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# Isolation and Evaluation of the Tolerance of Industrial Wastewater Bacteria to Heavy Metals Toxicity

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**Abstract:** In the present investigate, 123 bacterial isolates were isolated from different sources of industrial wastewater and polluted soil. These isolates were cultivated on different heavy metal ions ($Ni^{+2}$, $Zn^{+2}$, $Fe^{+3}$, $Al^{+3}$ and $Cr^{+5}$) at different concentrations. Among these isolates, 100 bacterial isolates were heavy metal tolerance and 10 isolates were selected which gave the highest growth on 0.56, 7.00, 61.00, 61.00 and 2.20 ppm of $Ni^{+2}$, $Zn^{+2}$, $Fe^{+3}$, $Al^{+3}$ and $Cr^{+5}$, respectively. Out of 10 isolates, 3 isolates namely C4, C6 and C12 were capable to grow (tolerated) at high concentrations of the tested metal ions and gave a high growth ranged from 1.02-1.29, 0.84-1.15 and 0.74-1.31 in presence of Ni (at 17.8 ppm), Zn (at 224.03 ppm) and Cr (at 70.4 ppm), respectively. Carbon and nitrogen sources influence were studied to optimize the growth of tolerance tested isolates on a high metal ions concentration. It was found that glucose followed by whey were the best one sole of carbon sources and beef extract and the mixture of beef extract and peptone were the best nitrogen sources for removal of Ni, Zn and Cr (at 70.4 ppm) by the tested isolates.

**Keywords:** Isolation, Bacteria, Heavy Metal Uptake, Growth, Nickel, Chromium

## 1. Introduction

Heavy metals are ubiquitous in the environment, as a result of both natural and anthropogenic activities, and humans are exposed to them through various pathways [1]. Excessive accumulation of heavy metals in agricultural soils through wastewater irrigation, may not only result in soil contamination, but also lead to elevated heavy metal uptake by crops, and thus affect food quality and safety [2]. Chronic level intake of toxic metals has adverse impacts on humans and the associated harmful impacts become apparent only after several years of exposure [3]. However, the consumption of heavy metal-contaminated food can seriously deplete some essential nutrients in the body that are further responsible for decreasing immunological defenses, intrauterine growth retardation, impaired psycho-social faculties, disabilities associated with malnutrition and high prevalence of upper gastrointestinal cancer rates [4] [5]. Scientists do a lot of studies to reclaim and reuse of wastewater which come from different human activities like sewage and industrial wastewater. In the paste the studies dealing with water quality in relation to microbiological criteria. During the last decade, chemicals have also been incorporated into the observation panel, thus enlarging the number of parameters to be determined for safe reuse of wastewater [6]. [7] applied ten different heavy metals (eight transition elements and two lead group elements) to a system established to biomonitor (based on the ISO 20079 protocol) the higher plant *Lemna minor*, clone St. The growth inhibition was quantitatively measured (effective dose required for the inhibition of growth rates by 50%, ErC50) on the basis of multiplication rate, fresh weight, dry weight,

chlorophyll *a*, chlorophyll *b* and total carotenoid content. Based on the averages of all tested parameters, the following phytotoxicity series was obtained:($Ag^{+1}$> $Cd^{+2}$> $Hg^{+2}$> $T1^{+1}$> $Cu^{+2}$> $Ni^{+2}$> $Zn^{+2}$> $Co^{+6}$> $Cr^{+5}$> $As^{+3}$> $As^{+5}$). The toxic effects of heavy metals it is necessary to recall two well-known facts [6]. First, a heavy metal is not toxic per se; it is only toxic when its concentration in the plant exceeds a certain threshold ("it is the dose that makes the effect"). This is especially important to the second fact: that some elements, called micronutrients, have essential functions in plant cells. This has been shown for Co, Cu, Fe, Mn, Mo, Ni and Zn. Only when the internal concentration exceeds a certain threshold do they demonstrate toxic effects, and then they are commonly termed "heavy metals". Micronutrients are essential for biosynthesis, growth, nucleic acids, growth substances, chlorophyll and secondary metabolites, carbohydrates and lipids, as well as for stress resistance. A supply of micronutrients is also essential for the integrity of membranes [8]. Biosorption capacity is influenced by many factors, including the status of microorganism (cell age), properties of metal ions (radius of ion, valence, etc.) in aqueous solution, cultural conditions (carbon source, nutrition supply, composition of growth media, etc.), biosorption conditions (such as pH, temperature, contact time, co-ions in solution, initial concentration of metal and biomass, availability of metal ions and micronutrition etc.) [9].

This work aims to study the ability of bacterial isolates, isolated from industrial wastewater and soil samples to tolerance of some heavy metals at different concentrations. The nutritional requirements were study also.

## 2. Materials and Methods

### 2.1. Samples Collection

Six samples were obtained from different ecological sources (Cement factory soil, Iron & Steel factory soil, canal water near to Iron & Steel factory, Cement factory wastewater, canal soil near to Iron & Steel factory and Iron & Steel factory wastewater) in sterilized plastic bags and bottles. These samples were collected from factories in Egypt. These samples were used as a source for isolation of heavy metals removing microorganisms.

### 2.2. Media Used

Medium 1: Nutrient agar medium [10]

It was used for maintenance, preservation and isolation of bacteria. It has the following composition (g/L):

| | |
|---|---|
| Beef extract | 3.00 |
| Peptone | 5.00 |
| Agar agar | 20.0 |
| Distilled water | 1000 ml |
| pH | 7.00 |

Medium 2: Glucose agar medium [10]

It was used for quantitative and qualitative estimation of heavy metals removing bacteria. It has the following composition (g/L):

| | |
|---|---|
| Glucose | 10.00 |
| Beef extract | 3.00 |
| Peptone | 5.00 |
| Agar agar | 20.00 |
| Distilled water | 1000 ml |
| pH | 7.00 |

a. The above medium composition was modified by addition of different metal ions in different concentration to study the biosorption of heavy metals.
b. Glucose broth medium was the same as glucose agar medium without adding agar.

### 2.3. Metal Solution Preparation

Stock metal solution contained 1000 mg/L concentration each of $Ni^{+2}$ ($NiCl_2.7H_2O$), $Zn^{+2}$ ($ZnSO_4.6H_2O$), $Fe^{+2}$ ($Fe_2(SO_4)_3.H_2O$), $Al^{+2}$ ($Al_2(SO_4)_3.18H_2O$) and $Cr^{+5}$ ($K_2Cr_2O_7$)) was prepared by dissolving heavy metals salt in distilled water. The working metal solution was prepared from the stock solution which ranged from 0.56 -17.76 ppm for $Ni^{+2}$, from 7 -224.03 ppm for $Zn^{+2}$, from 61 -1952 ppm for $Fe^{+3}$ and $Al^{+3}$ and from 2.2 -70.4 ppm for $Cr^{+5}$. Metal solutions were sterilized by filtration using 0.2 μm pore-size Millipore sterile filters.

### 2.4. Isolation of Heavy Metals Removing Microorganisms

Ten gram representative soil samples were suspended in 90 ml of sterile tap water and shaken thoroughly for 10 min. Meanwhile, the water sample used as it is. Heavy metals removing microorganisms were isolated from collected samples by streak and pour plate methods for bacteria isolation using medium 2. The plates were incubated at 30°C for 24 - 48 hr. Developed colonies were picked, purified and preserved at 5°C on agar slant for further studies.

### 2.5. Maintenance of the Isolated Cultures

Stocks culture slants were maintained at 5°C on preservation medium (medium 1) after incubation at 30°C for 24 - 48 hours.

### 2.6. Preparation of Standard Inoculum

Bacterial standard inoculum was prepared by inoculation of conical flask (250 ml in volume) containing 50 ml of nutrient broth (medium 1) with a loop of tested culture. The inoculated flasks were incubated on rotary shaker (150 rpm) for 24 h at 30°C. The content of these flasks was used as standard inoculum which 1 ml contained $2.1\times10^5$ colony forming units /ml.

### 2.7. Qualitative Estimation of Heavy Metals Removing Tolerant Bacterial Isolates

Bacterial isolates were inoculated on plate agar medium

(med. 2) supplemented with different heavy metal saltsand the concentration of the metal salts was maintained at 0.56 ppm for $Ni^{+2}$, 7 ppm for $Zn^{+2}$, 61 ppm for $Fe^{+3}$ and $Al^{+3}$ and 2.2 ppm for $Cr^{+5}$ of the medium. The plates were incubated at 30°C for 24 h and observed the bacterial growth on solid medium by naked eyes. The same method was carried out with control plates (plates without metal).

### 2.8. Quantitative Estimation of Heavy Metals Removing Tolerant Bacterial Isolates

Batch experiments were carried out in plugged Erlenmeyer flasks (250 ml) containing 100 ml of glucose broth medium (medium 2) supplemented with a range of heavy metals concentrations (from 0.56 to 17.76 ppm for $Ni^{+2}$, from 7.00 to 224.03 ppm for $Zn^{+2}$, from 61.0 to 1952 ppm for $Fe^{+3}$ and $Al^{+3}$ and from 2.20 to 70.4 ppm for $Cr^{+5}$). So, 5 sources of heavy metals such as $NiCl_2.7H_2O$, $ZnSO_4.6H_2O$, $Fe_2(SO_4)_3.H_2O$, $Al_2(SO_4)_3.18H_2O$ and $K_2Cr_2O_7$ were applied separately to give different concentrations of $Ni^{+2}$, $Zn^{+2}$, $Fe^{+3}$, $Al^{+3}$ and $Cr^{+5}$ ions, respectively. Theses flasks were inoculated with 2% of standard inoculum for the tested isolates and incubated 30°C on rotary shaker (150 rpm) for 48 h. At the end of incubation period samples (10 ml) were taken from the growing cultures for bacterial growth to determine the optical density of growth spectrophotometrically at 620 nm.

## 2.9. Statistical Analysis

The collected data were statistically analyzed using Microsoft excel to determine correlation coefficient (rc)

### 2.10. Influence of Carbon and Nitrogen Sources on Growth of Heavy Metals Removing Tolerant Bacterial Isolates

Different carbon sources (sucrose, fructose, dextran, mannitol, molasses and whey (as lactose)) were replaced with the original carbon source of the used medium (glucose) with equivalent carbon amount of each of the tested carbon source to eliminate errors which may occur as a result of differences in carbon concentrations in each source.

To detect the adequate nitrogen source for heavy metal removal by the selected microbial isolates, the prescribed nitrogen source of the medium (beef extract and peptone) was replaced by equivalent nitrogen amount of each of the tested organic nitrogen source (Beef extract, peptone, yeast extract, soybean extract, corn steep liquor and tryptone) and inorganic nitrogen source ($NH_4Cl$ (Ammonium Chloride), $(NH_4)_3PO_4.3H_2O$ (Tri-Ammonium Phosphate) and $(NH_4)_3C_6H_5O_7$ (Tri- Ammonium Citrate)).

# 3. Results and Discussion

### 3.1. Isolation of Heavy Metal Removing Bacteria

One hundred and twenty-three bacterial isolateswere isolated from different wastewater and soil samples (which collected from industrial factories in Egypt) on medium 1. The percentages of the distribution of bacterial isolates were illustrated by Figure 1. The highest figure of isolates percentage was shown in samples collected from Cement factory soil (E), being 22% followed by isolates obtained from Iron & Steel factory soil (S), canal water near to Iron & Steel factory (N), Cement factory wastewater (C), canal soil near to Iron & Steel factory (F) and Iron & Steel factory wastewater (M) being 19, 18, 15, 15 and 11%, respectively.

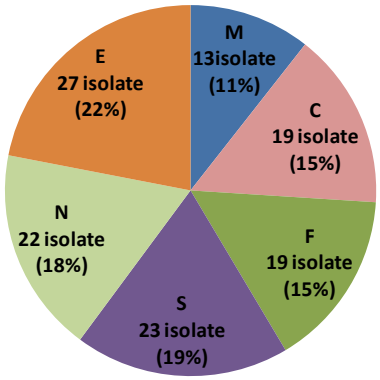


***Figure 1.*** *The percentage of the distribution of bacterial isolates obtained from different sources.*

### 3.2. Qualitative Determination of Heavy Metals Removing Bacterial Isolates

The primary selection of heavy metals removing bacterial isolates was based on their ability to grow on solid medium 2 supplemented with different heavy metal ions. After 48h of incubation period, growth of bacterial isolates in the presence of the metal ions such as $Ni^{+2}$, $Zn^{+2}$, $Fe^{+3}$, $Cr^{+5}$ and $Al^{+3}$ were detected and recorded in Table 1. Data show that among these 123 bacterial isolates, 100 bacterial isolates showed signs of growth on agar plats supplemented with heavy metal ions and demonstrated positive results from (+) to (++++) according to the density of growth from very low to high. In Figure 2 results exhibited the distribution number of metal ions tolerant bacterial isolates in the presence of different metal ions. Itwasobserved that out of 123 bacterial isolates only 120, 119, 108, 117 and 115 isolates were ability of grown in the presence of $Zn^{+2}$, $Ni^{+2}$, $Fe^{+3}$, $Cr^{+5}$ and $Al^{+3}$, respectively.

***Table 1.*** *Growth of bacterial isolates on solid medium 2 supplemented with different metal ions incubated at 30°C for 48 h.*

| source of isolation | isolates codes | Growth in presence of heavy metals | | | | | source of isolation | isolates codes | Growth in presence of heavy metals | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $Zn^{+2}$ | $Ni^{+2}$ | $Fe^{+3}$ | $Cr^{+3}$ | $Al^{+3}$ | | | $Zn^{+2}$ | $Ni^{+2}$ | $Fe^{+3}$ | $Cr^{+3}$ | $Al^{+3}$ |
| Iron & Steel | M1 | ++++ | ++++ | ++++ | ++++ | ++++ | Canal soil | F1 | + | ++ | ++ | + | ++ |
| factory | M2 | +++ | +++ | + | +++ | ++ | near to Iron | F2 | ++ | ++ | ++ | ++ | +++ |
| wastewater | M3 | +++ | +++ | ++ | +++ | ++ | & Steel | F3 | +++ | +++ | +++ | +++ | +++ |
| (M) | M4 | +++ | +++ | ++ | ++ | ++ | factory (F) | F4 | +++ | + | +++ | ++ | +++ |

| source of isolation | isolates codes | Growth in presence of heavy metals | | | | | source of isolation | isolates codes | Growth in presence of heavy metals | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $Zn^{+2}$ | $Ni^{+2}$ | $Fe^{+3}$ | $Cr^{+3}$ | $Al^{+3}$ | | | $Zn^{+2}$ | $Ni^{+2}$ | $Fe^{+3}$ | $Cr^{+3}$ | $Al^{+3}$ |
| Cement factory wastewater (c) | M5 | +++ | +++ | + | +++ | ++ | | F5 | +++ | +++ | +++ | +++ | ++ |
| | M6 | +++ | +++ | ++ | +++ | ++ | | F6 | ++ | - | ++ | - | - |
| | M7 | +++ | +++ | ++ | +++ | ++ | | F7 | ++ | ++ | ++ | ++ | +++ |
| | M8 | +++ | +++ | +++ | ++ | +++ | | F8 | ++ | + | + | +++ | +++ |
| | M9 | +++ | ++ | +++ | +++ | ++ | | F9 | +++ | +++ | +++ | ++ | +++ |
| | M10 | +++ | +++ | ++ | +++ | +++ | | F10 | +++ | ++ | +++ | ++ | ++ |
| | M11 | ++++ | ++++ | ++++ | ++++ | ++++ | | F11 | ++ | ++ | ++ | ++ | + |
| | M12 | +++ | ++ | - | - | - | | F12 | ++ | ++ | ++ | +++ | +++ |
| | M13 | +++ | + | ++ | +++ | + | | F13 | +++ | ++ | +++ | ++ | ++ |
| | C1 | +++ | +++ | + | + | + | | F14 | ++ | ++ | ++ | ++ | +++ |
| | C2 | +++ | +++ | +++ | ++ | ++ | | F15 | +++ | ++ | ++ | ++ | ++ |
| | C3 | +++ | +++ | ++ | +++ | +++ | | F16 | +++ | ++ | ++ | ++ | ++ |
| | C4 | ++++ | ++++ | ++++ | ++++ | ++++ | | F17 | ++ | +++ | ++ | ++ | +++ |
| | C5 | +++ | +++ | + | ++ | +++ | | F18 | +++ | ++ | +++ | ++ | ++ |
| | C6 | ++++ | ++++ | ++++ | ++++ | ++++ | | F19 | - | - | ++ | ++ | + |

***Table 1.*** *Continued.*

| source of isolation | isolates codes | Growth in presence of heavy metals | | | | | source of isolation | isolates codes | Growth in presence of heavy metals | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $Zn^{+2}$ | $Ni^{+2}$ | $Fe^{+3}$ | $Cr^{+3}$ | $Al^{+3}$ | | | $Zn^{+2}$ | $Ni^{+2}$ | $Fe^{+3}$ | $Cr^{+3}$ | $Al^{+3}$ |
| Cement factory wastewater (c) | C7 | +++ | +++ | +++ | +++ | ++ | Canal water near to Iron & Steel factory (N) | N10 | ++ | ++ | + | ++ | ++ |
| | C8 | ++++ | ++++ | ++++ | ++++ | ++++ | | N11 | ++ | ++ | ++ | + | +++ |
| | C9 | +++ | +++ | - | + | +++ | | N12 | +++ | ++ | ++ | ++ | +++ |
| | C10 | ++ | ++ | +++ | ++ | ++ | | N13 | ++++ | ++++ | ++++ | ++++ | ++++ |
| | C11 | + | +++ | + | +++ | + | | N14 | ++ | ++ | + | +++ | + |
| | C12 | ++++ | ++++ | ++++ | ++++ | ++++ | | N15 | ++ | + | - | - | + |
| | C13 | ++++ | ++++ | ++++ | ++++ | ++++ | | N16 | +++ | + | + | + | +++ |
| | C14 | ++ | ++ | ++ | ++ | + | | N17 | +++ | ++ | ++ | + | +++ |
| | C15 | ++ | ++ | ++ | + | ++ | | N18 | +++ | - | + | + | + |
| | C16 | +++ | ++ | - | ++ | + | | N19 | ++++ | ++++ | ++++ | ++++ | ++++ |
| | C17 | ++ | +++ | +++ | + | ++ | | N20 | +++ | + | + | ++ | + |
| | C18 | ++ | + | + | +++ | ++ | | N21 | + | + | ++ | ++ | +++ |
| | C19 | - | ++ | +++ | ++ | +++ | | N22 | - | +++ | - | +++ | ++ |
| Iron & Steel factory soil (S) | s1 | +++ | +++ | - | ++ | ++ | Cement factory soil (E) | E1 | +++ | +++ | + | +++ | +++ |
| | s2 | +++ | + | + | + | + | | E2 | +++ | +++ | +++ | +++ | +++ |
| | s3 | +++ | +++ | ++ | + | ++ | | E3 | +++ | +++ | +++ | +++ | +++ |
| | s4 | +++ | ++ | + | ++ | + | | E4 | +++ | +++ | +++ | +++ | +++ |
| | s5 | +++ | ++ | +++ | +++ | ++ | | E5 | +++ | +++ | +++ | +++ | +++ |
| | s6 | +++ | +++ | +++ | ++ | ++ | | E6 | +++ | +++ | ++ | ++ | +++ |
| | s7 | +++ | +++ | - | + | - | | E7 | +++ | ++ | +++ | +++ | +++ |
| | s8 | +++ | ++ | - | +++ | ++ | | E8 | +++ | +++ | ++ | +++ | ++ |
| | s9 | +++ | ++ | - | - | - | | E9 | +++ | +++ | +++ | +++ | +++ |
| | s10 | +++ | +++ | ++ | ++ | - | | E10 | +++ | +++ | +++ | +++ | +++ |
| | s11 | +++ | +++ | - | + | +++ | | E11 | +++ | ++ | ++ | +++ | ++ |
| | s12 | +++ | ++ | + | + | + | | E12 | +++ | ++ | - | +++ | ++ |
| | s13 | +++ | +++ | + | ++ | ++ | | E13 | ++ | ++ | +++ | +++ | +++ |
| | s14 | ++ | +++ | ++ | + | ++ | | E14 | +++ | +++ | ++ | +++ | +++ |
| | s15 | +++ | + | + | + | + | | E15 | +++ | +++ | ++ | +++ | +++ |
| | s16 | +++ | ++ | + | - | ++ | | E16 | +++ | +++ | +++ | ++ | +++ |
| | s17 | +++ | + | - | + | ++ | | E17 | ++ | ++ | + | +++ | ++ |
| | s18 | +++ | +++ | ++ | + | +++ | | E18 | + | + | +++ | +++ | +++ |
| | s19 | +++ | +++ | ++ | ++ | - | | E19 | +++ | +++ | +++ | +++ | +++ |
| | s20 | +++ | +++ | +++ | + | ++ | | E20 | ++ | ++ | +++ | +++ | +++ |
| | s21 | +++ | +++ | ++ | +++ | +++ | | E21 | ++ | ++ | +++ | +++ | +++ |
| | s22 | +++ | ++ | + | ++ | - | | E22 | +++ | ++ | +++ | +++ | ++ |
| | s23 | +++ | - | - | - | - | | E23 | +++ | +++ | +++ | ++ | ++ |
| Canal water near to Iron & Steel factory (N) | N1 | ++ | ++ | + | + | +++ | | E24 | +++ | +++ | ++ | +++ | +++ |
| | N2 | ++++ | ++++ | ++++ | ++++ | ++++ | | E25 | +++ | +++ | +++ | ++ | ++ |
| | N3 | +++ | +++ | + | +++ | + | | E26 | ++ | ++ | ++ | +++ | +++ |
| | N4 | +++ | ++ | ++ | + | + | | E27 | ++ | ++ | ++ | +++ | +++ |
| | N5 | ++ | +++ | + | +++ | +++ | | | | | | | |
| | N6 | ++ | ++ | ++ | + | +++ | | | | | | | |
| | N7 | ++ | ++ | - | +++ | +++ | | | | | | | |
| | N8 | +++ | ++ | - | + | +++ | | | | | | | |
| | N9 | +++ | + | - | +++ | +++ | | | | | | | |

+ = Very low growth, ++ = Low growth, +++ = Moderate growth, ++++ = High growth, - = No growth

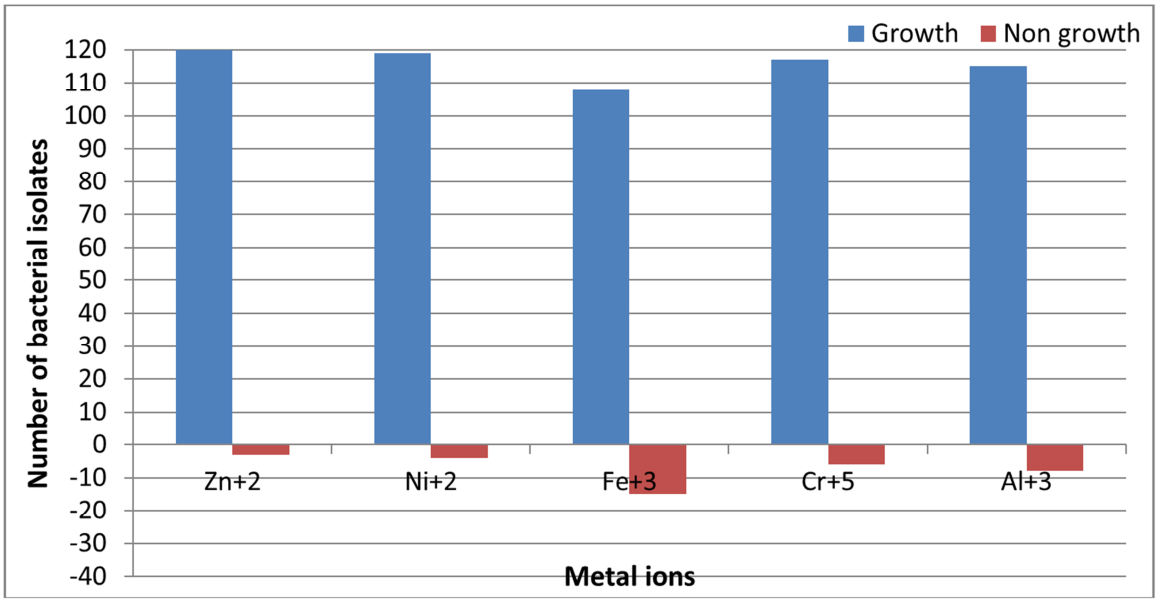


***Figure 2.*** *The distribution number of heavy metal removing bacterial isolates in the presence of different metal ions.*

Oves *et al* [11] collected 22 bacterial strainsfrom the rhizosphere of cauliflower which its ability to grow on medium nutrient agar supplemented with toxic metals (Cd, Cr, Cu, Pb and Ni). In addition, [12] found that the newly isolates of Micrococcussp. and *Aspergillus* sp. were tolerated chromium and nickel in growth medium and them gavegrowth. Moreover, [13] isolated heavy metal tolerant fungi from samples of sewage sludge and industrial effluent contaminated with heavy metals (Pb, Cr and Ni) which found many strains tolerant to Pb, some tolerant to Cr and some tolerant to Ni at 25 ppm. [14] found the ability of two bacterial species of*Bacillus subtilis* 117S and *Pseudomonas cepacia* 120S to biosorption of $Ni^{+2}$.

### 3.3. Quantitative Estimation of the Heavy Metals Tolerant Bacterial Isolates

Out 123, 10 bacterial isolates selected as efficientheavy metals tolerant isolates and cultivated in broth medium supplemented with different metal ions $Ni^{+2}$, $Zn^{+2}$, $Cr^{+5}$ and $Al^{+3}$ at different concentrations ranged from 0 to 17.76 ppm for $Ni^{+2}$, from 0 to 224.03 ppm for $Zn^{+2}$, from 0 to 1952 ppm for $Al^{+3}$ and from 0 to 70.4 ppm for $Cr^{+5}$. Results in Table 2 demonstrated that the biomass ofthe tested isolatesdecreased by increasing the metal ions concentrations. Whereas, all the tested isolates gavegrowth ranged from 0.005 to 2.13 as compared to control at 0 ppm. Three isolates of C4, C6 and C12 were gave a high growth being 1.20, 1.29 & 1.02, 1.15, 1.16 & 0.84 and 1.24, 1.31 &0.74 at high concentrations of Ni (17.8 ppm), Zn (224.03 ppm) and Cr (70.4 ppm). [15] stated that microorganisms play a significant role in bioremediation of heavy metal contaminated soil and wastewater. [16] Found that ten bacterial species and seven *Saccharomyces cerevisiae* strains showed more than 50% removal for 16 heavy metal ions including nickel. In other study, it has also been observed that with a decrease in metal ion concentration the biosorption rate increases rapidly while with higher metal ion concentrations a substantial decline in metal removal rate is reported which could probably be due to the saturation of a number of adsorption sites [17]. Similar results have been reported by others [18-20]. In the present study, it could be explained that the ability of the bacterial isolates to remove different heavy metals from the growth media was found to be depended on different chemical, physical and biological factors for example, the type of the tested microorganism, heavy metal type, charge and concentration, incubation period, pH, temperature and the composition of the growth media. [21] Suggested that at higher pH values, more ligands like carboxyl, phosphate, imidazole and amino group would be exposed and carry negative charges with a subsequent attraction of metallic ions with positive charge and biosorption onto the cell surface. Most of the living organisms have been shown to biosorb heavy metals such as Cd and Cu at a low pH, due to their physiological properties [22]. Moreover [23] also say the speciation, behavior, transport, and decisive fate of heavy metals in instinctive ecosystems depend mainly on the sorption with surface functional groups of microbial communities. Some fungi have broad range which bind and accumulate majority of heavy metals, while others are specific in their metal accumulation [24]. Also, several *Bacillus* species were able to accumulation of heavy metals such as copper, zinc, cadmium, and nickel. Heavy metal resistant isolates show no inhibition of growth for higher concentration of heavy metals [25, 26], whereas heavy metal-sensitive isolates show inhibition of growth for higher concentration of heavy metals [27].

From statistical analysis, it was observed that a negative correlation coefficient (rc) between the growth of bacterial isolates and metal ions concentrations for all bacterial isolates which r ranged from -0.47 to -0.98. A high r was recorded with M11 and C12 isolates at all the tested concentrations. Whereas, the tested isolates of C4, C6, M1

and C13 gave a high r for all the tested metals except $Al^{+3}$ was moderate r. Also, C8 and N2 isolates were high correlation coefficient with all the tested metalsexcept $Ni^{+2}$ was moderate. However, N13 and N19 achieved a moderate r for all the tested metals exceptwith $Ni^{+2}$ and $Zn^{+2}$ was low and moderate, respectively.

From all the previous data, it could be summarized that C4, C6 and C12 were the most efficient bacterial isolates for growth on medium supplemented with metal ions ($Zn^{+2}$, $Ni^{+2}$ and $Cr^{+5}$) at concentration (112.02, 8.91 and 35.2 ppm) respectively. And exclude $Al^{+3}$ and $Fe^{+3}$, because the low growth of bacterial isolates with $Al^{+3}$ and the difficulty of growth detection due to the interference between growth density and $Fe^{+3}$ turbidity. So, these isolates (C4, C6 and C12) were selected for subsequent studies with $Zn^{+2}$, $Ni^{+2}$ and $Cr^{+5}$.

***Table 2.*** *Growth density of bacterial isolates as influenced by different heavy metals concentrations for 48 h at 30°C using shake flasks as batch culture.*

| Metal ions | Ions Concs.(ppm) | Optical density (OD) of resistant bacterial isolates | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C4 | C6 | C8 | N13 | M1 | M11 | N19 | N2 | C12 | C13 |
| $Fe^{+3}$ | 0 | 2.17 | 1.57 | 1.17 | 2.78 | 1.04 | 1.12 | 2.75 | 0.05 | 1.19 | 0.84 |
| | 61 | Nd | | | | | | | | | |
| | 122 | | | | | | | | | | |
| | 244 | | | | | | | | | | |
| | 488 | | | | | | | | | | |
| | 976 | | | | | | | | | | |
| | 1952 | | | | | | | | | | |
| $Ni^{+2}$ | 0 | 2.17 | 1.57 | 1.17 | 2.78 | 1.04 | 1.12 | 2.75 | 0.05 | 1.19 | 0.84 |
| | 0.56 | 2.01 | 2.01 | 1.75 | 0.64 | 0.81 | 1.51 | 1.01 | 0.15 | 1.135 | 0.701 |
| | 1.11 | 1.86 | 1.94 | 1.61 | 0.52 | 0.79 | 1.24 | 0.98 | 0.05 | 1.16 | 0.656 |
| | 2.23 | 1.75 | 1.88 | 0.76 | 0.37 | 0.74 | 1.08 | 0.83 | 0.055 | 1.14 | 0.573 |
| | 4.46 | 1.64 | 1.85 | 0.75 | 0.31 | 0.65 | 0.96 | 0.57 | 0.031 | 1.128 | 0.557 |
| | 8.91 | 1.24 | 1.34 | 0.65 | 0.21 | 0.53 | 0.89 | 0.51 | 0.006 | 1.04 | 0.39 |
| | 17.76 | 1.2 | 1.29 | 0.63 | 0.19 | 0.5 | 0.86 | 0.49 | 0.005 | 1.02 | 0.36 |
| | rc | -0.90 | -0.78 | -0.66 | -0.47 | -0.82 | -0.71 | -0.54 | -0.62 | -0.93 | -0.86 |
| $Zn^{+2}$ | 0 | 2.17 | 1.57 | 1.17 | 2.78 | 1.04 | 1.12 | 2.75 | 0.05 | 1.19 | 0.84 |
| | 7.00 | 2 | 2.13 | 1.45 | 1.6 | 0.973 | 1.23 | 1.7 | 0.13 | 1.14 | 0.7 |
| | 14.00 | 1.96 | 2 | 1.34 | 1.36 | 0.946 | 1.17 | 1.69 | 0.11 | 1.05 | 0.67 |
| | 28.00 | 1.56 | 1.87 | 1.33 | 0.39 | 0.888 | 1.08 | 1.37 | 0.09 | 1.03 | 0.59 |
| | 56.01 | 1.46 | 1.64 | 1.3 | 0.38 | 0.868 | 1.08 | 0.78 | 0.02 | 0.96 | 0.59 |
| | 112.02 | 1.16 | 1.18 | 0.83 | 0.26 | 0.7 | 0.78 | 0.6 | 0.01 | 0.86 | 0.49 |
| | 224.03 | 1.15 | 1.16 | 0.8 | 0.24 | 0.069 | 0.75 | 0.59 | 0.01 | 0.84 | 0.47 |
| | rc | -0.84 | -0.81 | -0.85 | -0.64 | -0.98 | -0.91 | -0.74 | -0.70 | -0.87 | -0.81 |
| $Cr^{+5}$ | 0 | 2.17 | 1.57 | 1.17 | 2.78 | 1.04 | 1.12 | 2.75 | 0.05 | 1.19 | 0.84 |
| | 2.2 | 1.72 | 1.91 | 1.1 | 1.01 | 0.94 | 0.96 | 1.36 | 0.1 | 1.23 | 0.73 |
| | 4.4 | 1.64 | 1.88 | 1.04 | 0.99 | 0.87 | 0.92 | 1.16 | 0.08 | 1.17 | 0.63 |
| | 8.8 | 1.48 | 1.62 | 0.84 | 0.95 | 0.73 | 0.83 | 0.77 | 0.06 | 0.96 | 0.51 |
| | 17.6 | 1.32 | 1.58 | 0.77 | 0.64 | 0.62 | 0.76 | 0.73 | 0.01 | 0.82 | 0.34 |
| | 35.2 | 1.26 | 1.34 | 0.75 | 0.58 | 0.56 | 0.69 | 0.25 | 0.01 | 0.79 | 0.24 |
| | 70.4 | 1.24 | 1.31 | 0.74 | 0.57 | 0.56 | 0.68 | 0.24 | 0.01 | 0.78 | 0.23 |
| | rc | -0.70 | -0.79 | -0.74 | -0.53 | -0.78 | -0.78 | -0.69 | -0.71 | -0.78 | -0.82 |
| $Al^{+3}$ | 0 | 2.17 | 1.57 | 1.17 | 2.78 | 1.04 | 1.12 | 2.75 | 0.05 | 1.19 | 0.84 |
| | 61 | 0.4 | 0.9 | 0.57 | 0.53 | 0.4 | 0.8 | 0.98 | 0.1 | 0.66 | 0.22 |
| | 122 | 0.37 | 0.88 | 0.54 | 0.44 | 0.4 | 0.78 | 0.93 | 0.09 | 0.56 | 0.21 |
| | 244 | 0.32 | 0.3 | 0.45 | 0.22 | 0.25 | 0.55 | 0.69 | 0.08 | 0.26 | 0.14 |
| | 488 | 0.05 | 0.19 | 0.11 | 0.2 | 0.13 | 0.22 | 0.15 | 0.02 | 0.13 | 0.06 |
| | 976 | 0.02 | 0.09 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 |
| | 1952 | 0.02 | 0.08 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 |
| | rc | -0.50 | -0.69 | -0.73 | -0.49 | -0.67 | -0.83 | -0.64 | -0.74 | -0.70 | -0.56 |

rc= correlation coefficient between the heavy metal concentration and optical density, Nd= not detected

## 3.4. Optimization of Carbon Sources

An experiment was carried out to investigate the effect of different carbon sources such as glucose, sucrose, fructose, dextran, mannitol, molasses and whey (as lactose) on growth of tested bacteria in presence of different metal ions have been illustrated by Figure 3. It was found that glucose (as a control) was the best carbon source for removal of heavy metal expressed as growth in presence of metal ions by the tested bacterial isolates of C6 being 1.83, 1.35 and 1.40 in presence of $Ni^{+2}$, $Zn^{+2}$ and $Cr^{+5}$ respectively, C12 being 1.22, 0.9 and 0.80 in presence of $Ni^{+2}$, $Zn^{+2}$ and $Cr^{+5}$ respectively and C4 being 1.35, 1.24 and 1.30 in presence of $Ni^{+2}$, $Zn^{+2}$ and $Cr^{+5}$ respectively. The secondbest carbon source was whey (as lactose) for all the tested isolates in the presence of $Ni^{+2}$, $Zn^{+2}$ and $Cr^{+5}$. The lowest values of growth (O.D) was observed for C6 ranged from 0.07 to 0.43. On mannitol, molasses and fructose in presence of $Ni^{+2}$, $Zn^{+2}$ and $Cr^{+5}$, respectively, while for C12 isolate ranged from to on molasses in presence of $Ni^{+2}$, $Zn^{+2}$ and $Cr^{+5}$ and for C4 isolate ranged from to on molasses in presence of $Ni^{+2}$ and $Zn^{+2}$ but dextran gives lowest growth in presence of $Cr^{+5}$.

These results are agreement with those of [28, 29] who observed that glucose was increased the heavy metal reduction rate by *S. cerevisiae*and*C. sorokiniana*. The role of glucose in biosorption depends on the type of strains and the status of metal ions (free or complex), even for the same biomass and for the same metal ions [30]. The biosorption of $Na^{+}$ and $Mg^{++}$ by *A. nidulans* in medium supplemented with 1% sucrose was more favorable than 1% dextrose as carbon source [31]. Whereas, the highest reduction of chromium by *S. rubidaea* was recorded in broth medium supplemented with sucrose at 0.1% concentration [32]. Furthermore, it could be observed that the best carbon source was glucose followed by whey as lactose for growth of heavy metals tolerance tested bacteria. Whey as by-product waste contains lactose was selected as carbon source for further studies for low cost and eco-friendly medium.

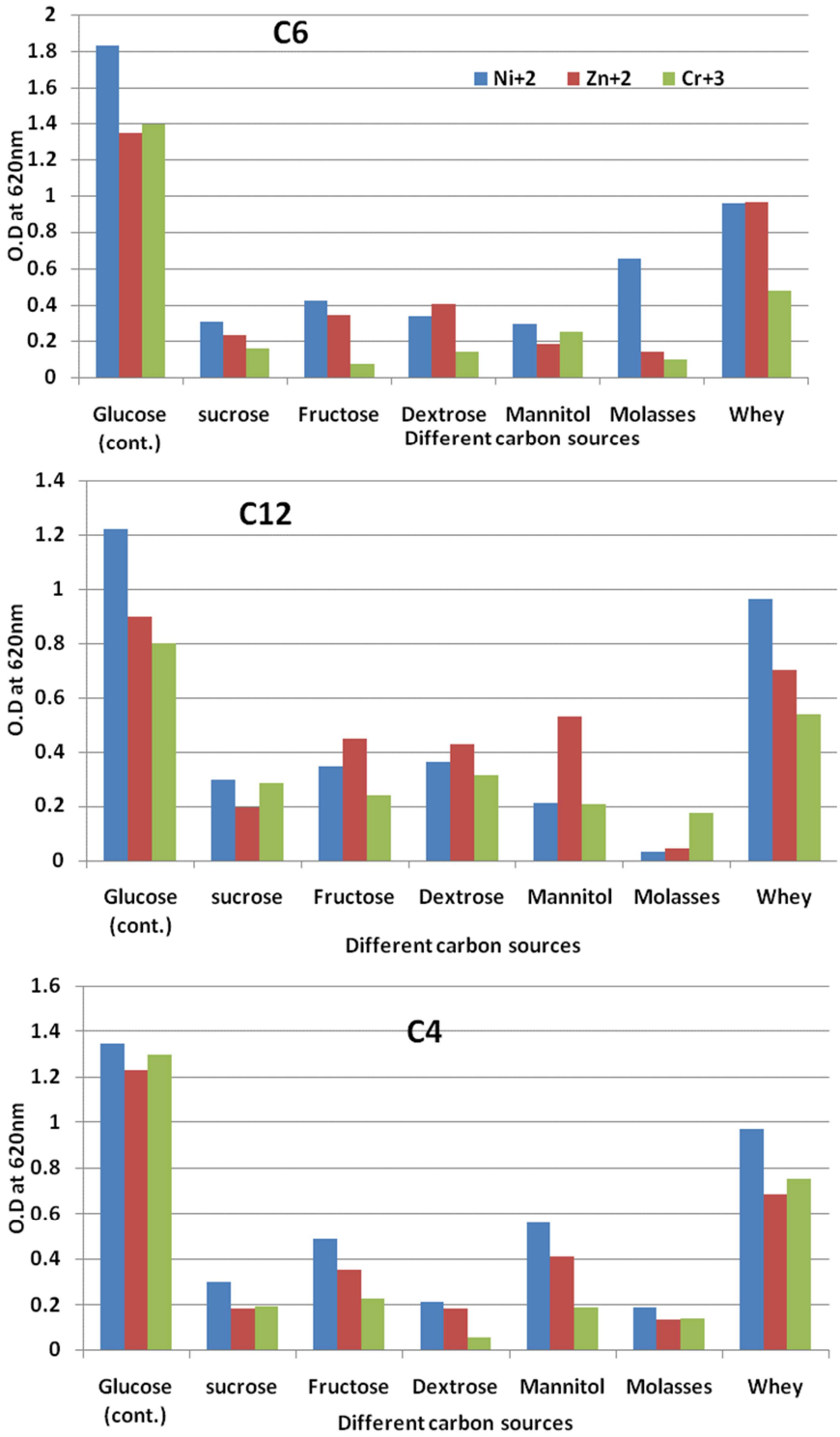


***Figure 3.*** *Biosorption of metal ions by the most tolerant bacterial isolates as influenced by different carbon sources at 30°C for 48h using shake flasks as a batch culture. cont.=control*

### 3.5. Optimal Nitrogen Source for the Selected Strains

To study the effect of nitrogen source on biosorption of heavy metals by tested bacterial isolates, it was cultivated on modified media containing different nitrogen sources with different heavy metal ions.

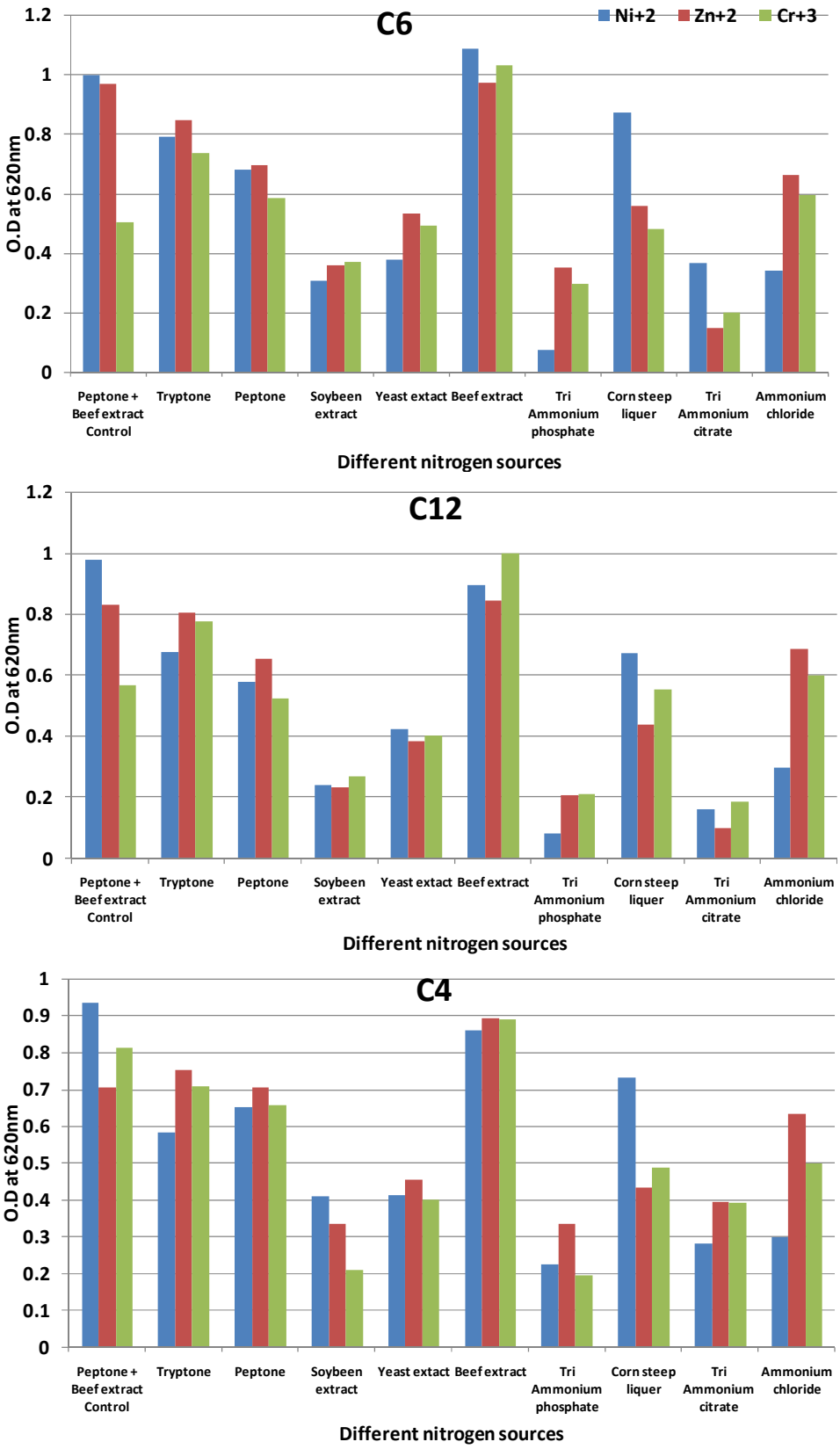


***Figure 4.*** *Biosorption of metal ions by bacterial isolatesas influenced by different nitrogen sources at 30°C for 48 h using shake flasks as a batch culture.*

Results illustrated by Figure 4 clearly show that the sources of nitrogen greatly affected the growth (removal of heavy metal) of bacterial isolates. The beef extract was the best nitrogen source for all selected bacterial isolates in the presence of used heavy metal ions except in case of isolates C4 and C12 with $Ni^{+2}$ the mixture of beef extract and peptone is the best source then beef extract. This resultes could be interrupted on the basis organic nitrogen such as beef extract and peptone not only as an organic nitrogen sources but also a source of growth factors and protein which play a vital role in enhancement of the microbial growth. And we can say that the organic nitrogen sources is the best from all applied nitrogen sources for nouriching the micobial growth because its easy to extracted by the bacteria and have other growth stimulator. The ability of *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp. and *A. niger* for biosorption different heavy metals of Co, Cd, Ni and Pb on medium containing mixture of peptone and beef extract as organic nitrogen sources [33]. From previous results, it could be stated that C6 isolate was more heavy metal tolerance isolate which gave a high growth on medium contained beef extract as nitrogen source in presence of $Ni^{+2}$ and $Cr^{+5}$ which increased about 1.39 & 1.35 folds in presence of $Ni^{+2}$ and 1.16 & 1.03 folds in presence of $Cr^{+5}$ as compared with C4 and C12, respectively. So, C6 isolate and beef extract were selected for further investigates.

## 4. Conclusions

Out of 123 bacterial isolates only 100 isolateswere tolerated different heavy metal ions. among these isolates only 10 were selected as a highlytoleranceisolatesfor different concentration of $Ni^{+2}$, $Zn^{+2}$, $Fe^{+3}$, $Cr^{+5}$ and $Al^{+3}$ at 30°C for 24h. Three tolerance heavy metal isolates (C4, C6 and C12) were selected as the most efficient isolates uptake of Ni, Zn and Cr. Glucose and whey (as by-product wastes) were the best carbon sources andbeef extract and the mixture ofbeef extract and peptone were the best nitrogen sources for removal the tested metal ions by the tested isolates. So, these isolates were selected for further studies for biosorption of the toxicity heavy metals.

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