

*SUMMARY AND
CONCLUSION*

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The present study entitled “Bioremediation of nickel electroplating effluent and its impact on green gram and cat fish” was conducted in five phases and the findings are summarized below:

PHASE I

The first phase of the study included the isolation and characterisation of metal tolerant bacteria and fungi from the soil contaminated with nickel electroplating effluent.

The metal tolerant bacterial isolates identified were *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Micrococcus luteus*. Based on the morphological characteristics, the fungal isolates identified were *Aspergillus niger*, *Fusarium solani* and *Cladosporium* species.

PHASE II

The second phase was performed in order to assess the efficiency of the selected live and dead bacterial and fungal isolates in the removal of nickel using different concentrations (25%, 50%, 75% and 100%) of effluent. The biosorption studies were further carried out with dead cells of bacterial and fungal isolates at various experimental conditions namely pH, temperature and incubation period in 25% concentration of nickel electroplating effluent.

The above study conducted on the removal of nickel by live and dead cells revealed that dead cells of both bacterial and fungal isolates were able to remove 90 and 96 per cent of nickel respectively in 25% concentration of effluent when compared to their live biomass with 82 per cent in bacteria and 85 per cent in fungi.

The nickel uptake by dead cells of the bacterial and fungal isolates was also influenced by pH, temperature and incubation period in 25% concentration of effluent. Maximum removal of nickel was found to occur at pH 7 in *P.aeruginosa* incubated at 35°C for 96 hrs of incubation. Removal of nickel was observed to be maximum at pH 5 using *A.niger* at 30°C for 9 days of incubation. The biosorption of nickel was high (99 per cent) in *A.niger* when compared to *P.aeruginosa* (93 per cent) in 25% concentration of effluent. Hence, further studies were carried out with *A.niger*.

The nickel removal capacity of *A. niger* was also supported by scanning electron microscopic study which proved the accumulation of nickel within the fungal mycelium.

PHASE III

In the third phase, physicochemical parameters of both raw and microbially treated nickel electroplating effluent were assessed.

The results of the study revealed the fact that the raw nickel electroplating effluent was green in color with unpleasant odour and turbid in nature. The physicochemical parameters analyzed in the effluent namely pH, Electrical Conductivity (EC), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), total hardness, total alkalinity, chlorides, sulphates, phosphates, ammoniacal nitrogen, calcium, magnesium, sodium, potassium, nickel, oil and grease exceeded the permissible limits specified by BIS and ISI. The levels of the above physicochemical parameters tested in the microbially treated effluent were within the permissible limits.

PHASE IV

The fourth phase of the study was attempted to assess the impact of tap water (T_1), untreated nickel electroplating effluent (T_2) and treated nickel electroplating effluent (T_3) on the biometric (germination percentage, shoot and root length, vigour index, fresh and dry weight, number and weight of pod per plant) and biochemical parameters (total protein, total carbohydrate, total chlorophyll and nickel contents) of green gram plants. The soil samples were also analyzed for their selected physicochemical parameters before and after the pot culture experiment.

Seven day old seedlings of the green gram plants grown in treated nickel electroplating effluent (T_3) showed maximum percentage of seed germination, shoot and root length, fresh and dry weight and high vigour index whereas the above parameters tested in seedlings grown in untreated nickel electroplating effluent (T_2) were minimum when compared to control.

All the growth parameters (shoot and root length, fresh and dry weight) assessed in T_3 plants showed an increase with those of T_2 plants which recorded a decrease on 30th and 60th day.

Number of pods / plant and pod weight showed a maximum increase (60.2%) in green gram plants grown using treated effluent whereas a decrease (40.9%) in the above yield parameters was noted in the plants grown in untreated nickel electroplating effluent when compared to control.

Percentage increase in the growth was found to be directly proportional to the number of days increased in T_3 plants.

Maximum increase in total protein, total carbohydrate and total chlorophyll contents was observed in green gram plants grown in treated nickel electroplating effluent (T₃) when compared to T₁ and T₂ plants.

Nickel content present in green gram plants and pods grown in untreated nickel electroplating effluent (T₂) exceeded the permissible limit of WHO indicating its toxicity to the plants whereas it was within the tolerance limit in T₃ plants.

The pH, EC, organic carbon, macronutrients (N, P, K, Ca, Mg and Na) and micronutrients (Zn, Fe, Mn, Cu and Ni) of the soil analysed before effluent treatment were within the tolerance limits. After treatment, the above soil parameters showed an increase in the soil irrigated with untreated effluent which exceeded the permissible limits, whereas in other two treatments (T₁ and T₃) the soil characters were within the tolerance limits specified.

PHASE V

The fifth phase of the study was designed to analyse the growth (length and weight) and biochemical constituents (proteins, carbohydrates, lipids and nickel) of the muscle, liver, gills, and kidney tissues of *Clarias gariepinus* exposed to tap water, untreated nickel electroplating effluent and treated nickel electroplating effluent. Histological changes were also observed in the gills, liver and kidney tissues of the fish exposed to the above treatments.

The length and weight gain was maximum in the fishes grown in treated nickel electroplating effluent whereas the gain recorded was minimum in the fishes grown in untreated nickel electroplating effluent to that of the control.

Maximum decrease in protein content was noted in different tissues (muscle, liver, gills and kidney) of *Clarias gariepinus* exposed to untreated nickel electroplating effluent on the termination of the experiment (60th day), whereas an increase in protein content was observed in fishes exposed to treated nickel electroplating effluent when compared to the fishes grown in tap water (control).

Carbohydrate content was increased in the fishes grown in treated nickel electroplating effluent while the level was decreased in fishes grown in untreated nickel electroplating effluent over their control.

Decline in the lipid content was observed in fishes exposed to untreated nickel electroplating effluent, whereas an increase was noted in the fishes grown in treated effluent when compared to that of the control.

Accumulation of nickel was maximum in all the tissues analysed in *C. gariepinus* exposed to untreated nickel electroplating effluent, whereas the level of nickel was within the tolerance limit in the fishes exposed to tap water and treated effluent.

Histological sections revealed maximum damage in the gills, liver and kidney tissues of *C. gariepinus* exposed to untreated nickel electroplating effluent (T₂). The gills of the fishes showed degenerative changes, necrosis, odema in the secondary lamella, clubbing and fusion of gill tips along with epithelial thickening and reduction in the interlamellar space and lamellar fusion. In liver, necrosis, degeneration of hepatocyte cells and formation of vacuoles were observed. In kidney, highly degenerative changes were found in lymphoid tissues which included infiltration of parenchymal cells,

congested glomeruli, severe necrosis and dilation of renal tubules and Bowman's capsule, whereas no such changes were observed in the tissue organization of T₁ and T₃ fishes.

From the findings of the present study, it may be concluded that the raw nickel electroplating effluent discharged from the industrial site contained various pollutants which exceeded the permissible limits specified by BIS and ISI. These pollutants were detoxified by *Aspergillus niger* (the fungal isolate), which performed better among the other isolates and was able to remove the maximum amount of nickel from the effluent which was also evident from the observations made through scanning electron microscopic study.

The green gram plants cultivated in microbially treated effluent showed higher growth, yield and biochemical constituents as compared to untreated effluent and control.

The microbially treated effluent might have enriched the soil nutrients and could have enhanced the growth, yield and biochemical components of green gram plants, whereas the untreated effluent retarded the plant growth and their yield.

Similarly, when the fishes were introduced into different treatments, a gain in length and weight and also an increase in the selected biochemical components (total proteins, total carbohydrates and lipids) in different organs (muscle, liver, gills and kidney tissues) were observed in those fishes that were grown in microbially treated effluent when compared with those grown in untreated effluent and control.

Histological studies conducted on the tissues of gills, liver and kidney revealed the fact that the fishes grown in untreated nickel electroplating effluent were adversely affected showing odema, necrosis, degenerative changes in the secondary lamella of gills, degeneration of hepatocyte cells of liver and dilation of renal tubules and Bowman's capsule of kidney, indicating toxicity of the metal. No such structural changes were noticed in the tissues of the fishes grown in microbially treated nickel electroplating effluent and tap water.

Hence, it is evident from the present study that the microbially treated effluent provides a nutrient-rich input for agriculture and aquaculture.

The present study, therefore provides a promising technology for the effective, economical and ecofriendly remediation of heavy metals and thereby promotes a pollution free environment.

SCOPE FOR FUTURE RESEARCH

The outcome of the study has opened up several promising insights of possible research. Some of them that can be suggested for future research are given below:

- ➡ Large scale treatment technology can be designed using the selected microorganisms for the bioremediation of nickel electroplating effluent.
- ➡ Role of mixed culture of selected microorganisms in the biosorption process can be studied.

- ➡ Studies can be carried out on cell wall modification of microbial cells in order to enhance the metal binding capacity of biomass.
- ➡ Processes can be developed for metal recovery using suitable desorbing agents without damaging the biosorbent so that it could be regenerated.
- ➡ The viable and nonviable cells of microorganisms can be immobilized into suitable matrices and studied for their efficiency in the removal of nickel.
- ➡ Rate of bioremediation can be further enhanced by developing engineered novel strains of microorganisms with increased nickel reducing efficiency.
- ➡ The sorption capacity can be assessed using selected agro waste absorbents.
- ➡ The bioaccumulation of nickel in the plants and animals grown in the effluent - discharged area can be studied.
- ➡ Field trails with safe levels of effluents for commercial production of plants and fish may be recommended.