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RESEARCH ARTICLE

Removal of Nickel (II) and Zinc (II) present in the Electroplating Industry Wastewater by Bioaccumulation Method

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ABSTRACT:

In the present work, the bioaccumulation study of electroplating industrial waste water using *Micrococcus cascolyticus* was carried out. The characteristics of the wastewater (pH, BOD, COD, TDS) were analyzed using standard method and it was found to be above permissible limit. The preliminary analysis for the bioaccumulation process was done by spread plate method and the concentration of the Nickel and Zinc was determined using standard method. After the bioaccumulation treatment, the aforesaid parameters were found to be below the permissible limit as prescribed by pollution control board. The removal percentage of Nickel and zinc present in the treated effluent was found to be 44.68 % and 48.76 % respectively. The maximum biomass for the Nickel and Zinc was found to be 5.8 g/l and 4.8 g/l respectively. For better bioaccumulation process, the parameters such as pH, Temperature, microbial volume, were optimized. The optimized temperature for the removal of Nickel and Zinc was found to be 50°C and 55°C respectively. Thus the *Micrococcus cascolyticus* has an ability to reduce the heavy metals concentration and other parameters (pH, BOD, COD, TDS) present in the waste water at the promising level.

KEYWORDS: Bioaccumulation, *Micrococcus cascolyticus,* electroplating industrial effluents, Spread plate assay.

1. INTRODUCTION:

Heavy metals are natural constituents of the environment, but indiscriminate use for human purposes has altered their geochemical cycles and biochemical balance (Marandi R 2011; Bais et al 2014). Rapid industrial development has led to the recognition and increased understanding of inter relationship between pollution, public health and environment. Industrial development results in the generation of industrial effluents, and if untreated, results in water sediments and soil pollution (Dixit et al 2015; Fakayode, 2005).

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Industrial wastes contain toxic and hazardous substances, most of which are detrimental to human health (Jimena *et al*., 2008; Ogunfowokan *et al*., 2005; Rajaram *et al*., 2008). Metals are non-biodegradable and are considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals (Patil et al 2011; Anil Kumar et al 2011).

The Natural components of the Earth's crust are heavy metals. They cannot be degraded or destroyed. To a small quantities they enter our bodies via food, drinking water and air. As trace elements, some heavy metals (e.g. Copper, selenium, zinc, etc.,) are essential to maintain the metabolism of the human body. However, at higher concentrations they can leads to harmful. Heavy metal poisoning could result from drinking water contamination (e.g. lead pipe), high ambient air concentrations near emission sources (Shi et al 2018.,

Miranda D and Rojas 2006). Heavy metals are hazardous because they tend to bioaccumulate. Bioaccumulation is the accumulation of heavy metals into the body of an organism and causes deleterious effects to the organism. Heavy metals which accumulate in living things that they are taken up and stored faster than they are broken down (metabolized) or excreted (Sethuraman P and Balasubramanian 2010, Sarabjeet et al 2007). Heavy metals can enter a water supply by industrial and consumer waste, or even from acid rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater. Heavy metals such as Lead, Copper, Chromium, Selenium, Mercury etc., are considered as pollutants and leads to very dangerous to human health and the environment (Ashfaq et al 2009., Acıkel and Tugba 2009).

Bioaccumulation is the accumulation of substances, such as pesticides, or organic chemicals in an organism. Bioaccumulation occurs when an organism absorbs a harmful substance at a rate greater than that at which the substance is lost. Thus, the longer the biological half-life of the substance the greater the risk of chronic poisoning, even if environmental levels of the toxin are not very high. Biotransformation can strongly modify bioaccumulation of chemicals in an organism (Ridvan Kızılkaya 2005). The excessive intake of copper by man leads to several mucosal irritations, wide spread capillary damage, hepatic and renal damage central nervous problem (Sharma 2011., Hassan et al 2015a).

2. MATERIALS AND METHODS:

2.1 MICROBIAL STRAIN:

Micrococcus cascolyticus, (strain no: 8555) ordered from MTCC (Chandigarh), Stock cultures were maintained on Nutrient agar medium.

2.2 PREPARATION OF SYNTHETIC SOLUTIONS (Ni and Zn)

2.2.1 Nickel Standard solution:

Nickel, standard solution was prepared by dissolving 0.4478 g $NiSO_4*6H_2O$ in distilled water with addition of 2 ml concentrated sulfuric acid. 1 ml of prepared solution consists 0.10 mg Ni. The solution was stable for a year. Nickel, working standard solution was prepared by dilution of 10 ml of basic standard solution in a volumetric flask (100 ml) and stirred. Dimethylglyoxime solution was done by dissolving dimethylglyoxime in ammonia and after adding water the solution was filtered. The solution had to be kept in dark bottle and it was stable for two weeks. Ammonium citrate solution was made from citric acid monohydrate which was dissolved in ammonia solution.

In order to prepare calibration curve the following volumes of nickel working standard solution 0.0, 1.0, 2.5, 5.0, 10.0, 25.0 and 50 ml (responding to 0.00, 0.01, 0.025, 0.05, 0.10, 0.25 and 0.50 mg Ni in standard solution) were placed in seven clean volumetric flasks. Afterwards, 50.0, 49.0, 47.5, 45.0, 40.0, 25.0, and 0.0 ml of distilled water was added, respectively. 10 ml citrate ammonia, 5 ml iodine solution and 20 ml dimethylglyoxime solution were added to each flask. The samples were mixed thoroughly. The prepared standards were ready to be used after 10 min but not later than after 30 min. Samples were transferred to the cuvette (absorption cell) and measured absorption at 530 nm.

2.2.2 Zinc Standard Solution:

Weigh exactly 4.40 g of zinc sulfate, and dissolve in water to make exactly 1,000 ml. Measure exactly 10 ml of the solution, and add water to make exactly 1,000 ml. One ml of this solution contains 0.01 mg of zinc (Zn).

2.3 CHARACTERIZATION OF WASTE WATER:

2.3.1 ELECTROPLATING INDUSTRIAL WASTE:

The waste was collected (Coimbatore) and characterized using standard procedure is used for further analysis.

2.3.2 CHEMICAL OXGEN DEMAND (COD):

Chemical Oxygen Demand gives us an idea about the amount of organic matter that is present in the effluent. This test allows the measurement of organic matter in terms of oxygen required for oxidation to CO_2 and water. It is based on the fact that all Organic matter can be oxidized by the action of powerful oxidizing agents under acidic conditions.

COD of the given sample can be calculated by the following formula (eqn. 1)

COD (mg/L) =

$$\frac{V.s - V.b * 0.1 * 8 * df * 1000}{V.S} \quad (1)$$

Where V.s = Volume of FAS consumed by the sample(ml).

V.b = Volume of FAS consumed by the blank. (ml).

df = Dilution factor (Volume of sample taken/ volume of total amount of sample to be added)

V.S = Total volume of sample to be added in the flask (ml).

Normality of FAS=0.1

Equivalent weight of Oxygen=8

2.3.3 TOTAL SUSPENDED SOLIDS:

Total suspended solids consist of both organic and inorganic solids. It is a measure of dry weight of suspended solids per unit volume. The procedure to determine the total suspended solids in the sample is as

follows (Rajender Kumar 2008).

A glass fiber filter was kept in an aluminum foil dish and dried in an oven, then kept in a desiccator and the paper, along with the dish was weighed. Then the sample was filtered and then the wet filter paper was kept in the dish and dried and then weighed again. The Total Suspended Solids can be calculated by the following formula (eqn. 2).

$$\frac{B-A}{V.s} * 1000 \quad (2)$$

Where B= weight of the filter paper and dish along with the dried sample (g).
A=weight of the filter paper and dish before filtration (g).

V.s=Volume of the sample (ml).

2.3.4 NICKEL:
Take 20 ml of waste water, add 80 ml of distilled water 10 ml citrate ammonia, 5 ml iodine solution and 20 ml dimethylglyoxime solution were added to each flask. The samples were mixed thoroughly. The prepared standards were ready to be used after 10 min but not later than after 30 min. Samples were transferred to the cuvette (absorption cell) and measured absorption at 530 nm.

2.4 SPREAD PLATE METHOD:
PRINCIPLE:
To inoculate a Petri dish, it provides the simple and the rapid method of diluting the sample by mechanical method as the loop is spread over agar by specialized glass rod, more and more bacteria are rubble off until individual bacteria is deposited on the agar. After inoculation the area at the bingeing of the spread pattern should show discreet colonies.

PROCEDURE:
- Label the sterile agar plate with the source of the culture
- Sterilize the loop using appropriate aseptic technique; remove the loop full of broth from the mixed culture tube
- Touch the loop full in the culture of the labeled Petri dish
- Spread the culture using the sterile specialized glass rod called Lbar prevents over crowding of the microbes (the Lbar is sterilized by dipping it in alcohol followed by 24to 48 hours
- Observe for growth and record your result

2.5: Determination of Removal Efficiency of Ni and Zn in Synthetic Medium:
2.5.1 Nickel:
Erlenmeyer flasks (250 ml) containing nutrient medium (100 ml) along with varying concentration of nickel such as 200 to 1000 ppm. To this add 2ml of culture and kept in incubator for 3 days under static condition. After 3rd day of incubation, the sample was collected and centrifuged at 5000 rpm for 15 min. Save the supernatant and pellets separately. The saved pellets were taken in Aluminum foil and keep it for drying. The initial and final weight of the aluminum foil was noted. Biomass present in the sample were calculated from the difference of aluminum foil.

2.5.2 Zinc:
Erlenmeyer flasks (250 ml) containing nutrient medium (100 ml) along with varying concentration of zinc such as 200 to 1000 ppm. To this add 2 ml of culture and kept incubator for 3 days under static condition. After 3rd day of incubation, the sample was collected and centrifuged at 5000 rpm for 15 min. Save the supernatant and pellets separately. The saved pellets were taken in Aluminum foil and keep it for drying. The initial and final weight of the aluminum foil was noted. Biomass present in the sample were calculated from the difference of aluminum foil (Pugazholi et al 2013).

2.6 Removal of Ni and Zi present in the electroplating waste water:
2.6.1 Nickel:
Erlenmeyer flasks (250 ml) containing nutrient medium (100 ml) along with varying concentration of nickel such as 200 to 1000 ppm. To this add 2 ml of culture and kept incubator for 3 days under static condition. After 3rd day of incubation, the sample was collected and centrifuged at 5000 rpm for 15 min. Save the supernatant and pellets separately. The collected supernatant were used for the estimation of heavy metals removal present in the waste. The concentration of nickel was determined by measuring the absorbance at 530 nm using UV-spectrophotometer.

2.6.2 Zinc:
Erlenmeyer flasks (250 ml) containing nutrient medium (100 ml) along with varying concentration of zinc such as 200 to 1000 ppm. To this add 2 ml of culture and kept incubator for 3 days under static condition. After 3rd day of incubation, the sample was collected and centrifuged at 5000 rpm for 15 min. Save the supernatant and pellets separately. The collected supernatant were used for the estimation of heavy metals removal present in the waste. The concentration of zinc was determined by measuring the absorbance at 220 nm using UV-spectrophotometer.

2.7 Effect of Temperature on Removal Efficiencies of heavy metals (Ni and Zn):

2.7.1 Nickel:

The effect of temperature on the nickel removal efficiency was studied by varying the temperature from 30 to 60°C. The sample was maintained at various temperature (30, 35, 40, 45, 50, 55, 60°C) incubator for 3 days. After the incubation period the samples were collected and centrifuged at 5000 rpm for 15 min. The supernatant used for the estimation of nickel concentration present in the treated waste water.

2.7.2 Zinc:

The effect of temperature on the zinc removal efficiency was studied by varying the temperature from 30 to 60°C. The sample was maintained at various temperature (30, 35, 40, 45, 50, 55, 60°C) incubator for 3 days. After the incubation period the samples were collected and centrifuged at 5000 rpm for 15 min. The supernatant used for the estimation of nickel concentration present in the treated waste water (Hassan et al 2015).

2.8 Analysis Biomass concentration in the degradation medium:

To study the biomass concentration of the *Micrococcus cascolyticus* on the degradation medium, the following procedure was adopted. The aluminium foil was made into a boat shape and its empty weight was measured. 1 ml of the culture was added to one of the flasks containing the medium. Immediately after this addition, 1 ml of the medium containing the newly added culture was taken out and was poured in the aluminium foil. The foil was then kept in a hot air oven at 100°C for 15 minutes. After the contents in the foil had dried, the foil was again weighed. The difference in weights gives the amount of biomass collected. This was found to be 1st day reading. 1 ml of the sample was taken out and put in an aluminium foil and then dried, then the difference in weight was noted continuously for the 5 days incubation (Rajeshkumar R and Kartic 2011).

2.9 Bioaccumulation studies:

During the incubation period, 1ml sample was taken from each flask at regular time intervals. The aliquots were centrifuged at 10,000rpm for 5min, and the supernatant fractions were collected to measure heavy metal. The residual heavy metal concentration (Ni and Zn) levels were measured by UV–vis spectroscopy at 530nm 220 nm respectively. The cells were washed after centrifugation and dried at 40 ◦C to measure the dry cell mass concentration. The bioaccumulation % of heavy metals was calculated using the following equation (3):

$$\text{Bioaccumulation\%} = \frac{Co - Cf}{Cf} * 100 \quad (3)$$

Where

C_0 is the initial concentration of metal (mg/L) and

C_f is the final concentration of metal (mg/L).

2.10 Effect of microbial volume for Ni (II):

In order to determine the optimum volume, 4 conical flasks of 150 ml capacity containing

100 ml of media were prepared with inoculums volume of 1, 2, 3, 4 ml/100 ml was used to inoculate in different flasks in a incubator shaker at 150 rpm. Samples were drawn after 12 hrs of incubation period and biomass and final concentration of nickel is noted (Preetha B and Viruthagiri 2007).

2.11 Effect of microbial volume for Zn (II):

In order to determine the optimum volume, 4 conical flasks of 150 ml capacity containing

100 ml of media were prepared with inoculums volume of 1, 2, 3, 4 ml/100 ml was used to inoculate in different flasks in a incubator shaker at 150 rpm. Samples were drawn after 12 hrs of incubation period and biomass and final concentration of zinc is noted.

2.12 Effect of pH for Ni (II):

Certain organisms have ionic groups on their active sites, and these ionic groups must be in a suitable form (acid or base) to function. Variations in the pH medium result in changes in the ionic form of the active site and changes in the activity of the organisms and hence the reaction rate. Bacterial isolates were inoculated into a series of 250 ml conical flask containing 100 mg/l of nickel. Initially the pH of the nickel in the medium was varied from 2 to 7. The medium pH was adjusted using dilute acids or alkalis. The cultures were shaken in a rotary shaker (150 rpm) in a temperature controlled water bath. After reaching the equilibrium incubation time, the concentration of the nickel was measured. Based upon the heavy metal removal, the optimal pH was determined (Poppuri and Guttikonda 2015).

2.13 Effect of pH for Zn (II):

Certain organisms have ionic groups on their active sites, and these ionic groups must be in a suitable form (acid or base) to function. Variations in the pH medium result in changes in the ionic form of the active site and changes in the activity of the organisms and hence the reaction rate. Bacterial isolates were inoculated into a series of 250 ml conical flask containing100 mg/l of zinc. Initially the pH of the nickel in the medium was varied from 2 to 7. The pH of the medium was adjusted using dilute HCl or NaOH. The cultures were shaken in a rotary shaker (150 rpm) in a temperature controlled water bath. After reaching the equilibrium incubation time, the concentration of zinc was measured. Based upon the heavy metal removal, the optimal pH was determined (Damini et al 2013).

3. RESULTS AND DISCUSSION:

3.1 CHARACTERISTICS OF ELECTROPLATING INDUSTRIAL WASTE WATER:

Table 1: Characterization of Electroplating Industrial Waste Water

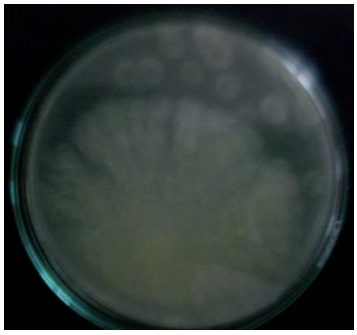
S. No	Parameters	Concentration (mg/l)
1	pH	2.7
2	TDS	28,534
3	COD	763
4	Zinc	242
5	Nickel	47
6	Chromium	32

From the Table 1, we inferred that the color of the waste was green in color and the pH was found to be 2.7 this is very acidic in nature. The optimum growth for *Micrococcus cascolyticus* was in the range of 2-4. Hence removal of heavy metal easier when compared to treatment using other organism. The concentration of Nickel and Zinc was found to be 47 mg/l and 242mg/l respectively. The concentration of Zinc was found to be very high when compared to other metal present in the waste. So the removal of these metal *Micrococcus cascolyticus* has the potential for the degradation of heavy metal. So removal of heavy metal can be done by bioaccumulation process.

3.2 SPREAD PLATE METHOD:

From the above Fig.1 and 2, we can see some white spots in the petridish for both Nickel and Zinc. These spots are bacterial colonies. The colonies confirm that of *Micrococcus cascolyticus* has ability to degrade both nickel and zinc.

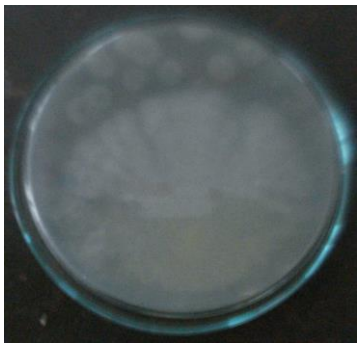
Control



Sample

Fig. 1: Spread plate assay for nickel

Control



sample

Fig. 2: Spread plate assay for zinc

3.3 Bioaccumulation studies of Ni and Zn in synthetic medium:

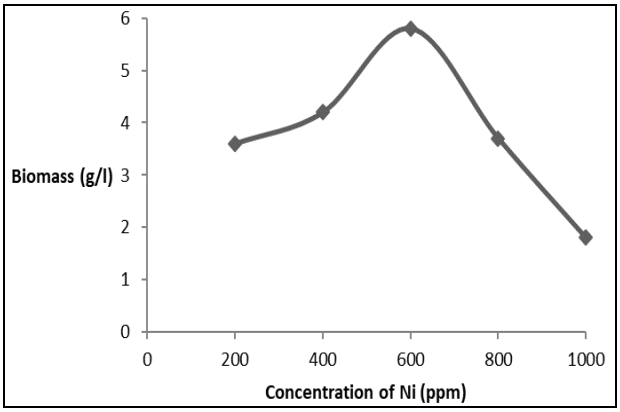


Fig. 3: Bioaccumulation of Ni using *Micrococcus cascolyticus*

From the Fig. 3 we can see that as the amount of biomass increases, the concentration of nickel increases till the point where biomass concentration is 5.8 g/l. On further increasing the biomass amount, the concentration of Nickel decreases since the organism can't able to grow beyond the concentration of 600 ppm

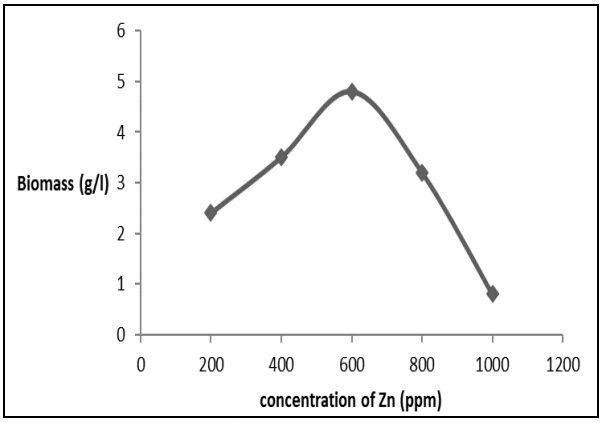


Fig. 4: Bioaccumulation of Zn using *Micrococcus cascolyticus*

From the Fig. 4, we can see that as the amount of biomass increases, the concentration of zinc increases till the point where biomass concentration is 4.8 g/L. On further increasing the biomass amount, the concentration of Nickel decreases since the organism can't able to grow beyond the concentration of 600 ppm.

3.4 Removal of Ni and Zn present in the electroplating waste water:

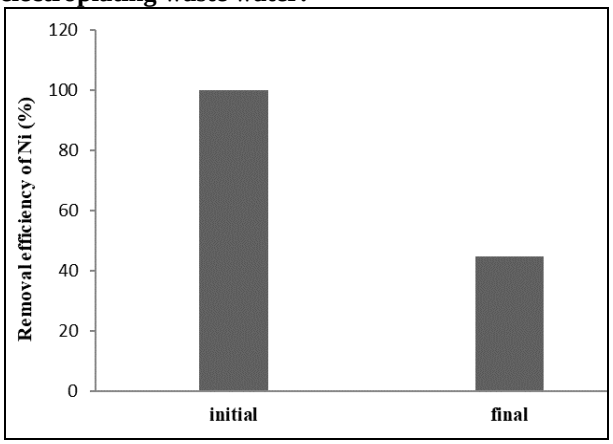


Fig. 5: Removal efficiency of Ni in treated waste water

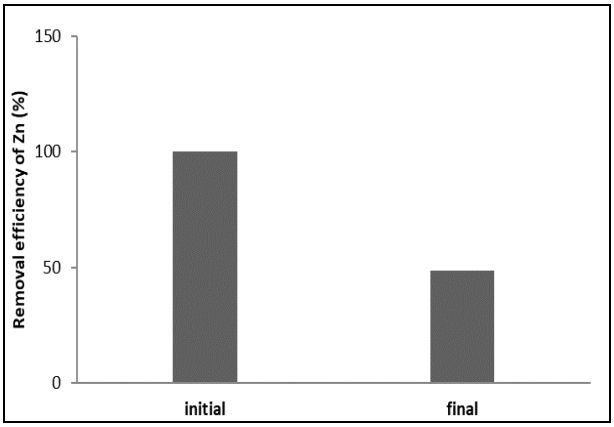


Fig. 6: Removal efficiency of Zn in treated waste water

From the Fig. 5 and 6, we inferred that *Micrococcus cascolyticus* can degrade Nickel from the concentration 47 mg to 21 mg and can degrade the Zinc from the concentration 242 mg to118 mg present in the effluent to a great extent effectively. Micrococcus has an ability to degrade the heavy metals present in the wastewater.

3.5 Effect of Temperature on Removal concentration of heavy metals:

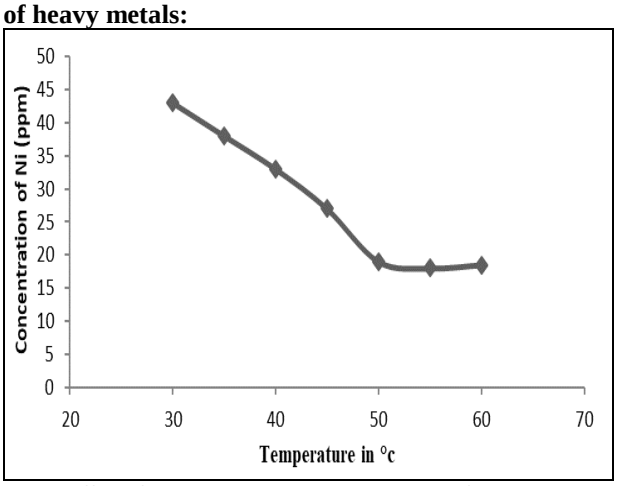


Fig. 7: Effect of temperature on bioaccumulation of nickel

From the Fig .7 it can be inferred that as the temperature increases, the concentration of Nickel in the effluent decreases till temperature reaches 50° C. Beyond this point, the Nickel concentration remains constant thereby reducing the removal efficiency of *Micrococcus cascolyticus* beyond 50°C.

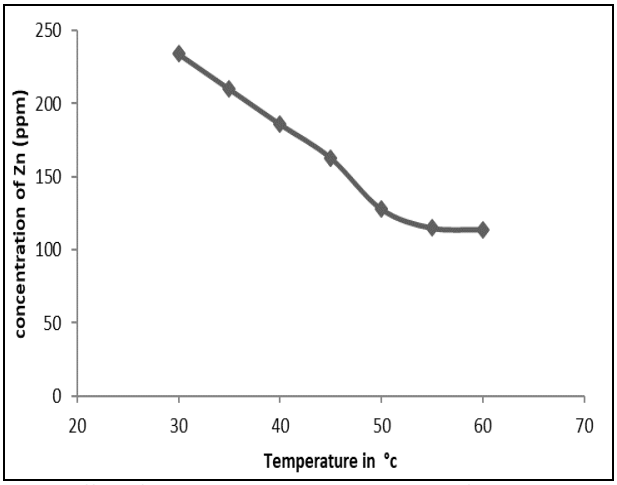


Fig. 8: Effect of temperature on bioaccumulation of zinc

From the Fig .8 it can be inferred that as the temperature increases, the concentration of Zinc in the effluent decreases till temperature reaches 55°C. Beyond this point, the Zinc concentration remains constant thereby reducing the removal efficiency of *Micrococcus cascolyticus* beyond 55°C.

3.6 Analysis Biomass concentration in industrial waste water

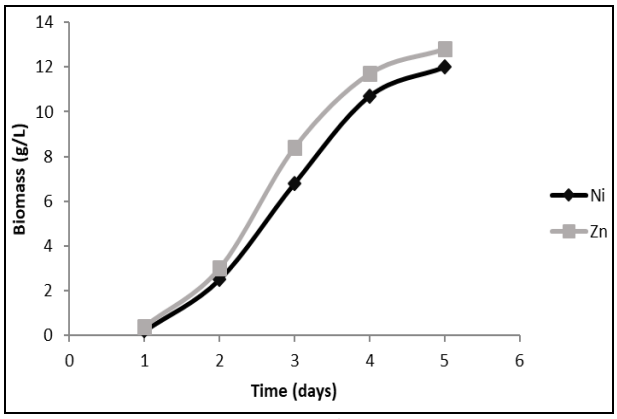


Fig. 9: Biomass concentration of *micrococcus cacolyticus* in wastewater

Figure 9. shows that the biomass concentration of *micrococcus cacolyticus* obtained by the cultivation from 1 to 5 days. There was an increase in the biomass concentration at approximately the 2nd day and increased till 4th day after which it attains saturation from 5th day. This implies that maximum growth was observed on the 4th day after which there was very little growth. A optimum biomass of 12 g/l and 12.2 g/l was obtained on 4th day for the both nickel and zinc respectively. There was no growth in the control flask indicating that the biomass concentration was only due to the bacterial growth. Increase in biomass concentration which leads to accumulation of heavy metals present in the waste water by micro-organisms.

3.7 Bioaccumulation Studies:

i. Nickel:

In a single system, the bioaccumulation of Ni (II) by *Micrococcus cascolyticus*. tropicalis was investigated at the empirically optimal pH of 5.0 in a electroplating industrial waste. The maximum percentage of bioaccumulation was found to be 67 (Fig. 10). The bioaccumulated metal exhibited a decreasing trend, indicating that media toxicity increased with increasing metal concentration. In the 4th day of incubation, the bioaccumulation (%) was found to be 67%, which was reduced to 52 %. The results suggest that bioaccumulation of Ni (II) exhibit similar trends with bioaccumulation of Cd (II) exhibited a higher bioaccumulation at all concentrations. Thus the data demonstrate that in a single system, the Ni (II) exhibit similar toxicity levels Ayten Ozturk (2007), whereas Cd (II) (Sahin Y, and Ozturk 2005) exhibited lower toxicity compared to other two pollutants.

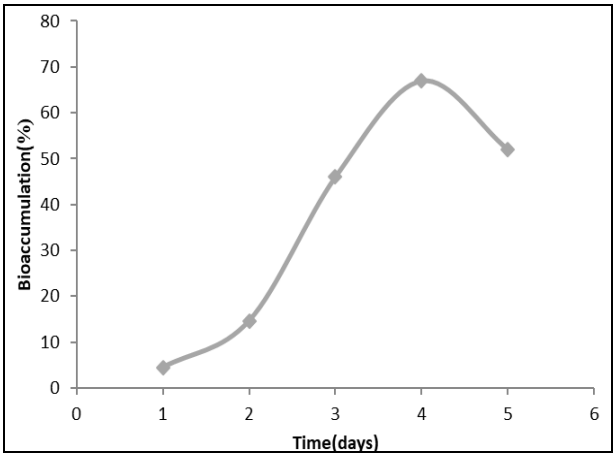


Fig. 10: Bioaccumulation of Nickel by *Micrococcus cascolyticus*

ii. Zinc:

In a single system, the bioaccumulation of Ni (II) by *Micrococcus cascolyticus*. tropicalis was investigated at the empirically optimal pH of 5.0 in a electroplating industrial waste. The maximum percentage of bioaccumulation was found to be 68. The bioaccumulated metal exhibited a decreasing trend, indicating that media toxicity increased with increasing metal concentration. In the 4th day of incubation, the bioaccumulation (%) was found to be 68%, which was reduced to 52 % (Fig. 11). The results suggest that bioaccumulation of Ni (II) exhibit similar trends with bioaccumulation of Cd (II) exhibited a higher bioaccumulation at all concentrations. Thus the data demonstrate that in a single system, the Ni (II) exhibit similar toxicity levels, whereas Cd (II) exhibited lower toxicity compared to other two pollutants (Ouadjenia-Marouf 2013).

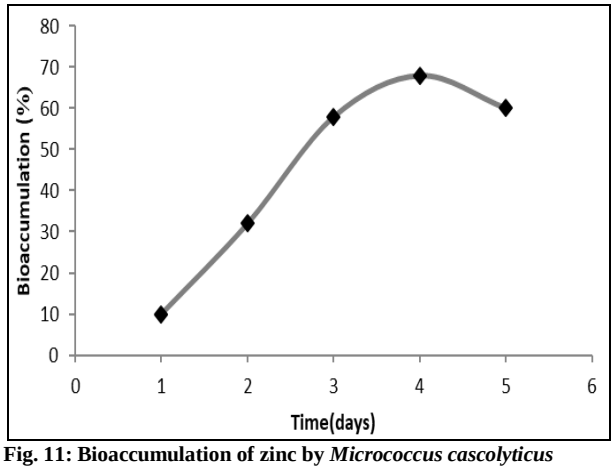


Fig. 11: Bioaccumulation of zinc by *Micrococcus cascolyticus*

3.8 Effect of Microbial volume:

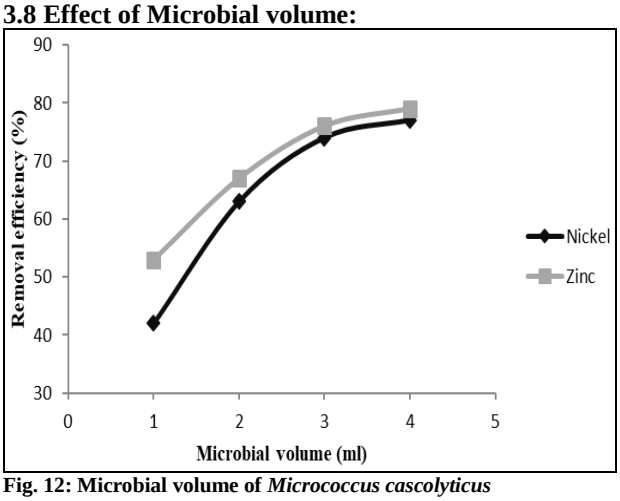


Fig. 12: Microbial volume of *Micrococcus cascolyticus*

From Fig. 12, the optimum microbial volume for the bioaccumulation process was found to be 3 ml for nickel the metal and 3ml was found to be maximum for zinc. since increase in microbial volume, it increases the bioaccumulation of heavy metal (Ni and Zn). At a particular volume it attains maximum absorption of heavy metal by the micro-organisam. Further increase in the microbial volume, there is no significant increase in the removal efficiency of heavy metal (Nanda et al 2011).

3.9 Effect of pH on heavy metal removal:

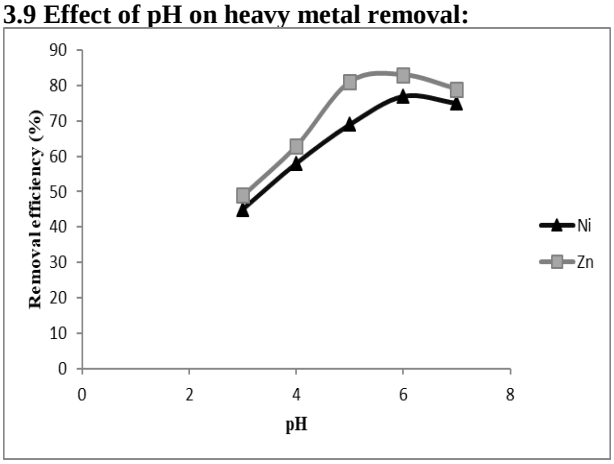


Fig. 13: Effect of pH on heavy metal removal

The effect of pH on the bioaccumulation of heavy metals, viz., Ni(II) and Zn(II), by *Micrococcus cascolyticus* was investigated by varying the pH of the growth medium from 2.0 to 7.0. The bioaccumulation of metal cations, viz., Ni(II) and Zn(II), by bacteria increased with increasing pH values up to 6.0 (Fig.13). Singh et al (2014) also reported maximum bioaccumulation levels for Copper, Lead, Chromium by *Aspergillus niger* at pH 4.0. Similar results were reported by Valecha (2015). In the binary system, the percentages of bioaccumulation of the nickel and zinc was found to be maximum at pH 5.0 and pH 6.0 respectively. Increasing the pH values increased the negative charges on the bacterial cell surface, thereby favouring the bioaccumulation of the metal cations, viz., Ni(II) and Zn(II) in the binary systems.

CONCLUSION:

From the above results, we concluded that the concentration of contaminants like Nickel and zinc present in the waste was determined using standard metal solution and it was found to be 47 mg/l and 242 mg/l respectively. The *Micrococcus cascolyticus* has an ability to degrade the Nickel and Zinc present in the Electroplating Industry waste water. The maximum biomass obtained for the Nickel and Zinc was found to be 5.8 g/l and 4.8 g/l respectively. The concentration of the Nickel and Zinc in the treated waste water was determined as 21 mg/l and 118 mg/l respectively. For the bioaccumulation process the optimized temperature for the removal of Nickel and Zinc found to be 50°C and 55°C respectively. the maximum biomass concentration for the Nickel and Zinc found to be 12 g/l and 12.2g/l respectively. The maximum bioaccumulation of Nickel 67% was found on 4th day of incubation. Similarly the maximum bioaccumulation of Zinc 68% were found on the 5th day of incubation. The optimum microbial volume for the bioaccumulation process for both Ni and Zn found to be 3ml and 3ml respectively. The effect of pH on bioaccumulation for the both Ni and Zn was found to be 6 and 5. Thus, *Micrococcus cascolyticus* is an efficient microorganism to degrade the heavy metals in the wastewater by bioaccumulation method.

REFERENCES:

1. Shi W., Fang X., Wu X., Zhang G., Que W. and Li F. Alteration of bioaccumulation mechanisms of Cu by microalgae in the presence of natural fulvic acids, Chemosphere.211;2018:717-725.
2. Popuri A K, Guttikonda P. Use of Agricultural Waste (Fly Ash) for Removal of Nickel Ions from Aqueous Solutions, Research J. Pharmacy and Technology. 8(12);2015:1665-1668.
3. Singh K, Bhartiya DK, Chauhan HK , Rai R , Singh RN. Bioaccumulation of Cobalt and Lead by Earthworm Eisenia fetida from Sewage sludge with different cattle dung during Vermicomposting, Research Journal of Science and Technology, 6(4); 2014:175-179.
4. Sharma D. Removal of Heavy Metals from Water/ Wastewater Using Agricultural and Industrial By-Products as Adsorbents, Asian J. Research Chemistry.4(9);2011:1432-1439.
5. Hassan TA, Abdalamir HT, Salah SH.A Novel Adsorbent Based on Lignin and Tannin for the Removal of Heavy Metals from Wastewater ,Research Journal of Pharmacognosy and Phytochemistry.7(1); 2015a: 38-48 .
6. Susheel KS, Yogender S and Reetu S. Potentiometric Determination of Heavy Metals Concentrations in Trace by Using Calixarene as Electroactive Materials, Asian J. Research Chemistry. 3(1);2010:229-233.
7. Ogunfowokan AO, Okoh EK, Adenuga AA, Asubiojo OI. An assessment of the impact of point source pollution from a university sewage treatment oxidation pond on a receiving stream – a preliminary study. Journal of Applied Sciences. 5(1);2005:36 – 43.

8. Dixit R, Wasiullah, Malaviya D, Pandiyan K, Singh UB, Sahu A, Shukla R, Singh BP, Rai JP, Sharma PK, Lade H. and Paul D. Bioremediation of Heavy Metals from Soil and Aquatic Environment: An Overview of Principles and Criteria of Fundamental Processes. Sustainability. 7(2);2015:2189-2212.
9. Ridvan Kızılkaya. The role of different organic wastes on zinc bioaccumulation by earthworm *Lumbricus terrestris* L. (Oligochaeta) in successive Zn added soil. Ecological Engineering. 25;2005:322–331.
10. Patil PM, Durugkar NJ, Kakolkar PP, Chaudhari PD. Bioaccumulation of Cadmium Chloride in the Fresh Water Fish Cattle Cattle, Research Journal of Pharmacy and Technology, 4(1); 2011:121-123.
11. Anil Kumar M, Keerthana S, Adyasa Pani, Suresh D and Seenuvasan M. Dynamic and Stability Criteria for a Continuous Bioaccumulation of Reactive Red C2G 29 Dye using *Penicillium chrysogenum* MTCC 6477.Research Journal of Engineering and Technology.2(3);2011:167-171.
12. Valecha SM, Synthesis and characterization of manganese(II), cobalt(II), nickel(II),copper(II), and zinc(II) mixed ligand complexes with [(1-phenyl-3- methyl- 5- hydroxopyrazol-4-yl) methylimino] 2',3' dimethylaniline and 2- hydroxy- 1-naphthaldehyde. Asian J. Research Chem. 8(11); 2015:693-696.
13. Damini D, Sukriti P, Subathra Devi C, Selvarajan E, Suganthi V and Mohanasrinivasan V, Removal of Heavy Metals from Leather Industry Effluent Using *Saccharomyces sp* In a Packed Bed Reactor. Research J Engineering and Technology. 4(2); 2013:53-56.
14. Pugazholi P, Babypriya A, Esai Kanaga Yadav KR. Phytoremediation: Removal of Heavy Metals from Soil using Helianthus Annuus. Research Journal of Engineering and Technology.4(4);2013:242-245.
15. Hassan TA, Abdalamir HT, Salah SH. Removal of Heavy metals from Wastewater by Novel Adsorbent based on Chitosan and Lignin. Research J. Science and Tech. 7(1);2015, 35-46.
16. Bais SK, Chandewar AV, Wargantiwar DW, Charjan SM. Comparative Evaluation of Heavy Metals in Marketed Haematinic Herbal Formulations by Atomic Absorption Spectroscopy. Asian J. Pharma Analysis.4(1) ;2014:11-16.
17. Sahin Y, and Ozturk A. Biosorption of chromium(VI) ions from aqueous solution by the bacterium *Bacillus thuringiensis*. Process Biochemistry. 40;2005:1895–1901.
18. Miranda D and Rojas R. Copper accumulation by bacteria and transfer to scallop larvae. Marine Pollution Bulletin. 52;2006:293–300.
19. Preetha B and Viruthagiri T. Bioaccumulation of chromium(VI), copper(II) and nickel(II)ions by growing *Rhizopus arrhizus.* Biochemical Engineering Journal. 34;2007:131–135.
20. Sarabjeet SA and Dinesh Goyal. Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresource Technology. 98;2007:2245-2257.
21. Ayten Ozturk. Removal of nickel from aqueous solution by the bacterium *Bacillus thuringiensis.* Journal of Hazardous Materials. 147;2007:518–523.
22. Rajender Kumar, Bishnoi R, Garima, Kiran Bishnoi. Biosorption of chromium(VI) from aqueous solution and electroplating wastewater using fungal biomass. Chemical Engineering Journal. 135;2008:202-208.
23. Jimena MG, Roxana O, Catiana Z, Margarita H, Susana M.and InesIsla M. Industrial effluents and surface waters genotoxicity and mutagenicity evaluation of a river of Tucuman, Argentina. Journal of hazardous Material, 155(3);2008:403– 406.
24. Rajaram T, and Ashutost D. water pollution by industrial effluents in India: discharge scenario and case for participatory ecosystem specific local regulation. *Envr. J.*, 40(1);2008:56– 69.
25. Ashfaq M., Sajjad Ali., Asif Hanif M. Bioaccumulation of cobalt in silkworm (Bombyx mori L.) in relation to mulberry, soil and wastewater metal concentrations. Process Biochemistry. 44;2009:1179-1184.
26. Acıkel U and Tugba Alp. A study on the inhibition kinetics of bioaccumulation of Cu(II) and Ni(II) ions using *Rhizopus delemar*. Journal of Hazardous Materials. 168;2009:1449–1458.
27. Fatima Ouadjenia-Marouf, Reda Marouf, Jacques Schott, Ahmed Yahiaoui. Removal of Cu(II), Cd(II) and Cr(III) ions from aqueous solution by dam silt. Arabian Journal of Chemistry. 6;2013:401-406
28. Sethuraman P and Balasubramanian N. Removal of Cr(VI) from aqueous solution using *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Enterobacter cloacae*. International Journal of Engineering Science and Technology. 2;2010:1811- 1825.
29. Marandi R.. Bioextraction of Cu (II) Ions from Acid Mine Drainage by *Bacillus Thuringiensis.* International Journal of Biological Engineering. 1;2011:11-17.
30. Rajeshkumar R and Kartic N. Removal of Cu2+ Ions from Aqueous Solutions Using Copper Resistant Bacteria. Our Nature. 9;2011:49-54.
31. Nanda M, Sharma D, Kumar A. Removal of Heavy Metals from Industrial Effluent using Bacteria. **I**nternational journal of environmental sciences. 2(2);2011:781-787.