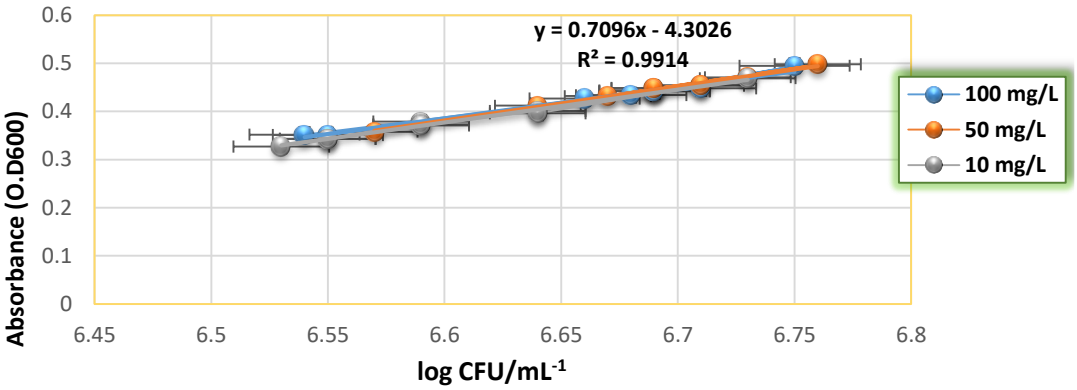


Figure 4- The correlation between viable cell count and the optical density for isolates in the presence Nickel

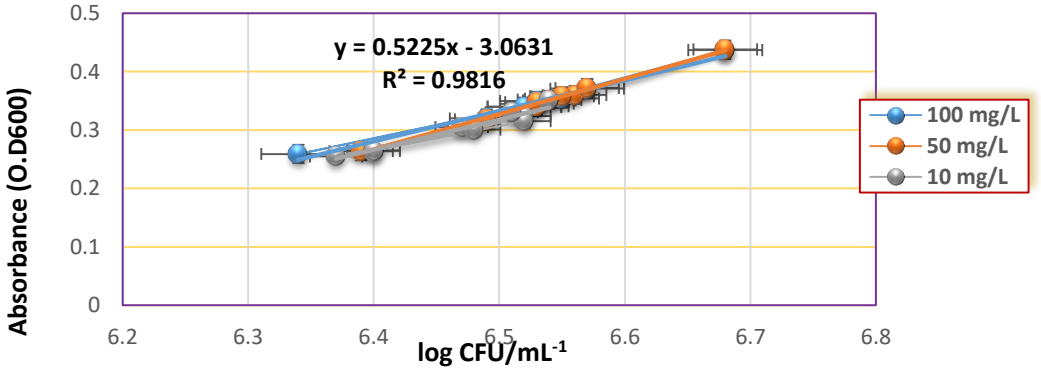


Figure 5-The correlation between viable cell count and the optical density for isolates in the presence chromium

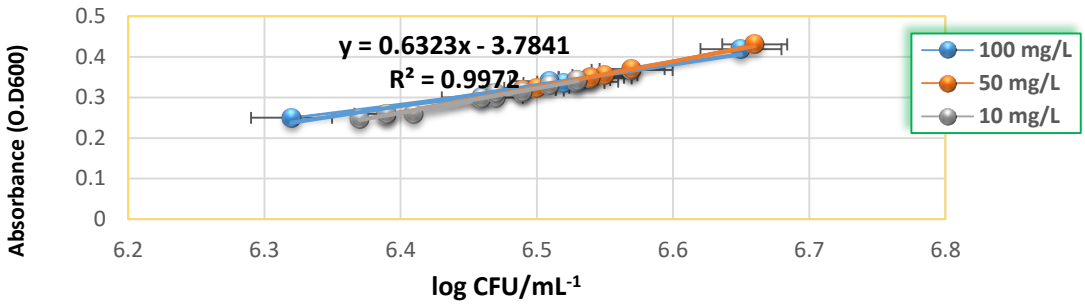


Figure 6 - The correlation between viable cell count and the optical density for isolates in the presence quaternary

Estimation of Kinetic Parameters of bacterial isolates during Exponential phase

The bacterial growth was specifically studied at exponential phase for bacterial isolates in the presence of the metals. Kinetic growth parameters such as specific growth rate, doubling time and biomass production rate, were used because the rate of growth is directly proportional to the concentration of cell. The purpose of measuring specific growth rate is to determine the rate of change in the cell number per unit time (minute, hour, d). The results as shown Table 1- 5 elucidated that the microbial growth within 18 hours in the presence of three concentrations was low compared to the control. In general the specific growth rate, doubling time and biomass production were relatively better at 10mg/L than others. The specific growth rate at 10, 50 and 100 mg/L were between 0.0861- 0.0869, 0.0849- 0.0858 and 0.0826- 0.0849 hr^{-1} respectively, for doubling time was the shortest 8 h at 10mg/L and the longest at 100 mg/L was 9h and 25 minutes. Whereas the produced biomass amount cell/hr was highest at 10mg/L then decreased with increase of metal concentrations. *B megaterium* was the highest produced of biomass, the produced amounts were 3.7 $x10^5$ cu, 4.7 $x10^5$ cr and 5.1 $x10^5$ quaternary, while showed *K rhizophila* the highest yield biomass with both zn and ni as were respectively 4.9 $x10^5$ zn and 5.0 $x10^5$ ni. These parameters with zinc and nickel showed that the growth kinetics of isolates were better suited to Zn and Ni metals individually as growth was enhanced, also they were more resistant to these two metals.

However, the growth kinetics of isolates with quaternary which represent mix of four metals were the best, which may be attributed to the fact that mixing metals may reduce their toxicity and its effect on cell growth and activity. Moreover, the results reveal that the bacterial growth was influenced with the chemical state of the metal or metal valence. That confirms the facts which were reported that the toxicity of heavy metals depends on a number of factors, including chemical forms of the metal, valence of metal ions and their compounds another aspect of metal form that may influence metal toxicity (Chibuike and Obiora, 2014).

Through the study of the growth kinetic of the isolates, it was showed that all isolates were able to produce biomass at all concentrations even with low specific growth rates. It was confirmed during one of studies that increasing toxic metal ion concentration in the growth medium usually lead to augment in the lag phase and minimize in microbial concentration (Mersin and Açıkel *et al*., 2021). These findings support also the fact that although specific bacterial growth reflects the amount of biomass and the ability of microbes to use the substrate, microbes may use the substrate without producing new cells (Niedhardt *et al*., 1990).

Bacterial isolates generated the highest specific growth rate with all metals were *Kocuria rhizophila* MIC-8, *Sphingobacterium ginsenosidimutans* A6MA-7, *Bacillus megaterium* BME-6 *Staphylococcus haemolyticus* STM-4 and *Sphingobacterium detergens* RMA-9. The effects of metals on living cells

which including increasing the doubling time and delaying growth represented as signal to toxicity level and respond by isolates. In general, there was no high inhibition of specific growth rate for all isolates and in all concentrations compared to control, although it was low and slowed, that was due to the high metal concentrations to which they were exposed. This highlights the resistance capabilities within all isolates. As was reported that decrease in growth is a response to metal stress and varies with different microorganisms (Mishra and Malik, 2013).

The biomass yields of cultures with most metals at 10mg/L were approximately more than half of the biomass produced without metals, but they remain less than control without metal ions. Ions of Zn and Ni promoted biomass production, it may be due to their use for metabolic reactions of bacterial cells with trace amounts and as cofactors of bacterial growth which may contribute to rise of the biomass yields (Valentine *et al.,* 1996). Despite the high concentrations of metals, the productions of biomass continued this may confirm the fact that under metal stress the physiological activities of cells may be affected, but without impairing the growth of these cells as previously reported (Silva *et al*., 2012). Statistically, no significant variation was observed of the effect different metals on growth kinetics of isolates, while there were significant variation compared to control, and with different metals in the same concentration at the 0.05 level. This confirms that the type of metal is one of the factors affecting the growth of bacteria. Moreover, the results of statistical analysis also appeared that zinc and nickel

Concentration	**10 mg/L**			**50 mg/L**			**100 mg/L**		
Isolates	**μ** hr^{-1}	t_d hr	r_x cell/hr	**μ** hr^{-1}	t_d hr	r_x cell/hr	**μ** hr^{-1}	t_d hr	r_x cell/hr
Microbacterium paraoxydans **C-1**	0.0847 0.0948	8.18 7.31	3.6×10^5 7.2×10^5	0.0834 0.0955	8.31 7.25	3.2×10^5 7.1×10^5	0.0754 0.0948	9.19 7.31	2.8 ×10^5 7.1×10^5
Streptomyces werraensis **C-2**	0.0818 0.0938	8.47 7.39	3.5×10^5 7.5×10^5	0.0806 0.0939	8.59 7.38	3.3×10^5 7.8×10^5	0.0778 0.0933	9.0 7.42	2.9 ×10^5 7.7 ×10^5
Microbacterium arabinogalactanolyticum **C-3**	0.0854 0.0953	8.11 7.27	3.4×10^5 6.9×10^5	0.0841 0.0945	8.24 7.23	3.1×10^5 6.9×10^5	0.0764 0.0949	9.00 7.30	2.7 ×10^5 7.0×10^5
Staphylococcus haemolyticus **C-4**	0.0865 0.0955	8.01 7.25	3.4×10^5 1.1×10^6	0.0843 0.0963	8.22 7.19	3.1×10^5 8.3×10^5	0.0817 0.0969	8.48 7.15	2.9 ×10^5 8.4×10^5
Bacillus paramycoides **C-5**	0.0854 0.0949	8.11 7.30	3.6×10^5 1.0×10^6	0.0844 0.0948	8.21 7.31	3.3×10^5 8.5×10^5	0.0785 0.0942	9.22 7.36	2.6 ×10^5 8.1×10^5
Bacillus megaterium **C-6**	0.0861 0.0934	8.01 7.41	3.7×10^5 8.2×10^5	0.0855 0.0942	8.11 7.35	3.6×10^5 8.3×10^5	0.0848 0.0939	8.17 7.38	3.4×10^5 8.1 ×10^5
Sphingobacterium ginsenosidimutans **C-7**	0.0866 0.0934	8.08 7.42	3.6×10^5 8.1×10^5	0.0849 0.0939	8.16 7.38	3.3×10^5 8.1×10^5	0.0845 0.0934	8.20 7.42	3.2 ×10^5 8.4 ×10^5
Kocuria rhizophila **C-8**	0.0858 0.0932	8.08 7.43	3.6×10^5 7.6×10^5	0.0851 0.0925	8.14 7.49	3.3×10^5 7.7×10^5	0.0839 0.0931	8.25 7.44	3.1 ×10^5 7.7×10^5
Sphingobacterium detergens **C-9**	0.0868 0.0934	8.0 7.42	3.6×10^5 7.9×10^5	0.0853 0.0935	8.12 7.41	3.4×10^5 8.1×10^5	0.0838 0.0941	8.27 7.36	3.1×10^5 8.2×10^5

growth kinetics were the most significant difference (P <0.05) compared to copper, chromium and nickel in the same concentration.

Table 1. The results of kinetic parameters of multi-metal resistant bacteria on free and incorporated medium with Copper metal

Specific Growth rate (h^{-1}), Doubling time ($t_{d)}$, Biomass production rate ($r_{x)}$, C control cultures without metal.

Table 2. The results of kinetic parameters of multi-metal resistant bacteria on free and incorporated medium with Nickel metal.

Concentration	**10 mg/L**			**50 mg/L**			**100 mg/L**		
Isolates	**μ** hr^{-1}	t_d hr	r_x cell/hr	**μ** hr^{-1}	t_d hr	r_x cell/hr	**μ** hr^{-1}	t_d hr	r_x cell/hr
Microbacterium paraoxydans	0.0836	8.29	4.2×10^5	0.0817	8.48	4.0×10^5	0.0811	8.54	3.2×10^5
C-1	0.0948	7.31	7.2×10^5	0.0955	7.25	7.1×10^5	0.0948	7.31	7.1×10^5
Streptomyces werraensis	0.0861	8.05	3.9×10^5	0.0840	8.25	4.4×10^5	0.0833	8.32	4.3×10^5
C-2	0.0938	7.39	7.5×10^5	0.0939	7.38	7.8×10^5	0.0933	7.42	7.7×10^5
Microbacterium arabinogalactanolyticum **C-3**	0.0844	8.21	4.4×10^5	0.0827	8.38	4.3×10^5	0.0812	8.53	3.4×10^5
	0.0953	7.27	6.9×10^5	0.0945	7.33	6.9×10^5	0.0949	7.30	7.0×10^5
Staphylococcus haemolyticus	0.0868	8.05	4.5×10^5	0.0844	8.21	4.0×10^5	0.0827	8.38	3.7×10^5
C-4	0.0955	7.25	1.1×10^6	0.0963	7.19	8.3×10^5	0.0969	7.15	8.4×10^5
Bacillus paramycoides	0.0864	8.02	4.3×10^5	0.0848	8.17	3.9×10^5	0.0829	8.35	3.6×10^5
C-5	0.0949	7.30	1.0×10^6	0.0948	7.31	8.5×10^5	0.0942	7.36	8.1×10^5
Bacillus megaterium	0.0865	8.01	4.8×10^5	0.0853	8.12	4.2×10^5	0.0795	8.25	3.4×10^5
C-6	0.0936	7.40	1.2×10^6	0.0933	7.42	1.1×10^6	0.0939	7.38	1.0×10^5
Sphingobacterium ginsenosidimutans	0.0871	8.00	4.6×10^5	0.0850	8.15	3.9×10^5	0.0838	8.26	3.8×10^5
C-7	0.0934	7.42	8.1×10^5	0.0939	7.38	8.1×10^5	0.0934	7.42	8.4×10^5
Kocuria rhizophila	0.0866	8.00	5.0×10^5	0.0849	8.16	4.8×10^5	0.0832	8.33	4.4×10^5
C-8	0.0932	7.43	7.6×10^5	0.0925	7.49	7.7×10^5	0.0931	7.44	7.7×10^5
Sphingobacterium detergens	0.0867	8.00	4.6×10^5	0.0854	8.11	4.2×10^5	0.0821	8.44	4.0×10^5
C-9	0.0934	7.42	7.9×10^5	0.0935	7.41	8.1×10^5	0.0941	7.36	8.2×10^5

Specific Growth rate (h^{-1}), Doubling time (t_d) Biomass production rate ($r_{x)}$, C control cultures without metal

Table 3. Kinetic parameters of multi-metal resistant bacteria on free and incorporated medium with Zinc metal

Concentration	**10 mg/L**			**50 mg/L**			**100 mg/L**		
Isolates	**μ** hr^{-1}	t_d hr	r_x cell/hr	**μ** hr^{-1}	t_d hr	r_x cell/hr	**μ** hr^{-1}	t_d hr	r_x cell/hr
Microbacterium paraoxydans **C-1**	0.0841 0.0948	8.24 7.31	4.2×10^5 7.2×10^5	0.0830 0.0955	8.35 7.25	4.0 ×10^5 7.1×10^5	0.0811 0.0948	8.55 7.31	3.7 ×10^5 7.1×10^5
Streptomyces werraensis **C-2**	0.0859 0.0938	8.0 7.39	3.5 ×10^5 7.5 ×10^5	0.0829 0.0939	8.3 7.38	3.2×10^5 7.8×10^5	0.0820 0.0933	8.45 7.42	3.1 ×10^5 7.7×10^5
Microbacterium arabinogalactanolyticum **C-3**	0.0843 0.0953	8.22 7.27	4.2×10^5 6.9×10^5	0.0832 0.0945	8.33 7.33	3.9 ×10^5 6.9×10^5	0.0813 0.0949	8.52 7.30	3.7 ×10^5 7.0×10^5
Staphylococcus haemolyticus **C-4**	0.0861 0.0955	8.05 7.25	4.6 ×10^5 1.1 ×10^6	0.0828 0.0963	8.25 7.19	4.0 ×10^5 8.3 ×10^5	0.0795 0.0969	8.37 7.15	3.7 ×10^5 8.4 ×10^5
Bacillus paramycoides **C-5**	0.0857 0.0949	8.08 7.30	4.4 ×10^5 1.0×10^6	0.0819 0.0948	8.46 7.31	3.8 ×10^5 8.5×10^5	0.0783 0.0942	9.25 7.36	3.2×10^5 8.1×10^6
Bacillus megaterium **C-6**	0.0861 0.0936	8.05 7.40	4.6×10^5 1.2×10^6	0.0849 0.0933	8.16 7.42	4.2×10^5 1.1×10^6	0.0838 0.0939	8.27 7.38	3.8×10^5 1.0 ×10^6
Sphingobacterium ginsenosidimutans **C-7**	0.0864 0.0934	8.02 7.42	4.8×10^5 8.1×10^5	0.0851 0.0939	8.14 7.38	4.2×10^5 8.1 ×10^5	0.0836 0.0934	8.28 7.42	4.0×10^5 8.4 ×10^5
Kocuria rhizophila **C-8**	0.0851 0.0932	8.14 7.43	4.9×10^6 7.6×10^5	0.0840 0.0925	8.25 7.49	4.7×10^5 7.7×10^5	0.0822 0.0931	8.43 7.44	4.4×10^5 7.7×10^5
Sphingobacterium detergens **C-9**	0.0863 0.0934	8.03 7.42	4.7×10^5 7.9×10^5	0.0852 0.0935	8.13 7.41	4.2×10^5 8.1×10^5	0.0790 0.0941	8.23 7.36	3.4×10^5 8.2×10^5

Specific Growth rate (h^{-1}), Doubling time (t_d) Biomass production rate (r_x), C control cultures without metal

Table 4. The results of kinetic parameters of multi-metal resistant bacteria on free and incorporated medium with Chromium metal.

Concentration	**10 mg/L**			**50 mg/L**			**100 mg/L**		
Isolates	**μ** hr^{-1}	t_d hr	r_x cell/hr	**μ** hr^{-1}	t_d hr	r_x cell/hr	**μ** hr^{-1}	t_d hr	r_x cell/hr
Microbacterium paraoxydans	0.0835	8.30	3.1×10^5	0.0815	8.50	3.0×10^5	0.0770	9.0	2.4×10^5
C-1	0.0948	7.31	7.2×10^5	0.0955	7.25	7.1×10^5	0.0948	7.31	7.1×10^5
Streptomyces werraensis	0.0818	8.47	3.3×10^5	0.0813	8.52	3.2×10^5	0.0808	8.58	3.2×10^5
C-2	0.0938	7.39	7.5×10^5	0.0939	7.38	7.8×10^5	0.0933	7.42	7.7×10^5
Microbacterium arabinogalactanolyticum **C-**	0.0839	8.26	3.3×10^5	0.0824	8.41	3.2×10^5	0.0758	9.14	2.0×10^6
3	0.0953	7.27	6.9×10^5	0.0945	7.33	6.9×10^6	0.0949	7.30	7.0×10^5
Staphylococcus haemolyticus	0.0852	8.13	4.5×10^5	0.0834	8.31	4.0×10^5	0.0791	8.7	3.1×10^5
C-4	0.0955	7.25	1.1×10^6	0.0963	7.19	8.3×10^5	0.0969	7.15	8.4×10^5
Bacillus paramycoides	0.0855	8.11	3.4×10^5	0.0828	8.37	3.1×10^5	0.0785	9.22	2.5×10^5
C-5	0.0949	7.30	1.0×10^6	0.0948	7.31	8.5×10^5	0.0942	7.36	8.1×10^5
Bacillus megaterium	0.0863	8.03	4.7×10^5	0.0852	8.13	4.4×10^5	0.0796	8.20	3.1×10^5
C-6	0.0936	7.40	1.2×10^6	0.0933	7.42	1.1×10^6	0.0939	7.38	1.0×10^6
Sphingobacterium ginsenosidimutans	0.0866	8.00	3.7×10^5	0.0847	8.18	3.3×10^5	0.0830	8.35	3.1×10^5 8.4×10^5
C-7	0.0934	7.42	8.1×10^5	0.0939	7.38	8.1×10^5	0.0934	7.42	
Kocuria rhizophila	0.0868	8.00	3.9×10^5	0.0850	8.15	3.6×10^5	0.0831	8.27	3.3×10^5
C-8	0.0932	7.43	7.6×10^5	0.0925	7.49	7.7×10^5	0.0931	7.44	7.7×10^5
Sphingobacterium detergens	0.0862	8.04	3.8×10^5	0.0842	8.23	3.4×10^5	0.0826	8.39	3.2×10^5
C-9	0.0934	7.42	7.9×10^5	0.0935	7.41	8.1×10^5	0.0941	7.36	8.2×10^5

Specific Growth rate (h^{-1}), Doubling time (t_d) Biomass production rate ($r_{x)}$, C control cultures without metal

Table 5. The results of kinetic parameters of multi-metal resistant bacteria on free and incorporated medium with quaternary

Concentration	**10 mg/L**			**50 mg/L**			**100 mg/L**		
Isolates	**μ** hr^{-1}	t_d hr	r_x cell/hr	**μ** hr^{-1}	t_d hr	r_x cell/hr	**μ** hr^{-1}	t_d hr	r_x cell/hr
Microbacterium paraoxydans C-1	0.0865 0.0948	8.01 7.31	3.9×10^5 7.2×10^5	0.0856 0.0955	8.09 7.25	3.8×10^5 7.1×10^5	0.0826 0.0948	8.39 7.31	3.6×10^5 7.1×10^5
Streptomyces werraensis C-2	0.0866 0.0938	8.00 7.39	3.7×10^6 7.5×10^5	0.0849 0.0939	8.16 7.38	3.6×10^5 7.8×10^5	0.0839 0.0933	8.25 7.42	3.5×10^5 7.7 ×10^5
Microbacterium arabinogalactanolyticum C-3	0.0863 0.0953	8.03 7.27	3.9×10^5 6.9×10^5	0.0858 0.0945	8.08 7.33	3.9×10^5 6.9×10^5	0.0814 0.0949	8.51 7.30	3.4×10^5 7.0 ×10^5
Staphylococcus haemolyticus C-4	0.0867 0.0955	8.00 7.25	4.5×10^5 1.1×10^6	0.0851 0.0963	8.14 7.19	4.3×10^5 8.3×10^5	0.0849 0.0969	8.16 7.15	4.1×10^5 8.4×10^5
Bacillus paramycoides C-5	0.0859 0.0949	8.07 7.30	3.6×10^5 1.0×10^6	0.0851 0.0948	8.14 7.31	3.4×10^5 8.5×10^5	0.0838 0.0942	8.28 7.36	3.2×10^5 8.1×10^5
Bacillus megaterium C-6	0.0865 0.0936	8.0 7.40	5.1×10^5 1.2×10^6	0.0854 0.0933	8.11 7.42	5.0×10^5 1.1×10^6	0.0841 0.0939	8.24 7.38	4.9 ×10^5 1.0 ×10^6
Sphingobacterium ginsenosidimutans C-7	0.0865 0.0934	8.0 7.42	3.7×10^5 8.1×10^5	0.0855 0.0939	8.11 7.38	3.6×10^5 8.1×10^5	0.0840 0.0934	8.25 7.42	3.1×10^5 8.4 ×10^5
Kocuria rhizophila C-8	0.0866 0.0932	8.00 7.43	4.3×10^5 7.6×10^5	0.0857 0.0925	8.10 7.49	4.1×10^5 7.7×10^5	0.0848 0.0931	8.17 7.44	4.0×10^5 7.7×10^5
Sphingobacterium detergens C-9	0.0869 0.0934	8.05 7.42	4.2×10^5 7.9×10^5	0.0850 0.0935	8.15 7.41	3.9×10^5 8.1×10^5	0.0845 0.0941	8.20 7.36	3.6 ×10^5 8.2×10^5

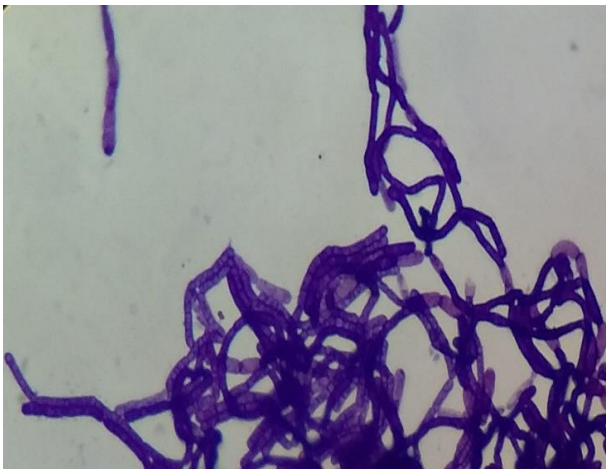
Specific Growth rate (h^{-1}), Doubling time (t_d) Biomass production rate ($r_{x)}$, C control cultures without metal

Determination of tolerance parameters and performance index depend on growth kinetic

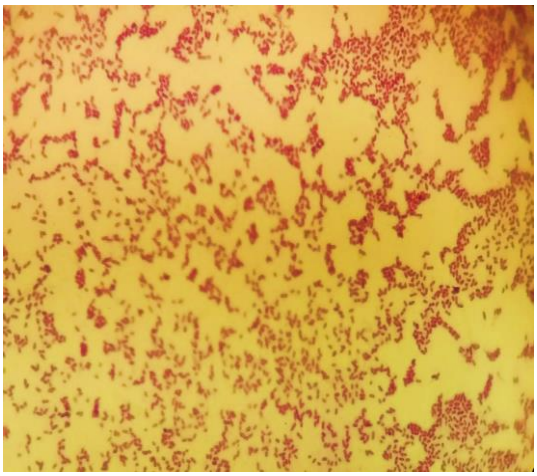
Tolerance parameters at 100 mg/L metal concentrations such as tolerance index (TI), and performance Index were used as indicators more accurate because they depend on specific growth rate(u) to quantify metal tolerance in the growth media and doubling time (t_d) (Escamilla-Rodríguez *et al*., 2021). Thus, the higher of the value of the Tolerance index (TI) larger the tolerance. After determining the tolerance index (TI) with single metals and quaternary, a performance index (PI) is determined with quaternary to choose the multimetal-isolates that can be used in a further study of biosorption and bioaccumulation capacity of metals, also physiological activity of cells under metallic stress. A Performance Index (PI) depend on Multiple Metal Resistance (MMR) value, the number of metals that isolates were able tolerate at the initial concentrations (a) to the total number of metals the isolates were tested against (b) (Kimiran-Erdem *et al*., 2015). It was considered 1.0 because the total of metals were 4 and all of them were able to be resisted by bacteria.

The results of TI and PI of bacterial isolates with individual and quaternary heavy metals are shown in Table 6 and 7. All isolates showed high levels of tolerance with certain metals individually and quaternary and may reinforce that the fact that the microbial communities clarify elevated level of tolerance to high concentrations of heavy metal in wastewater (Banerjee *et al*., 2018). At the high concentrations of quaternary 50 and 100mg/L the

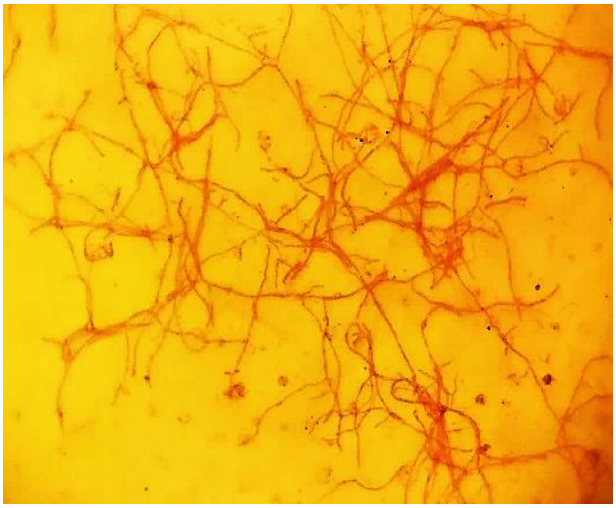
isolates *K rhizophila, S ginsenosidimutans, B megaterium and S werraensis* showed the highest TI which proved that these isolates were the most tolerant of all tested metals and multimetal tolerance. As well as, these isolates were appeared the highest PI, since performance index related to kinetic parameters these isolates achieved better specific growth rate and short double-time compared with the others in the presence quaternary of metals. So they were more adaptation to stressful conditions and better behaviour which advocate select them for further studies of metal tolerant strains, their activities and applicable for metals bioremediation.



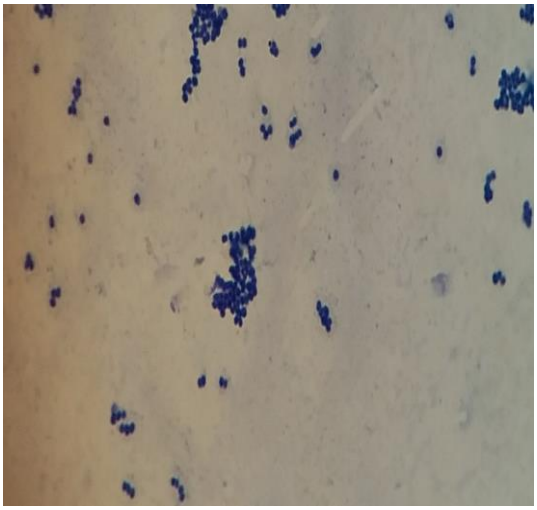
B megaterium



S gensenosidimutans



S werraensis



K rhizophila

Figure(7): Multimetal tolerant bacteria have the highest indexes of tolerance and performance

Table 6: Tolerance index of bacterial isolates with individual and quaternary heavy metal

Metals Concentrations	10 mg/L					50 mg/L					100 mg/L				
	Tolerance Index														
Isolates	Cu	Zn	Ni	Cr	Quaternary	Cu	Zn	Ni	Cr	Quaternary	Cu	Zn	Ni	Cr	Quaternary
Microbacterium paraoxydans	0.893	0.890	0.881	0.880	0.912*	0.873	0.869	0.855	0.853	0.896	0.795	0.855	0.854	0.812	0.871
Streptomyces werraensis	0.872	0.916	0.918	0.872	0.923*	0.858	0.882	0.894	0.866	0.904*	0.833	0.879	0.892	0.866	0.899
Microbacterium arabinogalactanolyticum	0.896	0.884	0.885	0.880	0.905*	0.889	0.880	0.875	0.871	0.907*	0.805	0.856	0.855	0.798	0.858
Staphylococcus haemolyticus	0.905	0.901	0.908*	0.892	0.908*	0.875	0.859	0.876	0.866	0.884	0.843	0.820	0.853	0.816	0.876
Bacillus paramycoides	0.899	0.903	0.910*	0.900	0.905*	0.890	0.863	0.894	0.873	0.898	0.833	0.831	0.880	0.833	0.889
Bacillus megaterium	0.921	0.919	0.924*	0.922	0.924*	0.908	0.909	0.914*	0.913	0.915*	0.903*	0.892	0.848	0.848	0.896
Sphingobacterium ginsenosidimutans	0.927*	0.925	0.932	0.927*	0.926	0.904	0.906*	0.905	0.902	0.910*	0.904	0.895	0.897	0.888	0.899
Kocuria rhizophila	0.920	0.913	0.929*	0.931*	0.929*	0.920	0.908	0.917	0.918	0.926*	0.901*	0.882	0.893	0.892	0.910*
Sphingobacterium detergens	0.929*	0.923	0.928*	0.922	0.930*	0.912	0.911	0.913*	0.900	0.909	0.890	0.839	0.872	0.878	0.897

Table 7: Index of resistance and Performance to bacterial isolates with quaternary of metals

Metals Concentrations	10 mg/L				50 mg/L				100 mg/L			
Isolates	MMR	$1/t_d$	TI	PI	MMR	$1/t_d$	TI	PI	MMR	$1/t_d$	TI	PI
Microbacterium paraoxydans	1.0	0.124	0.912	0.113	1.0	0.123	0.896	0.110	1.0	0.119	0.871	0.103
Streptomyces werraensis	1.0	0.125	0.923	0.115	1.0	0.122	0.904	0.110	1.0	0.121	0.899	0.109*
Microbacterium arabinogalactanolyticum	1.0	0.124	0.905	0.112	1.0	0.123	0.908	0.111	1.0	0.120	0.858	0.102
Staphylococcus haemolyticus	1.0	0.125	0.908	0.113	1.0	0.122	0.884	0.108	1.0	0.122	0.876	0.107
Bacillus paramycoides	1.0	0.123	0.905	0.111	1.0	0.122	0.898	0.109	1.0	0.120	0.889	0.100
Bacillus megaterium	1.0	0.125	0.924	0.116*	1.0	0.123	0.916	0.112*	1.0	0.121	0.896	0.108
Sphingobacterium ginsenosidimutans	1.0	0.125	0.926	0.116*	1.0	0.123	0.910	0.111	1.0	0.121	0.899	0.108
Kocuria rhizophila	1.0	0.125	0.929	0.116*	1.0	0.123	0.926	0.113*	1.0	0.122	0.910	0.111*
Sphingobacterium detergens	1.0	0.124	0.930	0.115	1.0	0.122	0.909	0.110	1.0	0.121	0.897	0.108

Conclusion

The effect of individual metals and quaternary at different concentrations on the growth kinetics of nine bacterial isolates was studied. The activity of these microbial groups that grew at exponential rates, their expected behaviour under metallic stress and their ability to produce biomass were determined. Growth kinetics analysis of selected isolates cultured with different metals concentrations demonstrated. Identify metal tolerance level of isolates and determination suitable isolates to metals remediation were carried out using the index of tolerance and performance. The results showed that bacterial isolates possess strong adaptive capabilities and grew in presence metals but the growth reduced relatively with increasing metals concentrations. Growth kinetics showed inhibition levels and interaction between minerals and bacterial isolates. The results concluded that even in case of the high concentrations of metals the isolates were able to produce new cells despite their lower rates compared to cells that were far from the metal stress. The level of biomass yield ranging approximately between one-third and one half of the maximum biomass produced with isolates without metals. These isolates were able to survive well in high metals concentrations and possess more efficient mechanisms of resistance. The results and conclusions of carried experiments are promising for use of these isolates in bioremediation of areas contaminated with heavy metals.

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