

*RESULTS AND
DISCUSSION*

4. RESULTS AND DISCUSSION

The results of the present study entitled “**Bioremediation of nickel electroplating effluent and its impact on the growth and biochemical constituents of green gram and cat fish**” are discussed as follows:

PHASE 1

4.1. CHARACTERISATION OF METAL TOLERANT BACTERIAL AND FUNGAL ISOLATES

Bacteria and fungi which were tolerant to nickel were isolated from the soil contaminated with nickel electroplating effluent.

4.1.1. Morphological and biochemical characterisation of the bacterial isolates

The morphological and biochemical characteristics of selected bacterial isolates observed are presented in Table I.

It is evident from the table that the isolate 1 observed in the nutrient agar medium was Gram negative, motile, rod shaped bacterium measuring 2.6 – 4.1µm in size and had diffusible green pigments. The biochemical characterisation of isolate 1 showed positive results for catalase, oxidase, citrate utilization, gelatin hydrolysis and nitrate reduction tests, whereas indole production, methyl red, voges proskauer, starch hydrolysis and urease tests were found to be negative. However, carbohydrate fermentation test showed that isolate 1 fermented maltose producing both acid and gas whereas only acid production was observed when glucose was used as a substrate. Acid and gas production was not observed in lactose and sucrose substrates. The bacterial isolate was streaked on cetrimide agar medium for confirmation. Based on the identifications the isolate 1 was found to be *Pseudomonas aeruginosa* (Plate VI a).

TABLE I
MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF
THE SELECTED BACTERIAL ISOLATES

Parameters	Isolate 1	Isolate 2	Isolate 3
A. Morphological Properties			
Shape	Rod	Rod	Cocci
Size	2.6 – 4.1 μm	1 – 1.6 μm	1.7 – 3.1 μm
Gram Staining	Negative	Positive	Positive
Motility	Motile	Motile	Non motile
Nutrient agar	Diffused green pigment	Raised opaque, dull, white colonies	Large, circular, smooth, yellow colonies
B. Biochemical Tests			
Catalase	+	+	+
Oxidase	+	-	-
Indole production	-	-	-
Methyl red	-	-	-
Voges Proskauer	-	+	-
Citrate utilization	+	-	-
Starch hydrolysis	-	+	-
Gelatin hydrolysis	+	+	+
Urease	-	-	+
Nitrate reduction	+	-	-
Carbohydrate Fermentation test			
Glucose	A ⁺ G ⁻	A ⁺ G ⁻	A ⁻ G ⁻
Lactose	A ⁻ G ⁻	A ⁻ G ⁻	A ⁻ G ⁻
Sucrose	A ⁻ G ⁻	A ⁺ G ⁻	A ⁻ G ⁻
Maltose	A ⁺ G ⁺	A ⁺ G ⁻	A ⁻ G ⁻
Isolate identified	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>	<i>Micrococcus luteus</i>

+ = positive
 - = Negative

A⁺G⁺ = Acid and Gas production
 A⁻G⁻ = No Acid and Gas production

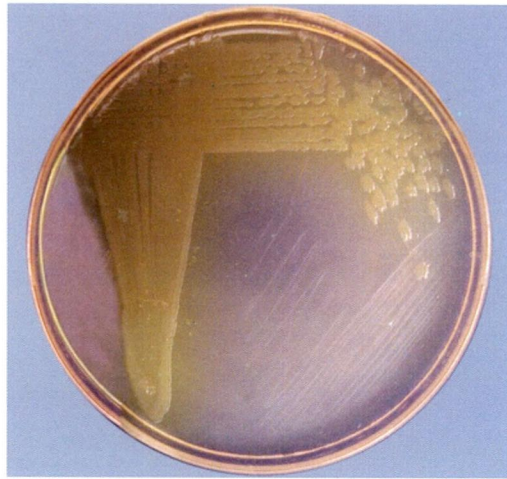
Isolate 2 was identified as a Gram positive, rod shaped and motile bacterium with the size ranging from 1 – 1.6 μm . White colonies were produced on nutrient agar medium. The biochemical characterisation of isolate 2 showed positive results for catalase, voges proskauer, starch and gelatin hydrolysis tests, whereas oxidase, indole production, methyl red, citrate utilization, urease and nitrate reduction tests showed negative results. In the carbohydrate fermentation test, isolate 2 produced only acid with glucose, sucrose and maltose as substrates whereas there was no acid and gas production with lactose as substrate. Based on these morphological and biochemical tests isolate 2 was identified as *Bacillus subtilis* (Plate VI b).

The morphological features of isolate 3 revealed that it was Gram positive cocci, non motile bacterium with yellow colonies on nutrient agar medium. The size of the bacterium was found to be between 1.7 and 3.1 μm . The biochemical characterisation of isolate 3 showed positive results for catalase, gelatin hydrolysis and urease tests. However, oxidase, indole production, methyl red, voges proskauer, citrate utilization, starch hydrolysis, nitrate reduction and carbohydrate fermentation tests were found to be negative. Based on these morphological and biochemical tests the isolate 3 was identified as *Micrococcus luteus* (Plate VI c).

The present investigation on the isolation of bacteria which could tolerate maximum amount of nickel may also be supported by the studies conducted by using *Micrococcus* species (Congeevaram *et al.*, 2007) and *Pseudomonas fluorescens* (Hussein *et al.*, 2004). Various metal tolerant microorganisms such as *Pseudomonas* species from electroplating effluent contaminated soil (Jasmine and Sasikumar, 2006 and Malekzadeh, 2005), *Bacillus*,

PLATE VI

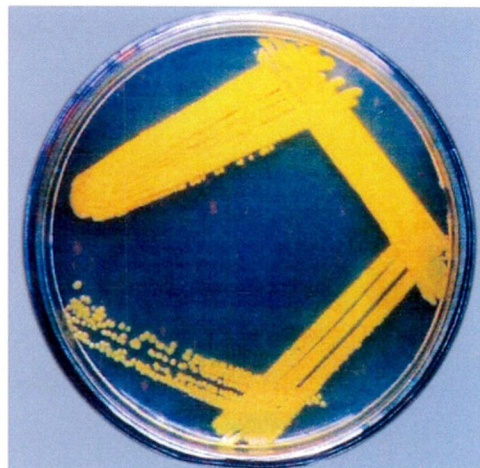
METAL TOLERANT BACTERIAL ISOLATES IDENTIFIED FROM
THE SOIL CONTAMINATED WITH NICKEL
ELECTROPLATING EFFLUENT



a. Pseudomonas aeruginosa



b. Bacillus subtilis



c. Micrococcus luteus

Pseudomonas and *Micrococcus* (hydrocarbon degrading bacteria) from the oil spilled area (Sivapriya and Nirmala, 2003), automobile waste water (Saravanan *et al.*, 2005), species of *Bacillus* and *Pseudomonas* from pharmaceutical (Prasanna *et al.*, 2008) and textile (Growther and Meenakshi, 2009) effluent contaminated soils were isolated and these studies may be an added support to the present investigation.

4.1. 2. Morphological characteristics of selected fungal isolates

Based on the plate morphology and characters, the fungal isolates were determined microscopically. Three fungal isolates namely, *Aspergillus niger*, *Fusarium solani* and *Cladosporium* species were identified. The interpretation drawn from the observations are detailed below:

Aspergillus niger

The surface of the colonies on rose bengal chloramphenicol agar was found to be black in colour. Immature colonies were found to be covered with white fluffy aerial mycelium and the mature colonies were black which had salt peppery effect. The reverse side of the plate was buff coloured. The vesicles were small (40 - 80 μ) and globose shaped. Sterigmata were found to be arranged in two series. The primary sterigmata were longer than the secondary sterigmata. The conidiophores were short and had thick smooth walls (Plate VII a).

Fusarium solani

The colonies were cottony white in colour and grew fast on rose bengal chloramphenicol agar medium. The colony on the reverse side of the plate was white in colour which turned to pinkish

red shades on maturation. Two types of conidia namely macroconidia and microconidia were present. Macroconidia are multicelled with 2 - 5 septa which were seen on aerial mycelium. These were sickle shaped with a distinct notched basal cell. The microconidia had one to two cells and were borne in short chains in aerial mycelium and were oval and globose in shape (Plate VII b).

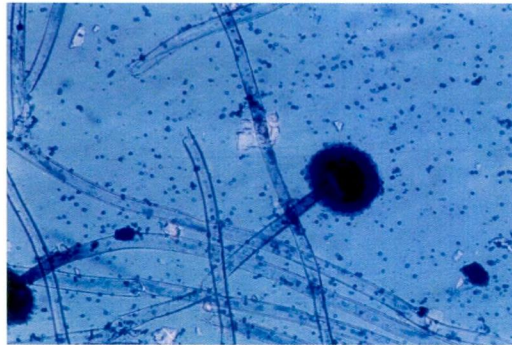
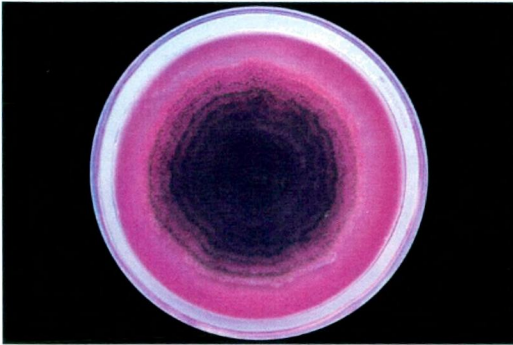
***Cladosporium* species**

The colonies were greenish black and powdery on rose bengal chloramphenicol agar medium. The hyphae were large and thick. The conidiophores were erect, branched, floccose and pigmented. The conidia were one celled, smooth, globose and ovate (Plate VII c).

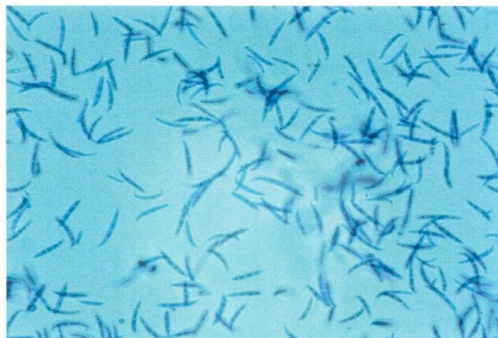
Different fungal isolates such as *Trichoderma viride* from electroplating industrial effluent contaminated soil (Bishnoi *et al.*, 2007), *Aspergillus niger* from textile effluent (Ali *et al.*, 2007) and metal contaminated soils (Magyarosy *et al.*, 2002) samples and species of *Rhizopus*, *Aspergillus* and *Penicillium* from textile effluent (Faryal and Hameed, 2005) contaminated soil were isolated and these findings are in accordance with the present study. The filamentous soil fungi like *Aspergillus terreus*, *Cladosporium cladosporioides*, *Fusarium oxysporium*, *Gliocladium roseum*, *Penicillium* sp. and *Trichoderma koningii* isolated from industrially polluted soils were reported (Massaccessi *et al.*, 2002) to remove cadmium which may support the present investigation.

PLATE VII

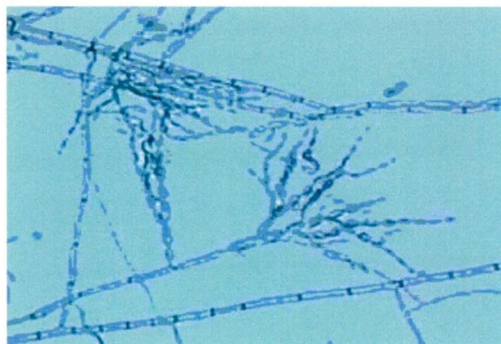
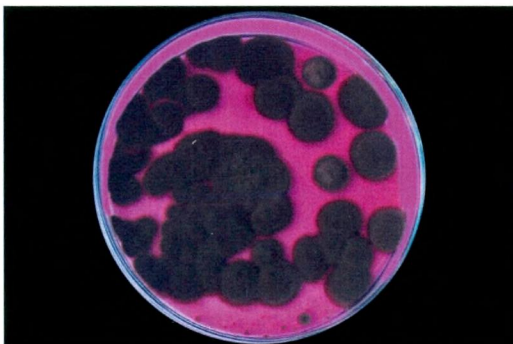
METAL TOLERANT FUNGAL ISOLATES IDENTIFIED FROM THE SOIL
CONTAMINATED WITH NICKEL ELECTROPLATING EFFLUENT



a. *Aspergillus niger*



b. *Fusarium solani*



c. *Cladosporium species*

PHASE II

4.2. REMOVAL OF NICKEL FROM THE EFFLUENT USING BACTERIAL AND FUNGAL ISOLATES

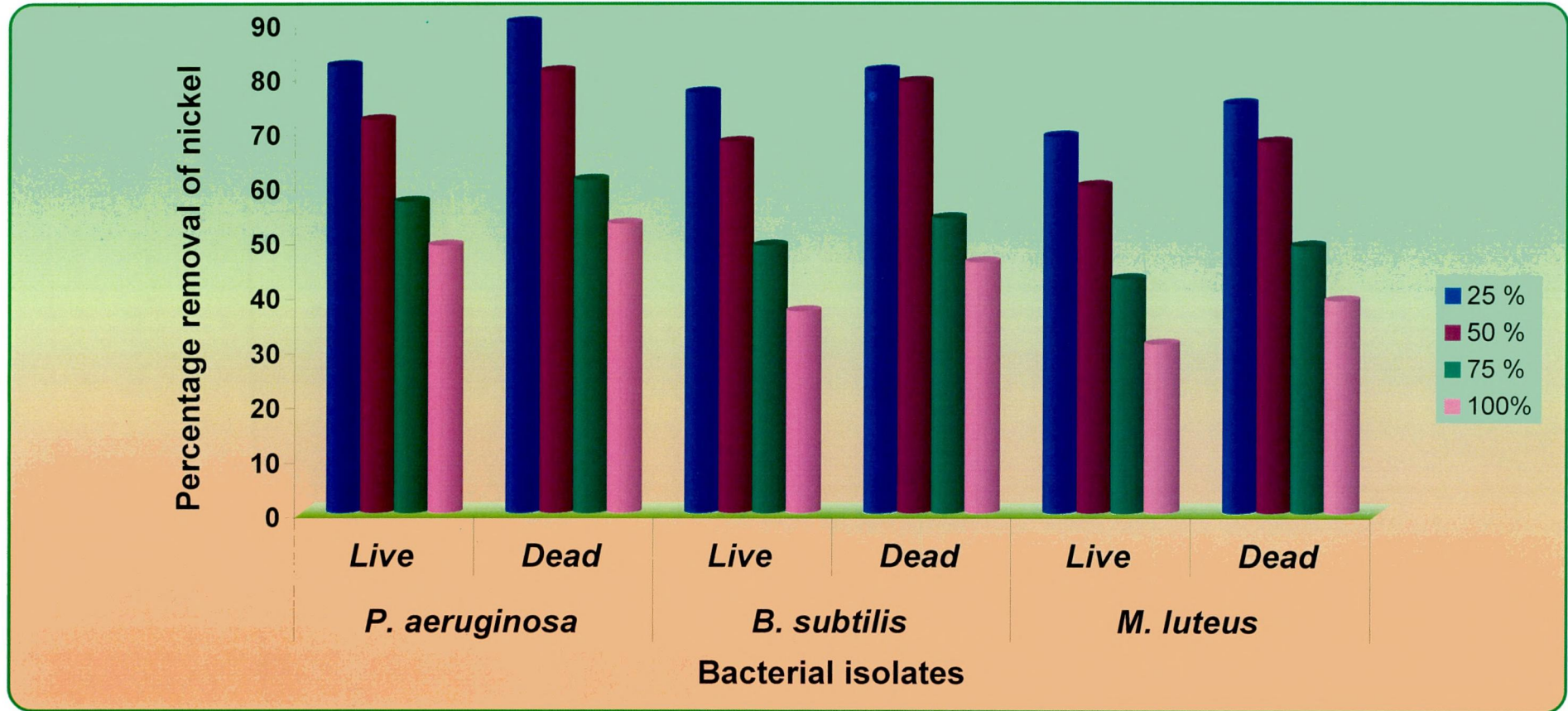
A comparative study was carried out using live and dead biomass of bacteria and fungi at varying concentrations of nickel electroplating effluent (25%, 50%, 75% and 100%) to find out their efficiency in the removal of nickel under various conditions.

It was observed from the Figure 1 that both live and dead biomass of all the three bacterial isolates exhibited maximum removal of nickel in the lower (25%) concentration of effluent. Among them, the dead biomass of *P. aeruginosa*, *B. subtilis* and *M. luteus* removed a maximum of 90, 81 and 75 per cent of nickel respectively from 25% concentration of effluent, whereas the removal of nickel was comparatively lesser in live bacterial isolates which was recorded to be 82 per cent in *P. aeruginosa*, 77 per cent in *B. subtilis* and 69 per cent in *M. luteus*.

In 50% concentration, removal of nickel was less when compared with 25% concentration of effluent. The effluent treated with dead biomass of *P. aeruginosa* removed 81 per cent of nickel which was higher when compared with its live biomass which removed only 72 per cent of nickel. Removal of nickel from the effluent by the dead biomass of *B. subtilis* and *M. luteus* was 79 and 68 per cent and that of live biomass was only 68 and 60 per cent respectively.

FIGURE 1

PERCENTAGE REMOVAL OF NICKEL BY LIVE AND DEAD BACTERIAL ISOLATES FROM DIFFERENT CONCENTRATIONS OF NICKEL ELECTROPLATING EFFLUENT



Followed by 50% concentration of effluent, the uptake of nickel was less in 75% concentration of effluent. The uptake of nickel by the dead biomass of *P. aeruginosa*, *B. subtilis* and *M. luteus* was 61, 54 and 49 per cent respectively. The removal efficiency of nickel was lesser in the live biomass of *P. aeruginosa* which was 57 per cent followed by *B. subtilis* with 49 per cent and *M. luteus* with 43 per cent.

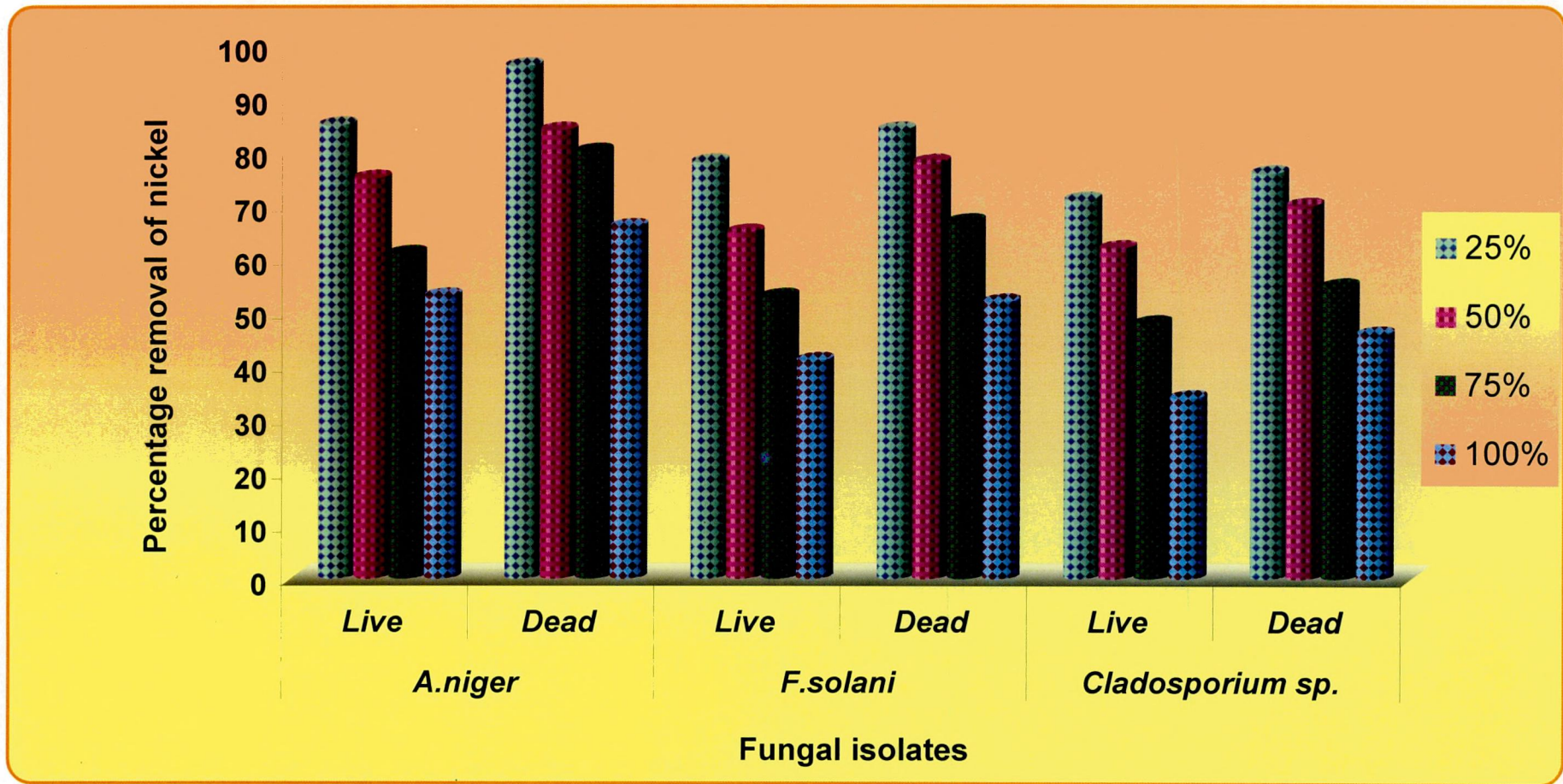
When compared to all the above concentrations, the raw effluent (100%) was found to exhibit minimum removal of nickel from the effluent. The removal of nickel by the dead biomass of *P. aeruginosa* was found to be 53 per cent and that by *B. subtilis* was 46 per cent. *M. luteus* removed only 39 per cent of nickel. The live biomass of *P. aeruginosa*, *B. subtilis* and *M. luteus* were able to remove only 49, 37 and 31 per cent of nickel from the effluent respectively.

The efficiency of nickel uptake by the three selected fungal isolates such as *A. niger*, *F. solani* and *Cladosporium* species was assessed five days after incubation in graded concentrations (25%, 50%, 75% and 100%) of the nickel electroplating effluent. The results obtained are presented in Figure 2.

The dead cells of *A. niger* recorded maximum uptake of 96 per cent of nickel at 25% concentration of effluent followed by *F. solani* and *Cladosporium* species which were able to remove 84 per cent and 76 per cent of nickel respectively. The uptake of nickel by the live cells of *A. niger* was 85 per cent. *F. solani* and *Cladosporium* species were able to take up 78 and 71 per cent of nickel respectively from the 25% concentration of effluent.

FIGURE 2

PERCENTAGE REMOVAL OF NICKEL BY LIVE AND DEAD FUNGAL ISOLATES FROM DIFFERENT CONCENTRATIONS OF NICKEL ELECTROPLATING EFFLUENT



Percentage removal of nickel from 50% concentration of the effluent treated with dead biomass of *A. niger*, *F. solani* and *Cladosporium* species was 84, 78 and 70 per cent respectively. Comparatively the uptake of nickel was low in the live biomass of *A. niger*, *F. solani* and *Cladosporium* species which was 75, 65 and 62 per cent respectively.

The efficiency of nickel uptake was found to be low in 75% and 100% concentrations of effluent. The effluent with 75% concentration when treated with dead biomass of *A. niger*, *F. solani*, *Cladosporium* species could remove 80, 67, 55 per cent and in 100% concentration the removal of nickel was 66, 52 and 46 per cent respectively. Percentage removal of nickel in 75% concentration of effluent treated with live biomass of *A. niger* was 61, *F. solani* was 53 and *Cladosporium* species was 48, whereas it was 53, 41 and 34 per cent respectively in 100% concentration of effluent.

It becomes evident from the above study that there was an inverse relationship between nickel uptake and the concentration of effluent among all the isolates. The removal efficiency of nickel was found to be greater in dead form when compared with the live biomass of bacteria and fungi.

The use of dead biomass has an additional advantage when compared with live biomass. In dead biomass, the metal removal system is not subjected to toxicity limitations and there is no requirement for growth media and nutrients. The biosorbed metal ions can also be easily desorbed and the biomass can be reused or stored for extended periods at room temperature without putrefaction (Kapoor and Viraraghavan, 1997). The percentage removal of heavy metals was high in dead cells which might be due to the permeability

of cells that allow the metals to enter and bind with the internal and external components (Gadd, 1992).

The reduction in the nickel uptake by living system may be due to metal toxicity and therefore healthy microbial populations are difficult to maintain in effluents containing metal ions. In addition, maintenance of suitable operational conditions like pH and temperature are also difficult. The living systems also require the addition of nutrients which increases the BOD and COD in the effluent. The potentiality for desorptive metal recovery may be restricted since metals may be intracellularly bound and form complexes which retain them in the solution. Recovery and regeneration of the biosorbent is more complicated (Hussein *et al.*, 2004).

The results of the present study corroborate with the findings of Gadd (1990) who indicated that although living and dead cells are capable of metal accumulation there might be differences in the mechanism involved in either case, depending on the extent of metabolic dependence and independent biosorption.

The studies conducted by various researchers on the removal of copper and lead by live and dead biomass of *Rhizopus arrhizus* (Subudhi and Kar, 2008) and *Aspergillus niger* (Ahluwalia and Goyal, 2003), chromium by *Neurospora crassa* (Kiran *et al.*, 2005), copper and nickel by *Cladosporium cladosporoides* (Patil and Paknikar, 1999), nickel, cadmium and zinc by *Penicillium digitatum* (Galun *et al.*, 1987), nickel by *Aspergillus niger* and *Aspergillus terreus* (Dias *et al.*, 2002) and decolourization of methyl orange by sixteen different fungi (Seyis and Subasioglu, 2008) revealed the fact that the dead biomass exhibited higher level of metal uptake than the

live biomass. The above studies may also be considered as evidence for the present study.

From the above results, it was observed that among the different concentrations of effluent used, the lowest concentration (25%) was found to be more effective in the removal of nickel. Hence, this concentration was used for further studies.

4.2.1 Effect of pH on nickel uptake by dead bacterial and fungal isolates

The biosorption capacities of dead microbial biomass for heavy metals were found to be strongly dependent on pH of the solution.

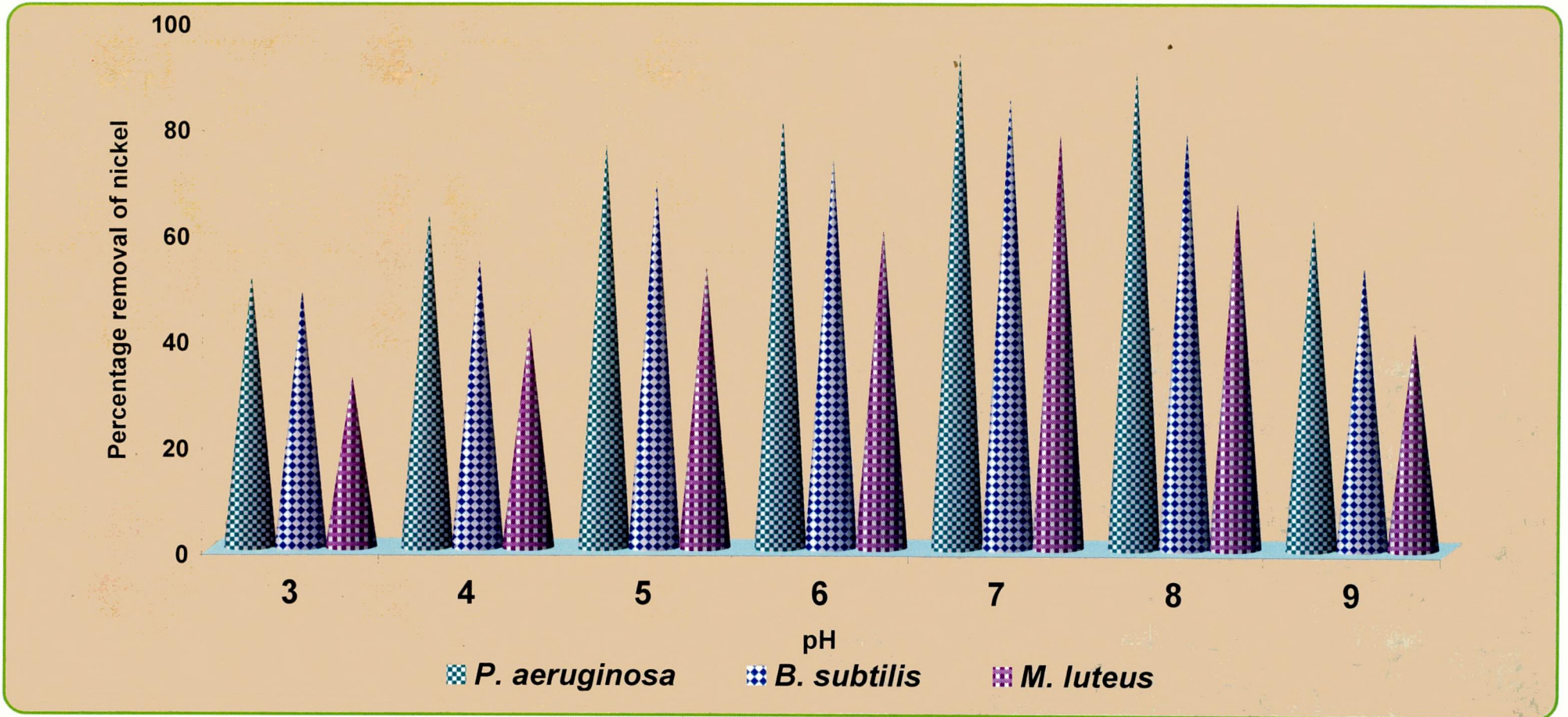
pH is the most important parameter in the biosorptive process which greatly affects the uptake of metal ions by microbes (Kapoor and Viraraghavan, 1995). Thus the effect of pH on nickel removal was studied with the isolated bacterial and fungal species. The results are graphically presented in Figure 3.

The maximum uptake of nickel by the three dead bacterial isolates was observed at pH 7. Among the three bacterial isolates *P. aeruginosa* was found to remove a maximum of 93 per cent nickel from the effluent compared to *B. subtilis* and *M. luteus* which could remove 85 and 78 per cent respectively. Percentage removal of nickel by these three bacterial isolates followed the same trend in all the other pH (3, 4, 5, 6, 8 and 9) levels also, but the removal was lesser than that of pH 7.

It was observed that the dead biomasses of all the three selected fungal isolates were able to absorb metal ions over a wide range of pH and the maximum uptake of nickel was found to occur at 5.

FIGURE 3

PERCENTAGE REMOVAL OF NICKEL BY THE DEAD BACTERIAL ISOLATES AT DIFFERENT pH



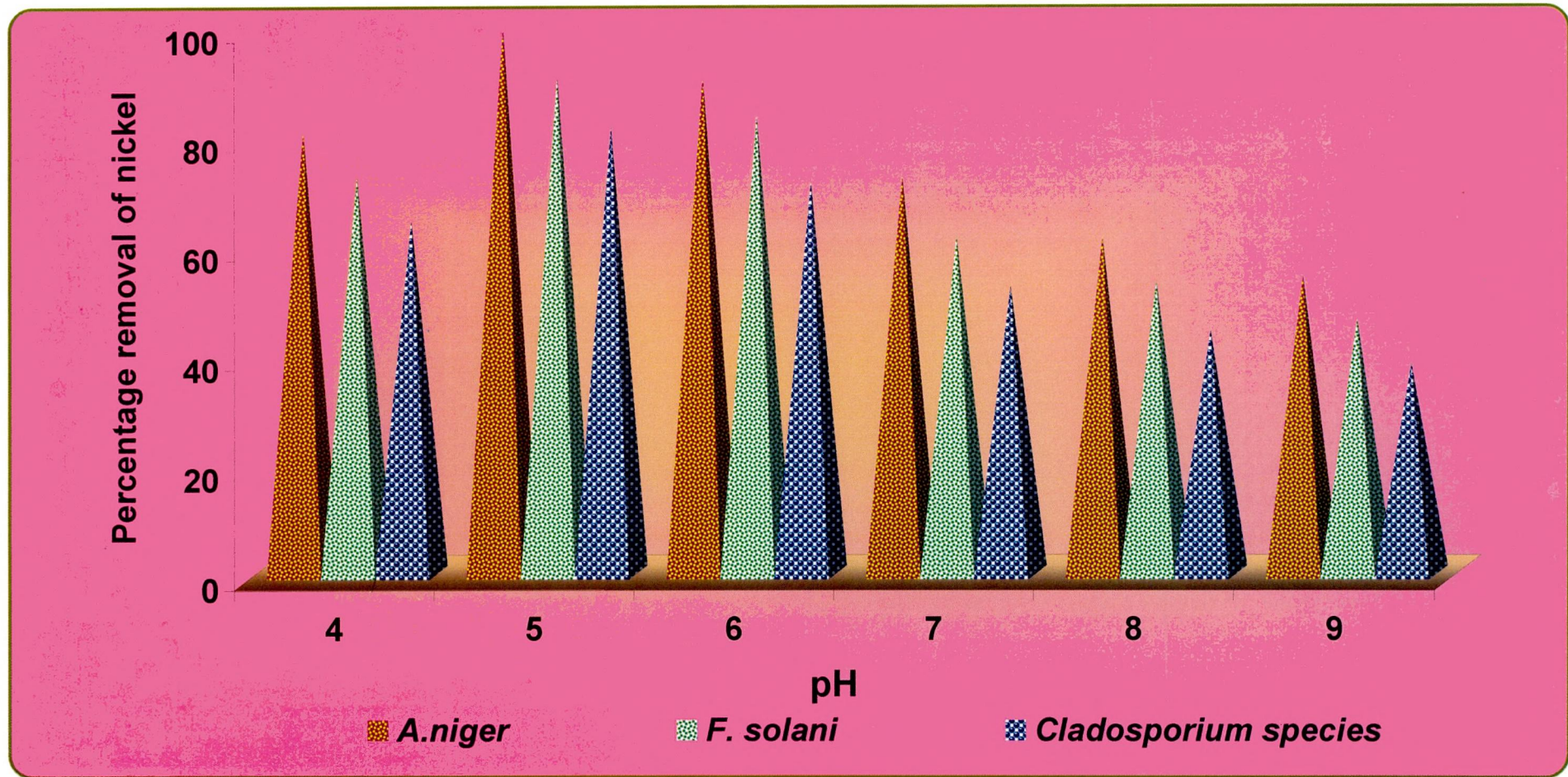
A. niger recorded a maximum uptake of 99 per cent of nickel at the optimum pH 5, followed by *F. solani* and *Cladosporium* species which remove 90 and 81 per cent of nickel respectively. Similar trend was also observed in other pH levels used in the present study (Figure 4). Minimum uptake of nickel was observed at pH 3 and 9 in bacterial and fungal isolates studied.

The minimum biosorption capacity by the microbes at lower pH may be attributed to high hydrogen ion concentration which forms ligands with cell wall making the metal cations and protons compete for binding sites of cell walls and reduce the metal uptake. As the pH increases, more negatively charged cell wall ligands are exposed and subsequent attraction of metallic ions with positive charge occurs. This increase in the biosorption with increased pH may be due to a strong relation of biosorption to the number of negative charges, which depends on the dissociation of functional groups (Delgado *et al.*, 1998) and also through an ion exchange type of mechanism (Torresey *et al.*, 1998). It was reported that at higher pH nickel might get transformed into hydroxide complex, interfering with the biosorption process and causing disturbance of equilibrium as reported by Chen *et al.* (2005).

The findings of the present study showed that the optimum pH for bacterial and fungal isolates were 7 and 5 respectively which was in accordance with the reports observed in *Pseudomonas* species which removed zinc (Jasmine and Sasikumar, 2006), lead (Panchanadikar and Das, 1994) at pH 7 and uranium at pH 6.5 (Malekzadeh *et al.*, 2000) and *M. luteus* removed lead and copper at pH 7 (Leung *et al.*, 2000).

FIGURE 4

PERCENTAGE REMOVAL OF NICKEL BY THE DEAD FUNGAL ISOLATES AT DIFFERENT pH



The biosorption of lead, cadmium, nickel and zinc was found to be inhibited at lower pH by *Penicillium digitatum* (Galun *et al.*, 1987), copper by *P. spinulosum* (Ross and Townley, 1986) and zinc by *S. cerevisiae* (Bardy *et al.*, 1994). Uptake of UO_2^{2+} and Pb (II) from aqueous solution by *Streptomyces* species (Golab and Orłowska, 1991) and the sorption of methyl violet and basic fuchsin by *A. niger* was maximum at pH 5 (Bhole *et al.*, 2004). All these reports support the findings of the present study.

4.2.3. Effect of temperature on nickel uptake by dead bacterial and fungal isolates

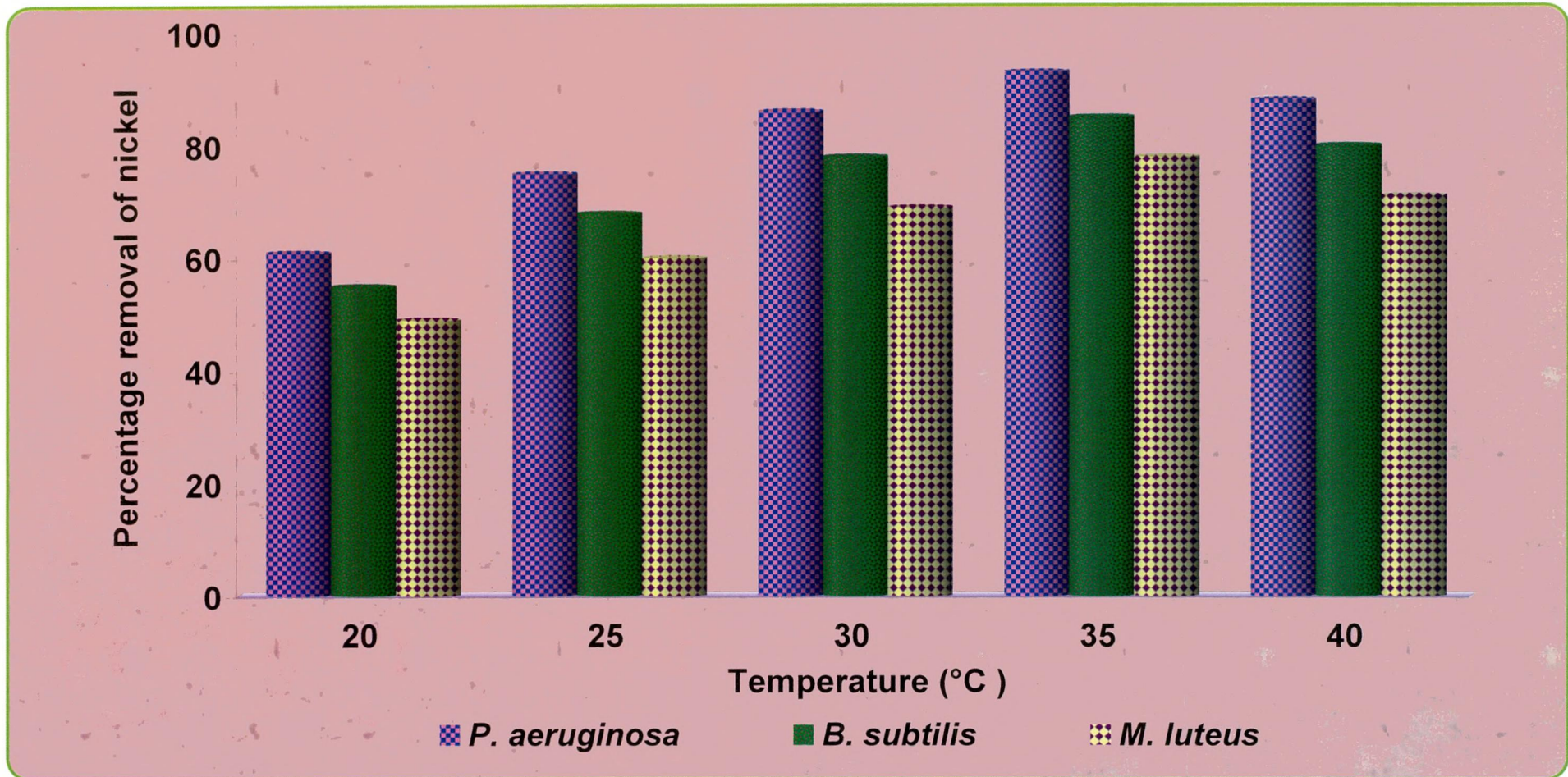
Nickel uptake by the dead biomass of the three bacterial and fungal isolates at different temperatures was studied and the results are shown in Figures 5 and 6.

As the temperature increased upto 35°C, there was an increase in the uptake of nickel by the dead biomass of all the bacterial isolates. No further increase in nickel uptake was noticed above this temperature. At 35°C the uptake of nickel from the effluent by *P. aeruginosa* was 93 per cent followed by *B. subtilis* and *M. luteus* with 85 per cent, 78 per cent respectively. The uptake of nickel at (20, 25, 30 and 40°C) by the three bacterial isolates exhibited the same effect as observed in 35°C which was depicted in Figure 5.

All the three fungal isolates were proved to be efficient in the removal of nickel at 30°C. Among the three fungal isolates examined *A. niger* was found to take up 99 per cent of nickel from the effluent whereas *F. solani* and *Cladosporium species* could remove only 89 per cent and 80 per cent respectively.

FIGURE 5

PERCENTAGE REMOVAL OF NICKEL BY THE BACTERIAL ISOLATES AT DIFFERENT TEMPERATURES



The removal of nickel by the three fungal isolates followed the same trend in other temperatures (20, 25, 35 and 40°C) which was graphically presented in Figure 6.

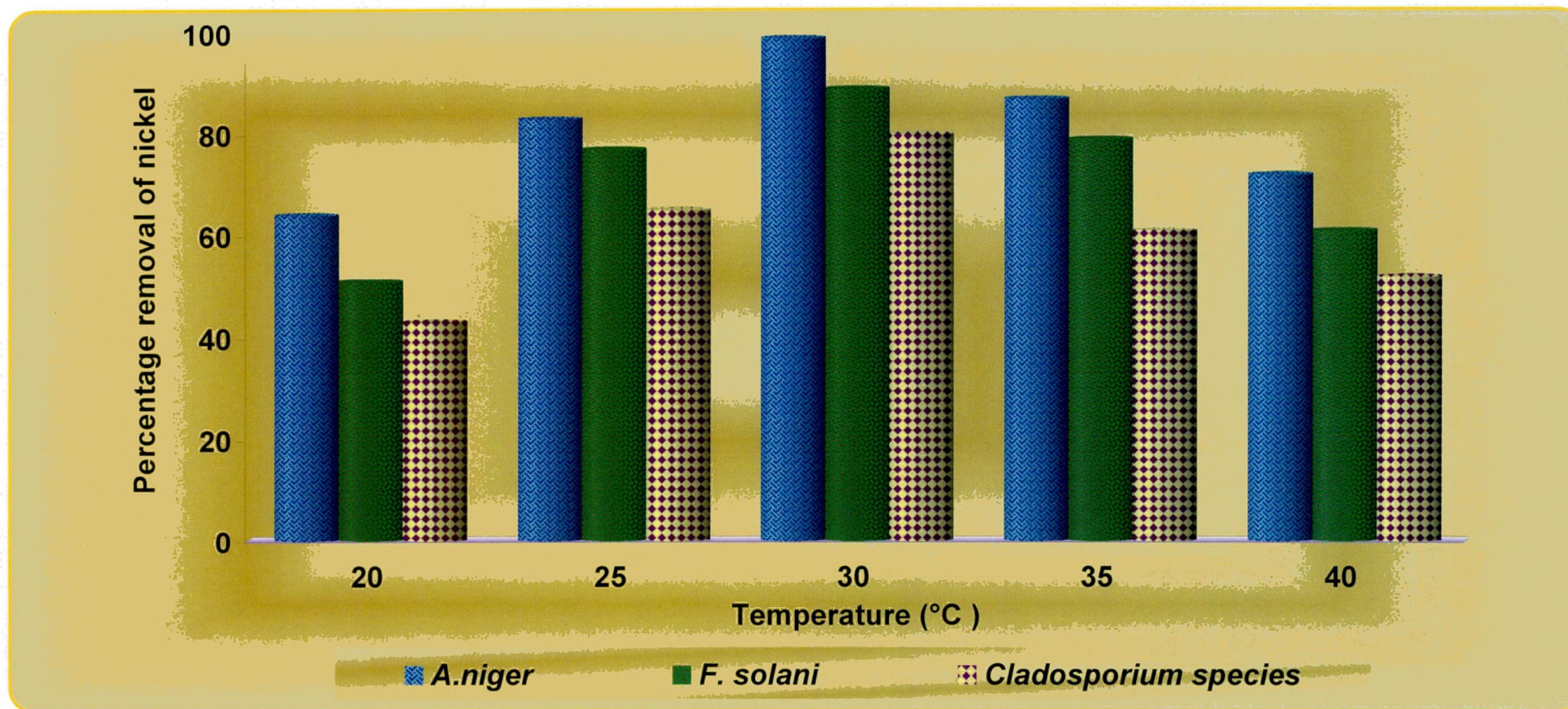
Temperature is an important parameter which is directly related with the chemical reaction in the solution and biochemical reactions in the microbes. The increase in biosorption with temperature indicates an endothermic process. Temperature affects a number of factors that are important for metal biosorption. This includes the stability of metal ion species, the ligand and ligand metal complex as well as the solubility of metal ions. In general higher temperature favours greater solubility of metal ions in solution and hence weakens the biosorption of metal ions (Lau *et al.*, 1999). Thermodynamically, biosorption will be favoured by high temperature if the binding is endothermic, but weakened if it is exothermic. It is reported by Singh (2007) that favouring or unfavouring of high temperature for the biosorption process is entirely dependent on the contribution of the carboxylate or amine ligands on the cell wall or surface.

Temperature is known to affect the stability of the cell wall, its configuration and also cause ionization of chemical moieties. These factors may simultaneously affect the binding sites of isolated fungal and bacterial species causing reduction in heavy metal removal. Energy independent mechanisms are less likely to be affected by temperature since the processes responsible for removal are largely physiochemical in nature (Gulay *et al.*, 2003).

The optimum temperature for all the bacterial and fungal isolates of the present study finds support from the works of various researchers who reported that the removal of chromium, nickel (Congeevaram *et al.*, 2007) and brilliant blue (Amutha, 2001) by

FIGURE 6

PERCENTAGE REMOVAL OF NICKEL BY THE ^{DEAD} FUNGAL ISOLATES AT DIFFERENT TEMPERATURES



Micrococcus species was maximum at 35° C; acid red by *A. niger* was maximum at 25°C (Ali *et al.*, 2007); dye house effluent at 30°C by *Trametes* species (Sukumar *et al.*, 2008); chromium by *Rhizopus nigricans* and *Aspergillus* species at 30°C (Bai and Abraham, 2001) and congo red by *Pleurotus roseus* at 30°C (Singh *et al.*, 2008).

The results of the present study showed that the optimum temperature for the selected bacterial and fungal species was 35°C and 30°C respectively.

4.2.3. Effect of incubation time on nickel uptake by dead bacterial and fungal isolates

The results of varying incubation periods was carried out to find the possibility of the involvement of metabolic processes in the uptake of nickel by dead isolates of bacterial and fungal species are presented in the Figures 7 and 8.

The rate of nickel removed from the effluent by all the three dead bacterial isolates showed a gradual increase from 6 to 96 hrs of incubation time whereas the removal efficiency showed a decreasing trend at 120 and 144 hrs of incubation time. Removal of nickel from the effluent by *P. aeruginosa* was maximum (95 per cent) followed by *B. subtilis* (89 per cent) and *M. luteus* (83 per cent) at 96 hrs of incubation.

The optimum incubation time required for the removal of maximum level of nickel from the effluent was observed to be 9 days by the fungal isolates studied. The uptake of nickel recorded at this period was 99, 89 and 81 per cent respectively by *A.niger*, *F. solani* and *Cladosporium* species. At 12 and 15 days of incubation period there was a sharp decline in the removal of nickel from the effluent by these species.

FIGURE 7

PERCENTAGE REMOVAL OF NICKEL BY DEAD BACTERIAL ISOLATES AT DIFFERENT INCUBATION TIME (HRS)

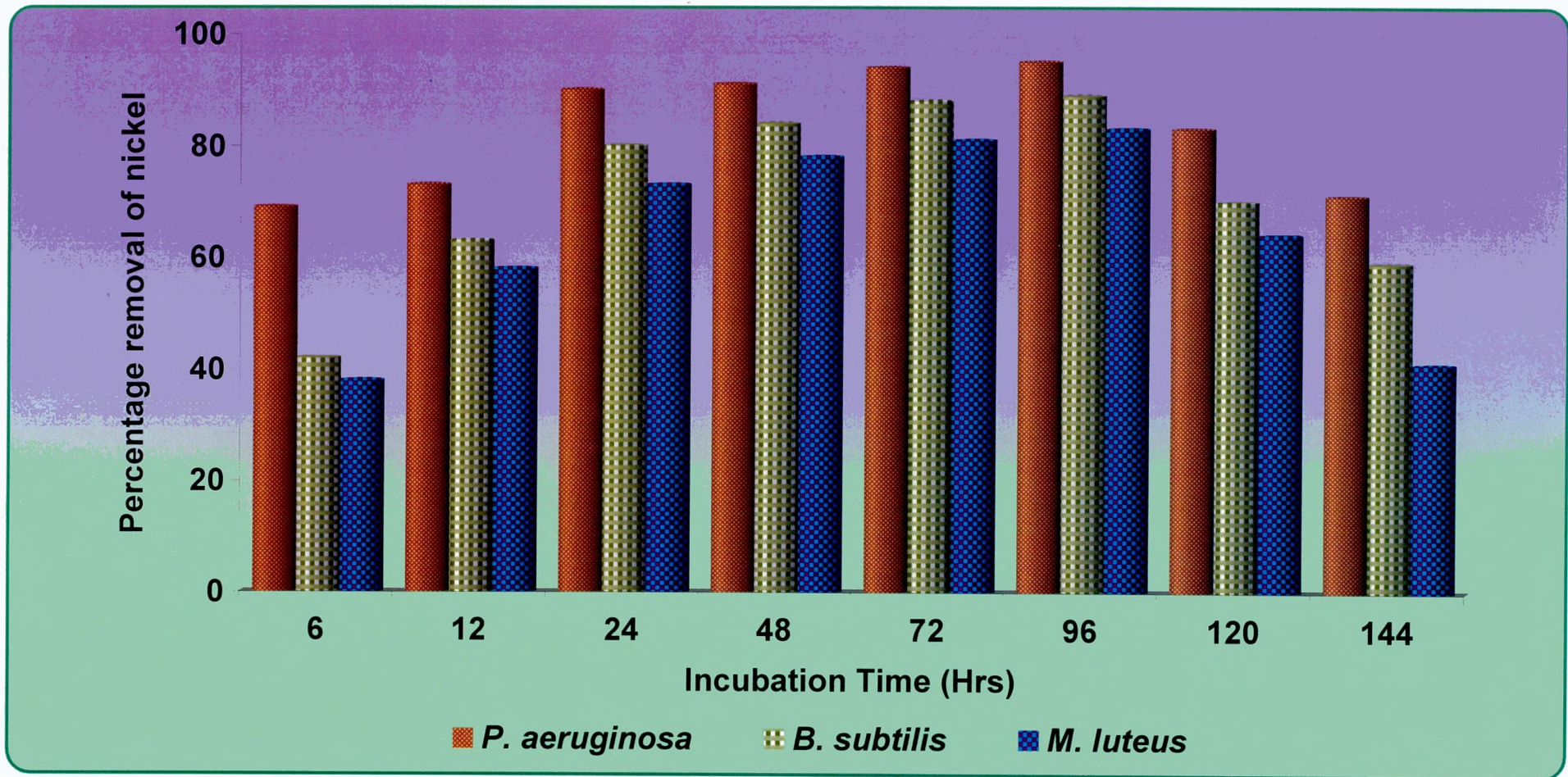
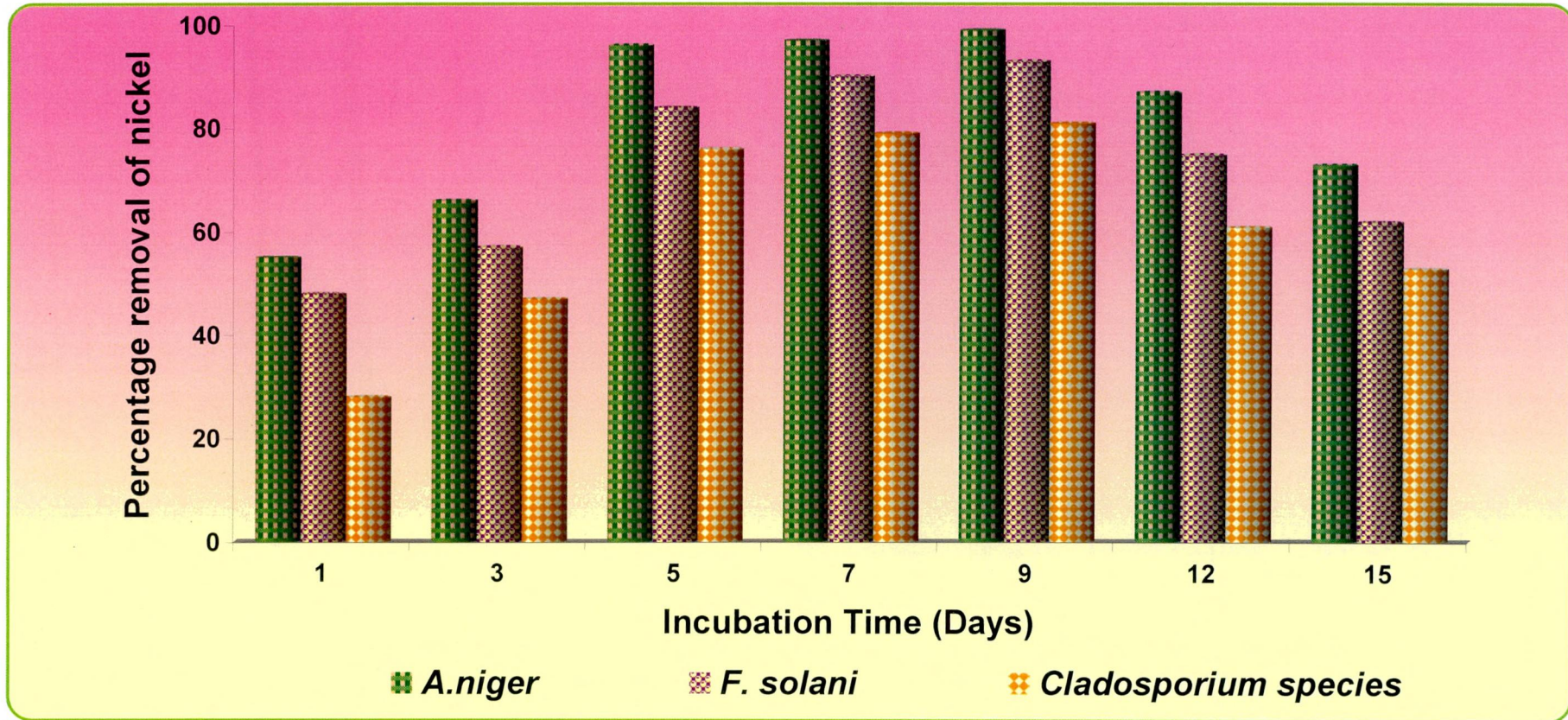


FIGURE 8

PERCENTAGE REMOVAL OF NICKEL BY DEAD FUNGAL ISOLATES AT DIFFERENT INCUBATION TIME (DAYS)



The sorption efficiency of the bacterial isolates used in the present study correlates with the results of Parameswari *et al.* (2009) who reported a maximum removal of chromium and nickel at 72 hrs of incubation by *Pseudomonas fluorescence* and *Bacillus* species. The maximum metal tolerance and binding capacity by the Gram negative bacterial forms might be due to their sedentary nature and metal precipitation in their peptidoglycan and lipopolysaccharide layers in the outer membrane (Mohanty *et al.*, 2004).

Studies conducted on the sorption of oil and grease by the species of *Pseudomonas*, *Micrococcus* and *Bacillus* recorded maximum uptake at 72 hrs of incubation and the removal rate showed a decrease when the incubation time was increased up to 120 hrs (Sivapriya and Nirmala, 2003).

Experiments conducted by Devi and Kaushik (2005) showed that treatment of textile dye effluent with *A. niger* resulted in 98 per cent decolourisation within 8 days of incubation. *Phanerochaete chrysosporium* could decolourise the phenolic paper mill effluent within seven days of incubation (Ali *et al.*, 2007). Sukumar *et al.* (2008) also reported that decolourisation of the dye effluent by fungal culture was significantly increased with incubation period from the 2nd to 7th day which was in accordance with the findings of the present study.

The maximum uptake of metals during the early incubation period might be due to the availability of abundant metal ions and empty metal binding sites in the microbes and the low absorption during the increased incubation period might be due to saturation of metal binding sites (Garnham *et al.*, 1992) which may support the findings of the present study.

Among the two groups of microbial isolates the dead biomass of fungal species were found to be the efficient ones in the removal of nickel when compared with dead bacterial isolates. From the results obtained in the optimization studies (pH, temperature and incubation time) it was found that the fungal isolates were efficient in the removal of nickel when compared with the bacterial isolates. Among the three fungal isolates, *A. niger* was found to be more effective than the others and further bioremediation studies were carried using the above fungus.

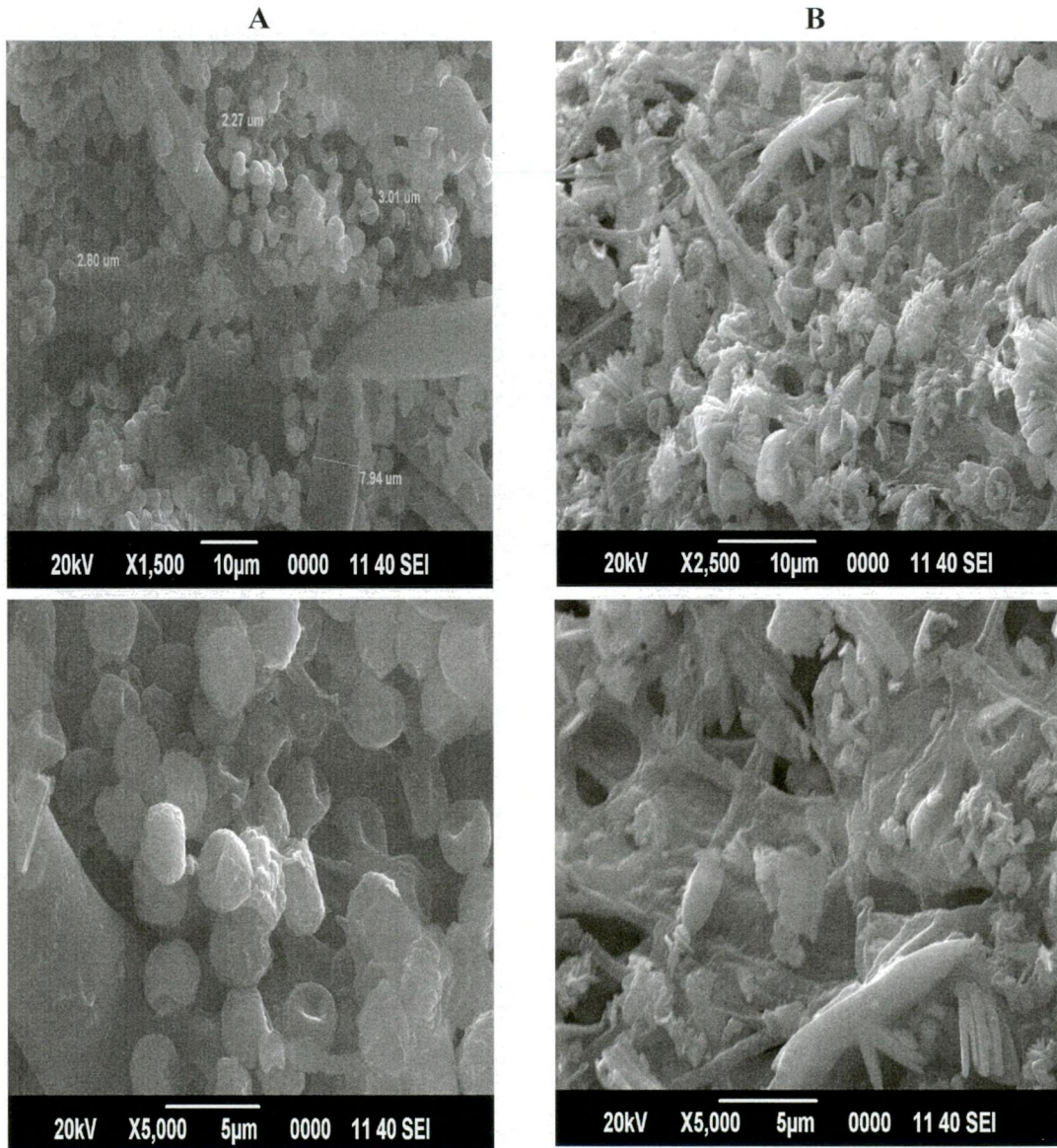
4.2.4. Evaluation of nickel absorption by scanning electron microscopy

The metal uptake in cells can induce different response mechanisms such as induction of molecules like siderophore, metallothioneins accompanied by specific changes in the cell morphology. The assessment of morphological changes in response to nickel accumulation in *A. niger* was performed by Scanning Electron Microscopy (SEM). From the SEM studies, it was observed that the mycelium of *A. niger* not exposed to nickel treatment (control) were cylindrical, septate and branched (Plate VIII A) and those treated with nickel were found to be flocculated and showed shrinkage in the cells (Plate VIII B)

Similar such SEM studies were conducted on the accumulation of chromium in the mycelium of *A. niger* seven days after incubation (Srivastava and Thakur, 2006) and lead ions in *Fusarium oxysporum* ten days after incubation (Sanyal, 2005) which provide supportive evidences for the present study.

PLATE VIII

SCANNING ELECTRON MICROGRAPHS DEPICTING THE EFFECT OF NICKEL ON THE CELL MORPHOLOGY AT DIFFERENT MAGNIFICATIONS



A – Scanning Electron Micrographs of *A. niger* before biosorption

B – Scanning Electron Micrographs of *A. niger* after biosorption

The higher efficiency of nickel removal by fungal biomass may be due to the high percentage of various components in the cell wall which show excellent metal binding properties (Gadd, 1990). They can be easily grown in substantial amount using unsophisticated fermentation techniques and inexpensive growth media. They are highly robust, tolerant to contaminants and could serve as an economical means for the removal and recovery of metal ions from aqueous solution (Kapoor *et al.*, 1999).

The high sorption capacity of heavy metals by *A. niger* might be due to the presence of neutral carbohydrates, hexosamine, acetyl content, lipids and phosphorus in their cell wall as suggested by Sag (2001) which may support the results of present study.

The results of the present study also falls in line with the findings of Malin and Bulow (2001) who reported that the detoxification of chromium by *A. niger* might be mediated either by the enzymatic antioxidant defense system (peroxidase, catalase and ascorbate peroxide) or phytochelatins which sequester metals inside the cells by binding through thiol coordination and limit damage to the metabolic process by reducing cytotoxic free metal ions.

It was also reported by Kapoor *et al.* (1999) that *A.niger* is known to produce citric acid during its growth. The citrate ion, being an efficient metal chelator remains on the surface of the biomass and helps in the removal of heavy metals. This study may give a strong support to the present investigation.

PHASE III

4.3. CHARACTERISATION OF NICKEL ELECTROPLATING EFFLUENT

4.3.1. Physical characteristics of raw and microbially treated nickel electroplating effluent

The results of the physical parameters analysed in raw and microbially treated nickel electroplating effluent are shown in Table II.

4.3.1. a. Colour and odour

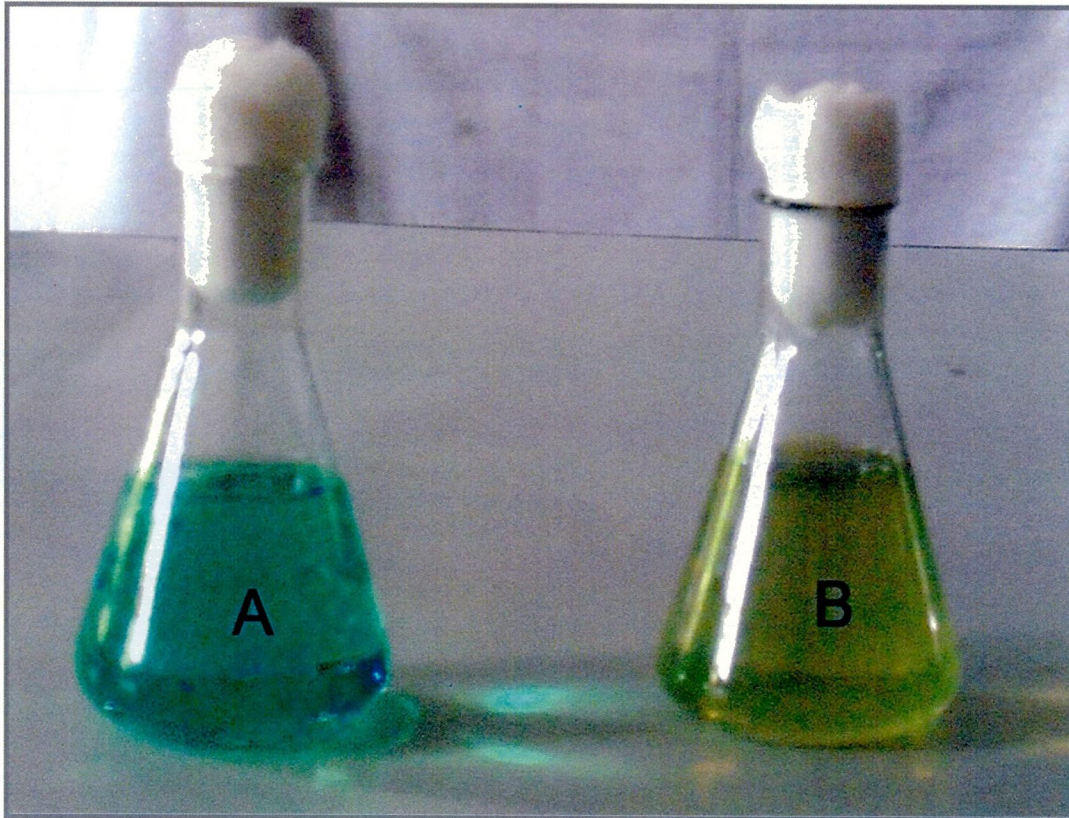
Observation of colour is the simplest test to determine the effectiveness of treatment of an effluent. It serves as a guide in deciding the quantity of chemicals to be used for the removal of colour and ensures economical treatment (Manivasakam, 1997).

It was observed from the Table II that the colour of the raw effluent was appeared to be green with an unpleasant odour and that of microbially treated nickel electroplating effluent was colourless and odourless (Plate IX).

The presence of colour is aesthetically undesirable and it increases the BOD and COD which leads to insufficient availability of oxygen to sustain aquatic life (Chaudhury *et al.*, 1998). The colour intensity of the effluent may depend on the pollutants that enter into it (Chukwu, 2006). Industrial wastes usually have their characteristic odour due to the presence of a large variety of contaminants or chemicals. Chlorine, added for disinfection of waters, may combine with certain impurities like phenolic compounds to produce high unpleasant odour (Goel, 1997). The results of the present study is in accordance with the findings of Prabhakaran (1990) who observed a maximum colour removal in the dye factory effluent treated with the species of *Aspergillus*, *Phanerochaete* and *Trametes*.

PLATE IX

BIOSORPTION OF NICKEL BY *ASPERGILLUS NIGER*



A – RAW NICKEL ELECTROPLATING EFFLUENT

B – NICKEL ELECTROPLATING EFFLUENT TREATED WITH *A. niger*

4.3.1. b. Turbidity

Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted with no change in direction or flux level through the sample (APHA, 1998).

The raw nickel electroplating effluent was observed to be turbid whereas the treated nickel electroplating effluent was clear. The turbidity of the raw effluent might be due to the discharge of large amounts of solids such as carbonates, bicarbonates, chlorides, sulphates, calcium and magnesium which are extensively used in the electroplating process.

4.3.1. c. pH

pH is a measure of hydrogen ion concentration or the hydrogen ion activity. pH is one of the most important parameters, as it indicates the acidity or alkalinity of an effluent. Based on the pH values all the treatment processes are carried out for further use of the effluents (Manivasakam, 1997).

The pH of the raw nickel electroplating effluent examined was 9.5 which exceeded the tolerance limits of 5.5-9.0 prescribed by the Bureau of Indian Standards. The pH of the treated nickel electroplating effluent was found to be 7.9 which fall within the limits specified by BIS.

Higher pH was also reported by Jeyanthi *et al.* (2001) when they analysed zinc electroplating effluent and their findings are in accordance with the present study. Begum and Noorjahan (2006) reported a high pH in untreated fertilizer effluent which after treatment with *Aspergillus niger* and *Phanerochaete chrysosporium* showed a

decrease. Similar such decrease was also observed in the present study in microbially treated nickel electroplating effluent.

4.3.1. d. Electrical Conductivity (EC)

Electrical conductivity is the ability of a substance to conduct the electric current. Water becomes a conductor of electrical current when substances are dissolved in it and the conductivity is proportional to the amount of dissolved substances which act as a conductor (Michael, 1984).

The electrical conductivity of the raw nickel electroplating effluent recorded a maximum of 5.32 mmhos / cm and that recorded in microbially treated nickel electroplating effluent was a minimum of 1.2 mmhos / cm. Higher EC in raw effluent indicates the presence of high amount of ionic substances.

Jeyanthi *et al.* (2001) reported a high EC value in the zinc electroplating effluent which is a supportive evidence for the present study. Jothimani and Elayarajan (2003) reported a reduction in the electrical conductivity by treating the raw textile dyeing effluent with fungal systems. Similar reduction was also recorded in the present study in microbially treated nickel electroplating effluent.

4.3.1. e. Total Suspended Solids (TSS)

The undissolved matter present in waste water is usually referred as suspended solids. It is one of the valuable parameters in judging the pollution potential of an effluent, pollution load on receiving streams and also to decide the efficiency of treatment units (Manivasakam, 1997).

TSS recorded in the raw nickel electroplating effluent of the present study was 600 mg/l which is six times greater than the

tolerance limits prescribed by the Bureau of Indian Standards (100 mg/l) whereas it was 87 mg/l in the microbially treated nickel electroplating effluent which satisfies the specified limits of BIS.

While analysing the metal finishing and plating industry waste waters Rao and Murthy (1992) also reported a high amount of total suspended solids. Higher amount of total suspended solids may elevate the density and turbidity of water, which in turn may affect the osmoregulation. Higher levels of TSS may also interfere with the photosynthesis by preventing sunlight (Kalita *et al.*, 2003). Total suspended solids when exceeding the limits are aesthetically unsatisfactory and may cause distress among human beings and livestock (APHA, 1985). High TSS level found in the raw nickel electroplating effluent in the present study was reduced after treatment with *A. niger*. Similar reduction in TSS was also reported (Lakshmi and Sridevi, 2009) in sugar factory effluent which was treated with *A. niger*.

4.3.1. f. Total Dissolved Solids (TDS)

Total dissolved solids are those which get dissolved in water and cannot be separated from water by filtration (APHA, 1985).

The amount of total dissolved salts present in the raw nickel electroplating effluent was recorded as 3200 mg/l which exceeded the tolerance limits (2100 mg/l) prescribed by the Bureau of Indian Standards (BIS) and that of microbially treated nickel electroplating effluent was only 1610 mg/l which was within the tolerance limits prescribed by BIS. Higher value of total dissolved solids is attributed to the presence of colloidal or finely divided suspended matter which does not readily settle. The presence of colloidal or finely divided suspended matter may be due to the direct discharge of solid wastes and construction activities around the catchment areas (Rajurkar *et al.*, 2003).

TABLE II
PHYSICAL CHARACTERISTICS OF THE RAW AND MICROBIALLY
TREATED NICKEL ELECTROPLATING EFFLUENT

Parameters	Nickel electroplating effluent		Tolerance limits
	Raw	Microbially Treated	
Colour	Green	Pale green to colourless	NM
Odour	Unpleasant odour	Odourless	Odourless
Turbidity	Turbid	Clear	-
pH	9.5	7.9	5.5 to 9.0 (BIS, 1981)
Electrical conductivity (mmhos/cm)	5.32	1.2	<2.25 (BIS, 1981)
Total Suspended Solids (mg / l)	600	87	100 (BIS, 1981)
Total Dissolved Solids (mg / l)	3200	1610	2100 (BIS, 1981)

BIS - Bureau of Indian standards.

High amount of TDS was also reported (Bachewer and Mehta, 2000) in zinc electroplating industrial effluent which may be in agreement with the present study.

The reduction of TDS in the effluent treated with *A. niger* in the present investigation is in accordance with the study conducted by Engade and Gupta (2007) who reported a maximum reduction of

TDS in the textile effluent treated with the dead biomass of *Saccharomyces cerevisiae*, *Aspergillus terreus* and *Rhizopus oligosporus*.

4.3.2. Chemical characteristics of raw and microbially treated nickel electroplating effluent

Table III presents the results of chemical parameters analysed in the raw and microbially treated nickel electroplating effluent.

4. 3. 2. a. Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand is taken as an indirect measure of water quality. It is infact a measure of the amount of oxygen required by microbes while stabilizing decomposable organic matter. Thus, the BOD values can be used as a measure of waste strength and also as an indicator of the degree of pollution (Sharma, 1997).

The biochemical oxygen demand in the raw nickel electroplating effluent was nil whereas in microbially treated effluent, it was found to be 24 mg/l which falls within the tolerance limits (30 mg/l) set by Bureau of Indian Standards for the discharge of effluents in inland surface waters.

The reduction in BOD was also reported in fertilizer effluent treated with *A. niger* and *Phanerochaete chrysosporium* (Begum and Noorjahan, 2006) and dye effluent treated with the species of *Aspergillus*, *Rhizopus* and *Geotrichum* (Anandapandian *et al.*, 2005). The results of the present study were found to be in accordance with these reports.

TABLE III
CHEMICAL CHARACTERISTICS OF RAW AND MICROBIALLY
TREATED NICKEL ELECTROPLATING EFFLUENT

Parameters	Nickel electroplating effluent		Tolerance limits
	Raw	Microbially treated	
Biochemical Oxygen Demand (BOD)	-	24	30 mg /l (BIS, 1981)
Chemical Oxygen Demand (COD)	1502	203	250 mg /l (BIS, 1981)
Total hardness	2729	196	250 mg /l (BIS, 1974)
Anions			
Total alkalinity	672	185	270 mg /l (BIS, 1993)
Chlorides	2749	543	1000 mg /l (BIS, 1981)
Sulphates	1449	833	1000 mg /l (BIS, 1981)
Phosphates	11	3	5 mg /l (BIS, 1981)
Ammoniacal nitrogen	81	31	50 mg /l (BIS, 1981)
Cations			
Calcium	273	56	75 (ISI, 1974)
Magnesium	255	34	50 mg /l (BIS, 1974)
Sodium	98	16	25 mg /l (BIS, 1974)
Potassium	43	7	20 mg /l (BIS, 1974)
Nickel	126	2	3 mg /l (BIS, 1981)
Oil and grease	28	7	10 mg /l (BIS, 1981)

All the values are expressed in mg / l

BIS - Bureau of Indian standards, ISI –Indian Standard Institutions

4. 3. 2. b. Chemical Oxygen Demand (COD)

Chemical oxygen demand is a test used to measure pollutants in natural and industrial waste waters. Chemical oxygen demand is the amount of oxygen required to oxidize the polluting chemicals to carbondioxide and water under controlled conditions (APHA, 1998).

The chemical oxygen demand estimated in the raw nickel electroplating effluent was 1502 mg/l and this was found to be about six times greater than the tolerance limit (250mg/l) prescribed by the Bureau of Indian Standards. The value of COD in microbially treated nickel electroplating effluent was 203 mg/l which was within the permissible limits of BIS.

Increase in COD was also reported (Suryawanshi *et al.*, 2004) in river samples where the electroplating effluent was discharged. The water with high level of COD when percolates into the ground, may affect its quality. The reduction in COD was also reported in paper mill effluent treated with *A.niger* (Kannan and Oblisami, 1990) and *Trichoderma versicolor* (Bajpai *et al.*, 1993). These studies may support the findings of the present investigation.

4.3.2. c. Total Hardness

Hardness of water is mainly due to the presence of calcium and magnesium ions, and is an important indicator of the toxic effect of poisonous elements (Tiwari, 2001).

The total hardness recorded in raw nickel electroplating effluent was 2729 mg/l which exceeded the tolerance limits prescribed by BIS (250 mg/l), whereas it was recorded as 196 mg/l in microbially treated nickel electroplating effluent which was below the specified limits.

Studies conducted by Borole and Patil (2004) in sugarcane industrial effluent also showed high total hardness which may support the present investigation. Hardness may be due to the presence of multivalent metal ions and the minerals dissolved in water. It has no hazardous effect but it increases the boiling point of water and inhibits lather formation with soap and causes scale formation in utensils (Janaki, 2001).

The results of the nickel electroplating effluent treated with *A. niger* is in accordance with the findings of Mala and Babu (2006) who reported the reduction of total hardness in the textile dyeing effluent treated with water hyacinth.

4.3.2.d. Anions {total alkalinity, chlorides, sulphates, phosphates and ammoniacal nitrogen}

Table III shows that the level of anions tested was greater in raw nickel electroplating effluent when compared to microbially treated nickel electroplating effluent.

Alkalinity is a measure of buffering capacity of the water and is important for aquatic life in a fresh water system because it equilibrates the pH ranges that occur naturally as a result of photosynthetic activity of aquatic plants (Kaushik and Saksena, 1999).

The level of total alkalinity estimated in the raw nickel electroplating effluent was 672 mg/l which is higher than the tolerance limits of 270 mg/l, whereas the value recorded in microbially treated nickel electroplating effluent was 185 mg/l which was found to be within the tolerance limits prescribed by BIS. Jothimani and Elayarajan (2003) reported higher level of total alkalinity in the dye factory effluent which may support the results of

the present study. Alkaline cleaning to remove oil and grease is a common practice prior to plating process. The cleaners contain carbonates, silicates, bicarbonates and phosphates which could have accounted for the increased level of total alkalinity in the effluent.

The concentration of chlorides in raw nickel electroplating effluent was 2749 mg/l which is almost three times higher than the prescribed limit (1000 mg/l) of BIS whereas that estimated in microbially treated nickel electroplating effluent was only 543 mg/l. Higher amount of chlorides was also reported in distillery effluent (Dhankhar and Singh, 2007). Large amounts of chlorides in the water leads to corrosiveness and may adversely affect water quality (Thresh *et al.*, 1994). Excess amount of chlorides in the effluent when discharged into the soil, may not be absorbed by the soil but they may move readily with soil water and are taken up by the crops and move in transpiration stream. When they get accumulated in the leaves, they may develop burns or drying off leaf tissue or even cause damages in the crops (Kalleshappa, 2008).

Sulphate is yet another parameter that has shown its contamination reporting 1449 mg/l in raw nickel electroplating effluent as against 1000 mg/l given by BIS. The amount of sulphates estimated in microbially treated nickel electroplating effluent was 833 mg/l which falls within the tolerance limits. Periyasamy and Rajan (2009) in their studies reported a high level of sulphates in electroplating industry effluent. High concentration of sulphates may be harmful to seedling stage and maturity of the plant (Chaudhary *et al.*, 2004). High amount of sulphates may cause laxative effect, diarrhoea and disorders of alimentary canal in human beings (Srinivas *et al.*, 2008). Less amount of sulphates present in the microbially treated effluent may corroborate with the results of

Mittal and Sengar (1989) who observed the removal of sulphate in higher level from the paper mill effluent using *Oscillatoria perornata* and *Scenedesmus quadricauda*.

The phosphate content in the raw nickel electroplating effluent was recorded as 11 mg/l which exceeded the tolerance limits of 5 mg/l prescribed by BIS. The amount of phosphates estimated in microbially treated nickel electroplating effluent was only 3 mg/l which falls within the tolerance limits specified. An increase in phosphate content was also reported in distillery effluent (Tharakeshwari and Jagannath, 2006) which may be a supporting evidence for the present investigation.

Removal of maximum amount of phosphates from domestic sewage was reported (Thanh and Simard, 1973) using the fungi such as *Trichothecium roseum*, *Cladosporium cladosporioides* and *Fusarium oxysporum*. The present findings on effluent treatment using *A. niger* also fall in line with the above results.

Ammoniacal nitrogen estimated in the raw nickel electroplating effluent was found to be 81 mg/l. The amount recorded in microbially treated nickel electroplating effluent was 31 mg/l which was within the tolerance limits (50 mg/l) prescribed by BIS. Manivasakam (1997) reported that in polluted waters, ammonia generally arises from the aerobic and anaerobic decomposition of the nitrogenous organic matter.

Higher levels of total alkalinity, chlorides, sulphates, phosphates and ammoniacal nitrates in dye effluent were found to be lower when treated with species of *Aspergillus*, *Rhizopus* and *Geotrichum* species (Anandapandian *et al.*, 2003). Similar result was obtained in the microbially treated effluent of the present study.

Studies conducted by Devi and Gowri (2007) on the removal of excess amount of ammoniacal nitrogen and phosphate present in the aquaculture waste water by using an alga *Enteromorpha flexuosa* may also support the present study.

4.3.2.e. Cations (calcium, magnesium, sodium, potassium and nickel)

The levels of calcium, magnesium, sodium, potassium and nickel estimated in the raw and microbially treated nickel electroplating effluent are presented in Table III.

The level of calcium and magnesium recorded in the raw nickel electroplating effluent were 273 mg/l and 255 mg/l respectively. These values were higher than the tolerance limits of 75 mg/l for calcium and 50 mg/l for magnesium as prescribed by ISI and BIS, whereas in microbially treated nickel electroplating effluent, the levels were 56 mg/l and 34 mg/l respectively for calcium and magnesium which falls within the permissible limits. High levels of calcium and magnesium were also reported in tannery effluent (Dadhich *et al.*, 2002) and in chromium electroplating effluent (Manju *et al.*, 2009). Excessive amount of calcium may cause problems related to urinary tract in human beings (Kumar and King, 2004). Higher concentration of magnesium results into encrustation of water supply structures and has an adverse effect on domestic use (Krishnamohan and Muthukrishnan, 1996).

In the present study 98 mg/l of sodium and 43 mg/l of potassium were recorded in the raw nickel electroplating effluent. These values exceeded the permissible limits. The levels of sodium and potassium in microbially treated nickel electroplating effluent were 16 mg/l and 7 mg/l respectively which were within the

permissible limits prescribed by BIS (25 and 20 mg/l). Higher amount of sodium and potassium were also reported in tannery effluent (Mariappan and Rajan, 2002) and in zinc electroplating industrial effluent (Bachewar and Mehta, 2000). Plating baths contain metal salts, acids, alkalies, ammonia, sodium and potassium as common cationic constituents which brightens the plating surface. This might be the reason for the increased levels of sodium, potassium and other cations in the electroplating industry effluents.

A significant reduction in the level of sodium, potassium, calcium and magnesium was observed in the paper mill effluent treated with *Westiellopsis prolifica* (Dash and Mishra, 1998) and this may be in accordance with the present study.

The level of nickel recorded in the effluent was 126 mg/l which exceeded the tolerance limit of 3 mg/l stipulated by the Bureau of Indian Standards. The nickel content in the microbially treated nickel electroplating effluent was estimated as 2 mg/l which was within the permissible limits prescribed by BIS. Higher level of nickel in the effluent may be due to the usage of nickel in the electroplating process which might have been let out in the spent water. Higher nickel content was also reported (Lokhande and Vaidya, 2004) in the industrial effluent of Kalyan-Dombivali (Maharashtra) and in nickel electroplating industrial effluent (Vijayaraghavan *et al.*, 2005). Excess quantities of nickel thrown into the biosphere might result in pollution hazards to the habitat in that area. Similar to the present investigation removal of excess amount of copper and nickel from the electroplating effluent using *A.niger* was also reported by Subudhi and Kar (2008). Higher amount of copper and zinc was reduced by *A. niger* in metal bearing effluent (Karavaiko *et al.*, 1996).

4.3.2.f. Oil and grease

The amount of oil and grease estimated in the raw nickel electroplating effluent was 28 mg/l which was higher than the permissible limits of Bureau of Indian Standards (10 mg/l). In the microbially treated nickel electroplating effluent, the amount of oil and grease was found to be 7 mg/l which was within the tolerance limits.

Oil is applied as lubricating medium in the machineries and also present as a surface active agent. Poonkothai and Parvatham (2005) observed a high level of oil and grease in automobile waste water. Anandapandian *et al.* (2003) reported that the amount of oil and grease in the dye effluent were reduced to a great extent when treated with species of *Aspergillus*, *Rhizopus* and *Geotrichum* which may support the results of the present study.

4.4. IMPACT OF NICKEL ELECTROPLATING EFFLUENT (UNTREATED AND TREATED) ON THE GROWTH OF GREEN GRAM PLANTS

A pilot study was conducted to assess the impact of tap water (T₁), untreated nickel electroplating effluent (T₂) and treated nickel electroplating effluent (T₃) on seed germination and vigour index of green gram seeds.

4.4.1. Effect of nickel electroplating effluent on seed germination and seedling development

The results of seed germination percentage, shoot and root lengths, vigour index, fresh and dry weights of green gram seedlings grown in tap water (T₁), untreated nickel electroplating effluent (T₂) and treated nickel electroplating effluent (T₃) are presented in Table IV. Plate X shows the growth of green gram seedlings seven days after sowing.

TABLE IV
BIOMETRIC PARAMETERS IN 7 DAYS OLD GREEN GRAM
SEEDLINGS GROWN IN DIFFERENT TREATMENTS

Treatments	Germination Percentage	Shoot length (cm)	Root length (cm)	Vigour index	Fresh weight (g)	Dry weight (g)
T ₁	93.3	16	5.33	1994	0.21	0.099
T ₂	86.6	10.1	2.96	1537	0.187	0.043
T ₃	96.6	19.3	7.7	2612	0.303	0.187
CD (5%)		0.2179	0.9901		0.0022	0.002

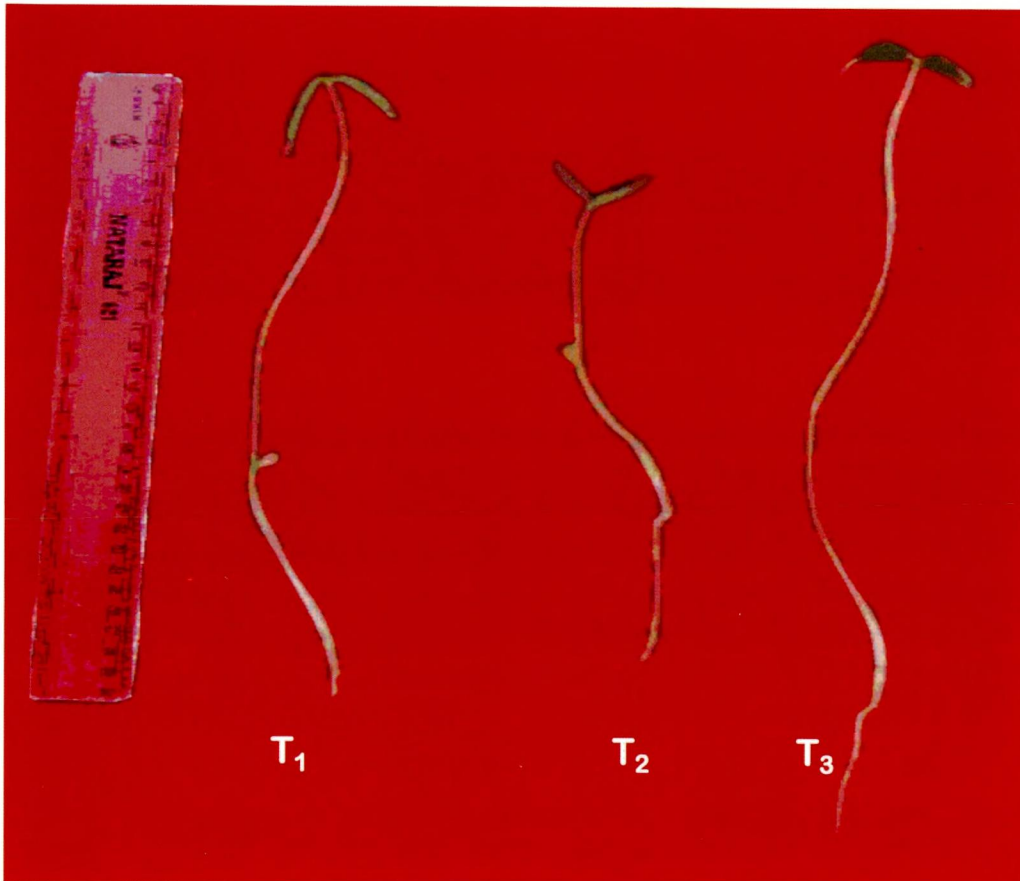
The values are mean of triplicates

T₁ -control, T₂ - untreated effluent, T₃ - treated effluent

4.4.1. a. Germination percentage

A maximum of 96.6 per cent germination was recorded in green gram seeds grown in microbially treated nickel electroplating effluent (T₃) followed by 93.3 per cent in tap water (T₁). A minimum of 86.6 per cent germination was recorded in seeds grown with untreated nickel electroplating effluent (T₂). This reduction in seed germination can also be supported with similar such observations made by several researchers in various agricultural crops grown in dyeing (Kumar and Bishwas, 2005 and Rajeswari, *et al.*, 2005), distillery (Virendra and Dhar, 2002 and Surendra *et al.*, 2005), dairy (Sangeetha *et al.*, 2005 and Dhanam, 2009), tannery (Thangavel and Balagurunathan, 2002), brewery (Pandey, 2006), rubber factory (Augusthy and Mani, 2001), paper (Choudhury *et al.*, 2004; Raina and Agarwal, 2003; Kavitha *et al.*, 2005; Bhargava and Sonali, 2005) and pulp mill (Medhi *et al.*, 2008) effluents.

PLATE X



**SHOOT AND ROOT LENGTHS OF 7 DAYS OLD GREEN GRAM
SEEDLINGS EMERGED FROM DIFFERENT TREATMENTS**

T₁ - CONTROL

T₂ - UNTREATED NICKEL ELECTROPLATING EFFLUENT

T₃ - TREATED NICKEL ELECTROPLATING EFFLUENT

This reduction in seed germination may be due to the presence of high amount of physical and chemical constituents in the effluent which might have caused physical and biological disturbance to the seeds (Debojit and Rao, 1994 and Umamaheswari *et al.*, 2003).

The germinating seeds may get less oxygen due to high COD in nickel electroplating effluent thereby restricting their energy supply through aerobic respiration which is essential for the growth and the development of young seedlings. It may be possible that different types of ions which are present in the effluent might have inhibited the activity of hydrolytic enzymes (peroxidase and acid phosphatase) required at the time of germination and early growth (Singh *et al.*, 2004 and Yadav and Minakshi, 2006).

Higher percentage of germination in green gram seeds grown in microbially treated nickel electroplating effluent might be due to maximum absorption of nickel ions by *A.niger*. Microbially treated effluent in the present study contained less toxic nutrients which might have induced maximum germination in the seeds of green gram. The results of the present study is in consonance with the findings of Jothimani and Elayarajan (2003) who reported the same in dye effluent treated with biological systems. Srivastava (2007) reported that trace level of nickel is necessary for the seeds to germinate to a minimum level.

4.4.1.b. Shoot length and root length

Shoot lengths of seven day old green gram seedlings after treatment with tap water (T_1), untreated nickel electroplating effluent (T_2) and treated nickel electroplating effluent (T_3) are recorded in Table IV. The values recorded for the shoot lengths of green gram seedlings were 16, 10.1 and 19.3 cm in T_1 , T_2 and T_3 respectively.

The values recorded for the seedlings grown in treated and untreated effluents are highly significant when compared with control.

Similar trend was also observed in root lengths of green gram seedlings. The root lengths of seedlings recorded seven days after treatment were 5.33 cm in T₁, 2.96 cm in T₂ and 7.7 cm in T₃ and these values show significant difference between the treatments and control.

4.4.1. c. Vigour index

It was evident from the Table IV that the maximum vigour index of 2612 was observed in T₃ followed by 1994 in T₁. T₂ exhibited minimum vigour index of 1537 on 7th day after sowing.

Decrease in the vigour of seeds irrigated with polluted water observed in the present study might be due to the interaction of different pollutants with the developing radical. In early stages of germination, the membrane of the embryonic axis could have increased permeability thereby permitting the entry of pollutants and this might have reduced the vigour of seeds. During germination, number of physiological processes contributes to the growth of plants which are responsible for loss of vigour. Seed vigour may also be correlated with physiological and chemical properties of axes than with those of whole seeds (Andersen and Abdul Baki, 1971).

The maximum vigour index in green gram seedlings grown using treated nickel electroplating effluent of the present study was in accordance with the results of Jothimani and Elayarajan (2003) who observed the same trend in green gram seedlings grown using microbially degraded dyeing effluent.

4.4.1. d. Fresh weight and dry weight

Fresh and dry weight of green gram seedlings grown using different treatments were presented in Table IV. The maximum fresh weight recorded in seven day old seedlings grown in treated nickel electroplating effluent (T_3) was 0.303g which is followed by 0.21g in tap water (T_1). A minimum weight of 0.187g was recorded in seedlings grown in untreated nickel electroplating effluent (T_2). The dry weight of the seedlings were found to be 0.187g in T_3 , 0.099g in T_1 and 0.043g in T_2 (Table IV). The weights (fresh and dry) of green gram seedlings also proved to be highly significant when compared with T_2 and T_1 .

4.4.2. Biometric parameters of green gram plants on 30th and 60th days

In continuation of the seed germination study, a pot culture experiment was conducted to assess the growth performance of green gram plants using different treatments (tap water - T_1 , untreated nickel electroplating effluent - T_2 and treated nickel electroplating effluent - T_3) on 30 and 60 days after sowing.

4.4.2. a. Shoot length and root length

Table V, Plate XI and XII represent the shoot and root lengths of green gram plants grown in tap water (T_1), untreated nickel electroplating effluent (T_2) and treated nickel electroplating effluent (T_3) 30 and 60 days after sowing.

Thirty days after treatment with tap water (T_1), untreated nickel electroplating effluent (T_2) and treated nickel electroplating effluent (T_3), the shoot lengths of green gram plants were measured and recorded in Table V.

TABLE V

GROWTH PARAMETERS (SHOOT LENGTH, ROOT LENGTH, FRESH WEIGHT AND DRY WEIGHT) OF GREEN GRAM PLANTS GROWN IN DIFFERENT TREATMENTS

Treatments	Length (cm)				Weight (g)			
	Shoot		Root		Fresh		Dry	
	30 th day	60 th day	30 th day	60 th day	30 th day	60 th day	30 th day	60 th day
T ₁	23.11	31.14	8.71	15.73	1.77	6.05	0.91	2.71
T ₂	14.7 (-36.39)	22.54 (-27.61)	5.48 (-37.09)	11.42 (-27.4)	1.02 (-42.37)	3.98 (-34.21)	0.71 (-21.98)	2.29 (-15.50)
T ₃	25.44 (10.08)	36.41 (16.92)	10.76 (23.54)	20.33 (29.24)	2.96 (67.23)	10.7 (76.85)	1.71 (87.91)	5.29 (95.20)
CD (5%)	0.04256		0.07901		0.04546		0.0478	

The values are mean of triplicates

Figures in the parentheses are percentage decrease / increase over control

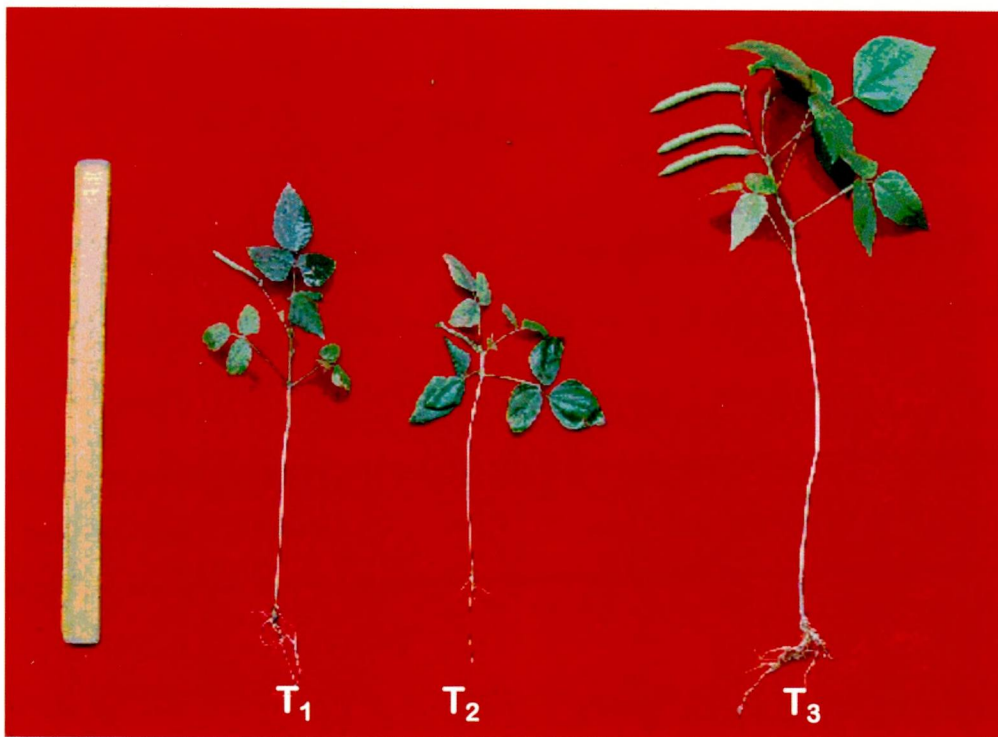
T₁ - Control, T₂ - Untreated effluent, T₃ - Treated effluent

PLATE XI



SHOOT AND ROOT LENGTH OF 30 DAYS OLD GREEN GRAM PLANTS GROWN IN DIFFERENT TREATMENTS

PLATE XII



SHOOT AND ROOT LENGTH OF 60 DAYS OLD GREEN GRAM PLANTS GROWN IN DIFFERENT TREATMENTS

The plants grown in treated effluent (T_3) recorded a maximum shoot length of 25.44 cm which is followed by 23.11 cm and 14.7 cm respectively in plants grown in tap water (T_1) and untreated effluent (T_2). At the end of the 60th day the shoot lengths of green gram plants showed an increase when compared with 30 days old plants. The values recorded were 36.41 cm in T_3 , 31.14 cm in T_1 and 22.54 cm in T_2 . Statistically there was a significant increase in shoot length of T_3 plants when compared with T_1 and T_2 plants.

The shoot lengths of green gram plants grown using treated effluent (T_3) showed an increase of 10.08 per cent over the control on 30th day and 16.92 per cent on 60th day. Decrease in shoot length was recorded in plants grown using untreated effluent (T_2) which was 36.39 per cent on 30th day and 27.61 per cent on 60th day.

Maximum root lengths of 10.76 cm were observed in T_3 plants on 30th day and 20.33 cm on 60th day. Followed by T_3 , the root lengths recorded in T_1 plants were 8.71 cm on 30th day and 15.73 cm on 60th day. The root lengths were found to be minimum in green gram plants grown using untreated effluent (T_2). The values recorded were 5.48 cm on 30th day and 11.42 cm on 60th day. There was a significant increase in the root length of T_3 plants when compared with control (T_1) and T_2 plants (Table V).

The root lengths of T_3 plants showed an increase of 23.54 per cent on 30th day and 29.24 per cent of increase on 60th day over the control. Decrease in the root length was recorded in plants grown using untreated effluent (T_2) which was 37.09 per cent on 30th day and 27.4 per cent on 60th day.

From the above results it was observed that the reduction in shoot and root length of seedlings grown using T_2 might be due to the

presence of high amount of salts in the nickel electroplating effluent. Suppression of shoot and root lengths of green gram plants in the present investigation is in accordance with the earlier studies conducted in *Vigna radiata* grown using zinc (Selvaraju, 1999), distillery (Subramani *et al.*, 1995 and Surendra *et al.*, 2005) and paper mill (Joshi and Tandon, 2003 and Luna *et al.*, 2005) effluents, wheat treated with aluminium (Shen *et al.*, 1993), *Triticum aestivum* with cadmium (Kalita *et al.*, 1993 and Singh *et al.*, 2005), *Pennisetum americanum* with cobalt (Burhan and Tahira, 2001), *Phaseolus aureus* with paper mill effluent (Suresh, 2005), *Phaseolus mungo* with steel factory effluent (Suresh, 2006) and *Cicer arietinum* with fertilizer factory effluent (Pratap *et al.*, 2006).

The reduction in shoot and root length of T₂ plants might be due to the heavy metal stress which could have restricted the rooting by increasing the soil osmotic pressure (Evers *et al.*, 1997) or by inhibiting the transport of nutrients and water to the plant parts (Shanker *et al.*, 2005).

The results of the shoot and root lengths obtained from the plants grown in treated effluent is in accordance with the findings of Jothimani and Elayarajan (2003) who recorded a maximum root and shoot lengths in black gram and green gram plants grown in microbially degraded dyeing effluent.

4.4.2. b. Fresh and dry weight of green gram plants

Fresh and dry weights of green gram plants treated with T₁ (tap water), T₂ (untreated nickel electroplating effluent) and T₃ (treated nickel electroplating effluent) on 30th and 60th days are presented in Table V.

Green gram plants grown using treated nickel electroplating effluent (T₃) recorded a maximum fresh weight of 2.96 g and 10.7 g and a dry weight of 1.71 g, 5.29 g on 30th and 60th days respectively. Followed by this the plants grown in T₁ (tap water) recorded 1.77 g of fresh weight and 0.91 g of dry weight on 30th day. On 60th day the fresh and dry weights recorded were 6.05 g and 2.71 g respectively. The values recorded for the fresh and dry weights of T₂ plants on 30th day were 1.02 g and 0.71 g and that recorded on 60th day was 3.98 g and 2.29 g respectively. A significant increase in fresh and dry weight was noted in T₃ plants when compared with control (T₁) and T₂ plants.

An increase of 67.23 per cent and 87.91 per cent of fresh and dry weight on 30th day and 76.85 per cent and 95.20 per cent on 60th day over control was observed in T₃ plants. Percentage decrease in fresh and dry weight of green gram plants grown using untreated effluent (T₂) were 42.37 and 21.98 on 30th day and 34.21 and 15.50 on 60th day respectively over the control.

A decline in the fresh and dry weights of green gram plants grown using untreated effluent (T₂) in the present study was in confirmation with the observations made in Urd bean treated with rubber factory effluent (Sharma *et al.*, 1997), rice with cardboard factory effluent (Dixit *et al.*, 1986), cow pea with fertilizer factory effluent (Subramani *et al.*, 1998), *Cicer arietinum* with dyeing effluent (Rao and Nandhakumar, 1983), mung bean (Bindhu and Bera, 2001) and barley (Aery and Rana, 2003) with cadmium, black gram with cobalt (Jayakumar and Vijayarengan, 2006), green gram with zinc (Balashouri and Prameeladevi, 1995) and pigeon pea with turpentine factory effluent (Antaryam *et al.*, 1997). The reduction in the fresh and dry weight of the green gram plants selected for the present study may be due to the toxic effect of nickel in the untreated effluent which might have inhibited water uptake resulting in the retardation of plant growth (Ganesh *et al.*, 2006).

Increase in fresh and dry weight of plants grown in microbially treated effluent is in consonance with the results of Ramakrishnan *et al.* (2001) in paddy grown in sugar mill effluent treated with yeast.

4.4.2. c. Number and weight of pods/plant

The number and weight of pods per green gram plant on 60th day using tap water (T₁), untreated nickel electroplating effluent (T₂) and treated nickel electroplating effluent (T₃) are presented in Table VI.

The number of pods per plant recorded was 5.18 in T₁, 3.06 in T₂ and 8.3 in T₃ plants on 60th day. Number of pods showed an increase of 60.23 per cent in T₃ and a decrease of 40.92 per cent in T₂ over control. The values of T₃ showed a significant increase over control.

TABLE VI
YIELD PARAMETERS (NUMBER AND WEIGHT OF PODS/PLANT)
OF GREEN GRAM PLANTS (60th day)

Treatments	Number of pods / plant	Pod weight / plant (g)
T ₁	5.18	3.2
T ₂	3.06 (-40.92)	0.98 (-69.38)
T ₃	8.3 (60.23)	4.73 (47.81)
CD (5%)	0.2578	0.3716

The values are mean of triplicates

Figures in the parentheses are percentage decrease / increase over control

T₁ - Control, T₂ - Untreated effluent, T₃ - Treated effluent

Table VI represents the weight of the pods produced by green gram plants grown in different treatments on 60th day. The weight of the pods/plant was observed to be 3.2g, 0.98g and 4.73g in T₁, T₂ and T₃ plants respectively. An increased weight of 47.81 per cent was observed in T₃ plants and the weight of the pods decreased to 69.38 per cent in T₂ plants. Difference in pod weight was highly significant between the treatments and the control.

Reduction in the yield and weight of pods was also observed in various plants treated with different types of heavy metal effluents. Reports on *Vigna mungo* grown using aluminium (Kumar and Bhargava, 2006), mustard with zinc (Chatterjee *et al.*, 2005), sunflower with cadmium (Agarwal *et al.*, 1995) and brinjal with copper (Neelima and Reddy, 2004) supported the findings of the present investigation, whereas an increase in dry weight and grain yield was reported (Saxena and Singh, 1974 and Shrikrishna and Singh, 1992) in pulses when zinc was applied.

4.4.3. Analysis of selected biochemical components in green gram plants grown in different treatments

Various biochemical components (total proteins, total carbohydrates, total chlorophyll and nickel) are analysed in green gram plants on 30 and 60 days after treatment with tap water (T₁), untreated nickel electroplating effluent (T₂) and treated nickel electroplating effluent (T₃) and the results obtained are presented in Table VII.

4.4.3. a. Total protein content

The total protein content analysed from the green gram plants grown in the soil treated with tap water (T₁), untreated nickel

electroplating effluent (T₂) and treated nickel electroplating effluent (T₃) is presented in Table VII.

It was observed from the Table VII that the green gram plants grown with treated effluent (T₃) showed increased amount of total protein when compared to control (T₁) and untreated effluent (T₂). The amount of total proteins recorded in T₃ plants on 30th day and 60th day was 22.83 mg/g and 37.95 mg/g respectively. The plants treated with tap water (T₁) was found to contain 15.18 mg/g and 24.43 mg/g of total proteins and that of T₂ plants was estimated to be 8.62 mg/g and 16.21 mg/g respectively on 30th and 60th days. As per the values presented in the table, the total protein content of the green gram showed a significant increase in T₃ plants when compared with T₁ and T₂ plants.

An increase of 50.4 and 55.34 per cent of total proteins was observed in plants grown using treated effluent (T₃) on 30th and 60th days respectively. Percentage decrease in total protein content in the plants grown using untreated effluent (T₂) was 43.21 per cent on 30th day and 33.65 per cent on 60th day.

The results of the present study agree with the reports of Dikshit and Pathak (1992) in gooseberry, Ashraf (1994) in *Fruca sativa* who had stated a decrease in total protein content when they treated the selected variety with saline. Decrease in protein content was also reported in soyabean treated with increasing concentrations of textile effluent (Vijayakumari, 2003), *Vigna radiata* treated with copper (Manivasagaperumal and Vijayarengan, 2005), *Cicer arietinum* with distillery effluent (Pandey and Neraliyu, 2002) and *Phaseolus mungo* seedlings treated with sewage water (Muthuchelian *et al.*, 1998). These reports may support the findings of the present study.

The heavy metals when transported into plants may reduce protein content by causing leakage of the plant material, induce the catabolic enzymes to destroy the proteins, disrupt the nitrogen metabolism of plants, reduce the sulphhydryl group of proteins and cause deleterious effect to the normal form of proteins (Rai *et al.*, 1992).

TABLE VII

TOTAL PROTEIN AND CARBOHYDRATE CONTENTS OF GREEN GRAM PLANTS GROWN IN DIFFERENT TREATMENTS

Treatments	Total protein content (mg/g)		Total carbohydrate content (mg/g)	
	30 days	60 days	30 days	60 days
T ₁	15.18	24.43	13.38	23.72
T ₂	8.62 (-43.21)	16.21 (-33.65)	7.34 (-45.14)	15.61 (-34.2)
T ₃	22.83 (50.4)	37.95 (55.34)	18.53 (38.63)	33.86 (42.74)
CD (5%)	0.04497		0.0434	

The values are mean of triplicates

Figures in the parentheses are percentage decrease / increase over control

T₁ - Control, T₂ - Untreated effluent, T₃ - Treated effluent

The total protein content of the present study was found to be high in green gram plants grown using treated effluent which is in accordance with the findings of Paneerselvam *et al.* (2008) who

reported the same in *Phaseolus trilobus* grown using dairy effluent treated with *Glomus fasciculatum* (Arbuscular Mycorrhizal Fungi).

4.4.3. b. Total carbohydrate content

Table VII represents the amount of total carbohydrates present in the green gram plants grown using tap water (T₁), untreated nickel electroplating effluent (T₂) and treated nickel electroplating effluent (T₃).

It was observed from the Table VII that the amount of total carbohydrates was found to be high in green gram plants grown using treated effluent (T₃) which was 18.53 mg/g and 33.86 mg/g respectively on 30th and 60th days. The plants grown in tap water (T₁) recorded an amount of 13.38 mg/g and 23.72 mg/g of total carbohydrates whereas a minimum of 7.34 mg/g and 15.61 mg/g was estimated in plants grown using untreated effluent (T₂) on 30th and 60th days respectively.

Decrease in the amount of total carbohydrates was found to be 45.14 per cent and 34.2 per cent in the plants grown using untreated effluent (T₂). An increase of 38.63 per cent and 42.74 per cent was recorded in the plants grown using treated effluent (T₃) on 30th and 60th days respectively. All the values recorded for the two treatments showed a significant difference over control.

Reduction in total carbohydrate content was also observed in various crops like *Beta vulgaris* grown in cadmium (Greger and Lindberg, 1986), ragi (Lakshmi and Sundramoorthy, 2000) and raddish (Indira and Mohanty, 2006) treated with sugar mill effluent. The above observations are in agreement with the present study.

The decrease in total carbohydrate content of the plants grown using untreated effluent might be due to deranged metabolism and poor translocation of sugars and other metabolites to the growing parts of the plants (Lakshmi and Sundramoorthy, 2000).

Increased levels of total carbohydrate content recorded in plants grown using treated effluent (T_3) in the present study is in confirmity with the findings of Paneerselvam *et al.* (2008) who reported similar such increase in *Phaseolus trilobus* grown in dairy effluent treated with Arbuscular Mycorrizhal Fungi.

4.4.3. c. Total chlorophyll content

Table VIII shows the amount of total chlorophyll content present in the leaves of the experimental plant (green gram) grown using tap water (T_1), untreated nickel electroplating effluent (T_2) and treated nickel electroplating effluent (T_3).

Green gram plants grown using treated nickel electroplating effluent (T_3) recorded a high total chlorophyll content of 0.34 mg/g and 0.77 mg/g respectively on 30th and 60th days, followed by the plants grown in tap water (T_1) which recorded 0.22 mg/g on 30th day and 0.49 mg/g on 60th day. Minimum total chlorophyll content of 0.14 mg/g and 0.34 mg/g was recorded in plants grown using untreated nickel electroplating effluent on 30th and 60th days respectively. Total chlorophyll levels were significantly increased in green gram plants grown using treated effluent in comparison with that of plants grown in untreated effluent and tap water.

The percentage increase in total chlorophyll content was 54.54 on 30th day and 57.14 on 60th day in green gram plants grown using treated nickel electroplating effluent (T_3). The green gram plants grown using untreated nickel electroplating effluent (T_2) recorded a

decrease of 36.36 per cent and 30.61 per cent of total chlorophyll content respectively on 30th and 60th days.

TABLE VIII
TOTAL CHLOROPHYLL CONTENT IN THE LEAVES OF GREEN GRAM GROWN IN DIFFERENT TREATMENTS

Treatment	Total chlorophyll content (mg/g)	
	30 days	60 days
T ₁	0.22	0.49
T ₂	0.14 (-36.36)	0.34 (-30.61)
T ₃	0.34 (54.54)	0.77 (57.14)
CD (5%)	0.00484	

The values are mean of triplicates

Figures in the parentheses are percentage decrease / increase over control

T₁ - Control, T₂ - Untreated effluent, T₃ - Treated effluent

The increase in seedling growth of the green gram plants grown using treated effluent (T₃) had stimulatory effect on chlorophyll content whereas untreated effluent (T₂) showed adverse effect when compared with control (T₁).

Leaves are the main assimilatory organs chiefly concerned with photosynthesis and the leaf area is a reliable index for determining the over all metabolic efficiency of a plant (Singh, 1992).

The decrease in chlorophyll content might be due to inhibition of photosynthetic electron transport (Bohner *et al.*, 1980), the unavailability of iron for the production of chlorophyll precursor (Mengal and Kirkby, 1987), direct interference in the incorporation of iron in enzyme porphyrin (Assche and Clijsters, 1999) and interaction of the metal with functional sulphhydryl (-SH) groups of the enzymes of chlorophyll biosynthesis (Prasad and Prasad, 1987). Treatment of plants with higher concentrations of effluent might impair photosynthetic ability of the plants, causing chlorosis in them (Baneerjee *et al.*, 2006).

Higher concentration of nickel and other heavy metals were reported to decrease the chlorophyll content of tomato and barley (Stobart *et al.*, 1985), vegetable crops (Veer, 1998), groundnut (Bhanumathi *et al.*, 2005), some medicinal plants (Sandhya *et al.*, 2005) and soybean and pigeon pea (Chikile and Sharma, 2008).

Higher amount of chlorophyll content in T₃ plants may be supported by the works of Subramaniam *et al.* (1999) who observed the same trend in green gram plants grown in *Ceratophyllum demersum* treated distillery effluent.

4.4.3. d. Nickel content in the plants and pods of green gram

The results obtained from the present study on the nickel content in the plants and pods of green gram are presented in Table IX.

Nickel content estimated on 30th day in green gram plants grown using untreated effluent (T₂) was found to be a maximum of 2.53 mg/kg which is followed by the plants grown using treated effluent (T₃) which was 1.72 mg/kg. A minimum of 0.28 mg/kg of

nickel was estimated in plants grown using tap water (T₁). On 60th day after treatment the amount of nickel estimated was 0.39 mg/kg in T₁, 7.2 mg/kg in T₂ and 4.11 mg/kg in T₃ plants. The values of T₁ and T₃ were within the tolerance limits (0.02 - 5 mg/kg) prescribed by WHO (1989). Statistical analysis showed an increased nickel content in T₂ plants when compared with T₁ and T₃ plants.

TABLE IX

NICKEL CONTENT IN THE GREEN GRAM PLANTS AND PODS

Treatments	Nickel content		
	Plant sample (mg / kg)		Pods (mg / g)
	30 days	60 days	60 days
T ₁	0.28	0.39	0.26
T ₂	2.53	7.2	1.5
T ₃	1.72	4.11	0.72
CD (5%)	0.0349		0.1273

The values are mean of triplicates

T₁ - Control, T₂ - Untreated effluent, T₃ - Treated effluent

The amount of nickel estimated in the pods of green gram was 0.26 mg/g in T₁, 1.5 mg/g in T₂ and 0.72 mg/g in T₃. The values of T₁ and T₂ were within the tolerance limits (1 mg/g) prescribed by WHO (1989). Various researchers have reported the accumulation of higher levels of heavy metals namely copper (Sagoo *et al.*, 2004), chromium, iron, manganese, copper and zinc

(Shailendra *et al.*, 1993) in the leaves of amaranthus irrigated with industrial effluents. Higher level of chromium was accumulated in brinjal and chillies (Banu *et al.*, 1998) and in *Bacopa monnieri* (Shukla *et al.*, 2005), cadmium, zinc and nickel in stems, roots and leaves of *Lycopersicum esculentum* (Harikrishan and Kumar, 2009), cadmium, lead and mercury in corn (Saleh, 2001 and Chikile and Sharma, 2008), zinc in the rhizome of *Nelumbo nucifera* (Arab and Donia, 2000) and nickel in *Raphanus sativus* and *Spinacia oleracia* (Pandey, 2006). The above studies may support the findings of the present investigation.

Application of nickel electroplating effluent in soil reduces the dry matter yield of green gram pods. Similar such observation was made by Zupancic *et al.* (2004) in the yield of tomato and egg plant. Nickel in soil might have disturbed the uptake of nutrient and decreased its content in plants. Khan and Moheman (2006) investigated the effect of cadmium and nickel on the sunflower and chilly plants. They reported that the accumulation of these metals was found to be high in the seeds of the above plants. The above studies are supportive evidences for the present findings.

The accumulation of heavy metals in the pods of green gram grown using treated nickel electroplating effluent was within the tolerance limit and this may be due to the metal ions that are held by the chelators, thereby they induce the defence mechanism to reduce metal toxicity in the cells. The present study falls in line with the findings of Indra and Singaram (2009) who reported that the textile wash water treated with the species of *Anabaena*, *Plectonema* and *Westilopsis* enhanced nickel uptake and increased the growth of plants.

The results of the present study coincide with the observations of Chen *et al.* (2005) who reported that mixed Arbuscular Mycorrhizal Fungi (AMF) inocula enhanced lead uptake and increased the growth of *Ixeris denticulate*. Joner and Leyval (1997) reported that the uptake of cadmium and lead by AMF from polluted soils had enhanced the growth of *Trifolium subterraneum*, *Pinus sylvestris* (Jonarath and Finlay, 2001) and *Ixeris denticulate* (Chen, 2005).

4.4.4 Impact of untreated and treated nickel electroplating effluent on the soil characters

The soil before treatment with tap water, untreated and treated nickel electroplating effluent was analysed for selected physicochemical characters and the results obtained are presented in Table X.

pH of the soil was recorded as 6.8 (slightly acidic) and electrical conductivity as 0.51 mmhos/cm. The organic carbon analysed was 1.02 per cent. The macronutrients estimated in the above soil are shown in Table X. An amount of 285 kg/ha nitrogen, 11.75 kg/ha phosphorous, 191.34 kg/ha potassium, 190.30 kg/ha sodium, 10.16 kg/ha calcium and 5.3 kg/ha magnesium were recorded from the soil analysis. The values of micronutrients namely zinc, iron, manganese and copper in the soil recorded were 0.54 ppm, 2.34 ppm, 0.89 ppm and 0.26 ppm respectively. The amount of nickel contained in the soil was 0.93 mg/kg. All the values recorded were found to be within the tolerance limits specified (Arnold, 1984; Gupta, 2007; FAO, 1980; WHO, 1989).

TABLE X
PHYSICOCHEMICAL CHARACTERS OF THE PRETREATED SOIL

Parameters	Quantity	Tolerance limits
*pH	6.8	6.5 – 8.4
*Electrical conductivity (mmhos/cm)	0.51	<1 (normal), 1-4 (critical), >4 (injurious to crop)
*Organic carbon (%)	1.02	<1 (low-deficient), 1-3 (normal), >3 (high-excess)
**Available Macronutrients (kg / ha)		
Nitrogen	285	0-280 (low), 280-450 (medium), >450 (high)
Phosphorous	11.75	0-11 (low), 11-22 (medium), >22 (high)
Potassium	191.34	0-118 (low), 118-280 (medium), >280 (high)
Sodium	190.30	0-118 (low), 118-280 (medium), >280(high)
Calcium	10.16	0.5 (low), 5-10 (medium), 10-20(normal), >20 (high-excess)
Magnesium	5.3	0.5 (low), 5-10 (medium), 10-20 (normal), >20 (high-excess)
***Micronutrients (ppm)		
Zn	0.54	1.2
Fe	2.34	6.3
Mn	0.89	2
Cu	0.26	0.72
Ni (mg / kg)	0.93	2-5 (WHO, 1989)

* denotes the limits prescribed by Arnold (1984)

** denotes the limits prescribed by Gupta (2007)

*** denotes the limits prescribed by FAO (1980)

WHO - World Health Organization

4.4.4.1. Physicochemical characters of untreated and treated soil at the end of the experimental period (60th day)

At the termination (60th day) of the pot culture experiment, the soil samples treated with tap water (T₁), untreated nickel electroplating effluent (T₂) and treated nickel electroplating effluent (T₃) were collected and subjected for various physicochemical analyses. The results obtained from the analyses are recorded and presented in Table XI.

4.4.4.1. a. pH

From the values recorded for pH it is clearly evident that there was a significant change in soil pH in all the treatments used. The pH recorded was 7.23 in T₁, 9 in T₂ and 8.13 in T₃. The pH of T₁ and T₃ were within the tolerance limits (6.5-8.4) prescribed by Arnold (1984) whereas it exceeded the tolerance limit in T₂. The pH of the soil sample differed significantly in T₂ and T₃ treatments when compared with control.

The measure of soil pH is an important parameter which helps in the identification of chemical nature of the soil. The pH of the soil induces the activity of beneficial microorganisms which in turn enhances the plant growth. Fungi are reported to tolerate the toxicity of metals better than other microbes (Shalini *et al.*, 2003).

Continuous irrigation of nickel electroplating effluent in the soil might have resulted in the accumulation of large amount of salts which might be the reason for an increase in the pH of the post harvested soil samples (Baskar *et al.*, 2004). The findings of the present study coincides with the studies of Aggarwal and Kumar (1990), Sweeney and Graetzm (1991), Kannan and Oblisami (1992),

Rao and Rao (1992), Palaniswami and Ramulu (1994) and Sandana (1995) who reported that the addition of effluent had increased the pH of soil due to the presence of high levels of potassium, calcium, magnesium and sodium. The increase in pH of the soil irrigated with untreated effluent could be either due to alkaline reaction of the effluent used or due to the increase in soil moisture with increasing C:N ratio of the waste water (Devagi *et al.*, 2000).

Variation in pH value of the present study may be attributed to a variety of factors such as soil formation, water content of the sampling site and cropping practices (Gupta, 2007).

4.4.4.1. b. Electrical Conductivity (EC)

From the Table XI it was observed that the electrical conductivity of the soil treated with T₁ was 0.78 mmhos/cm, T₃ was 0.98 mmhos/cm and T₂ recorded a maximum value of 1.32 mmhos/cm. Significant difference was found between the treatments (T₂ and T₃) and with control. The electrical conductivity of T₁ and T₃ were within the normal level (<1 mmhos/cm) whereas in T₂ it exceeded the specified limit (Arnold, 1984).

Electrical conductivity is the measure of total salt content present in the solid substance (Aziz *et al.*, 1994). The increase of electrical conductivity in the post harvested soil of T₂ could be attributed to higher concentration of the soluble salts present in the nickel electroplating effluent. Similar increase in electrical conductivity was reported in the soil irrigated with dye effluent (Someshekar, 1984 and Himabindhu and Reddy, 2008) and sugar mill effluent (Ramakrishnan *et al.*, 2001). These findings may be in accordance with the results of the present investigation.

TABLE XI

PHYSICOCHEMICAL CHARACTERISTICS OF THE TREATED SOIL AT THE END OF THE EXPERIMENTAL PERIOD

Treatments	pH	EC (mmhos/ cm)	Organic Carbon (%)	Available Macronutrients (Kg/ha)						Micronutrients				
				Nitrogen	Phosphorous	Potassium	Sodium	Calcium	Magnesium	Zinc (ppm)	Iron (ppm)	Manganese (ppm)	Copper (ppm)	Nickel (mg/kg)
T ₁	7.23	0.78	1.69	358.33	15.54	238.33	220.66	12.46	10.36	0.95	3.33	1.04	0.42	2.3
T ₂	9	1.32	3.72	480.66	28.29	284.66	282.33	26.84	24.9	2.13	7.07	3.1	0.95	5.6
T ₃	8.13	0.98	2.15	409.66	21.38	262.33	251	18.3	16.44	1.07	5.23	1.81	0.59	4.27
CD (5%)	0.6985	0.0771	.0932	6.4226	0.0901	6.2120	7.6227	0.807	0.840	0.3733	0.5411	0.0584	0.0480	0.5328

The values are mean of triplicates

T₁ - Control, T₂ - Untreated Effluent , T₃ - Treated Effluent

4.4.4.1. c. Organic carbon

The soil irrigated with untreated effluent (T_2) showed a maximum of 3.72 per cent of organic carbon followed by 2.15 per cent in treated effluent (T_3) and a minimum value of 1.69 per cent in tap water (T_1). The organic carbon content increased significantly in T_2 and T_3 when compared with control (T_1). The amount of organic carbon in untreated effluent (T_2) exceeded the tolerance limit (3%) prescribed by Arnold (1984).

The organic substances are major determinants of soil structure, moisture and pH which increase the soil fertility status, controls erosion and run off from the soil and water.

Presence of high percentage of organic carbon content was also reported by various workers in the soil samples treated with distillery effluent (Saritha *et al.*, 2009) and sugar mill effluent (Nemade and Shrivastava, 1998). This increase in organic carbon may cause deleterious effects on the soil which inturn would affect the plant growth (Pragasam and Kannabiran, 2004). These reports support the findings of the present study.

4.4.4.1. d. Available Macronutrients

It was observed from the Table XI that the available nitrogen content in T_2 treated soil was 480.66 kg/ha which was high and exceeded the tolerance limit of 450 mg/g whereas in T_3 it was 409.66 kg/ha and in T_1 it was 358.33 kg/ha. The values of T_1 and T_3 fall within the tolerance limits specified.

The amount of available phosphorus estimated in T_2 soil was 28.29 kg/ha and that estimated in T_3 soil was 21.38 kg/ha and in tap water (T_1) it was 15.54 kg/ha. The values of T_1 and T_3 are

within the tolerance limits (22 kg / ha) whereas in T₂ it exceeded the specified limits.

Table XI revealed the fact that the potassium, sodium, calcium and magnesium availability in T₂, T₃ and control (T₁) soil samples followed the same trend as that of the other two macronutrients tested.

Maximum amount of 284.66 kg/ha potassium, 282.33 kg/ha of sodium, 26.84 kg/ha of calcium and 24.9 kg/ha of magnesium were estimated in soil treated with T₂ which was higher than the values recorded for the soil treated with tap water which was 238.33 kg/ha, 220.66 kg/ha, 12.46 kg/ha and 10.36 kg/ha respectively. The amount of the above macronutrients estimated in the soil samples treated with T₃ was 262.33 kg/ha, 251 kg/ha, 18.3 kg/ha and 16.44 kg/ha respectively and these values are within the tolerance limits as shown in Table X.

All the macronutrients viz., nitrogen, phosphorous, potassium, sodium, calcium and magnesium in the soil increased significantly in T₂ and T₃ treatments when compared with control (T₁).

Soil provides the essential macronutrients required for the plant growth and development. These nutrients are ionized or solublized from solid phase of the soil and should be available for plant uptake. When the macronutrient levels exceed the tolerance limit there could be disruption in the normal growth and development of plants (Nwoko *et al.*, 2007).

Plants utilize most of their nitrogen as ammonium or nitrate ion. Nitrogen is a major component of proteins and nucleic acids. It is a limiting nutrient for plant growth and chlorophyll synthesis. The maximum amount of available nitrogen in the post harvested soil

treated with nickel electroplating effluent might be due to the mineralization of organic matter in the soil which was also reported (Rajukkannu *et al.*, 1996) in distillery effluent treated soil. This is in agreement with the above findings.

When excess amount of nitrogen is present in the soil it may react with the phosphate to form sodium phosphate which inhibits the root growth (Singh *et al.*, 2005). High amount of phosphorous in the soil may induce compaction and reduce the aeration and pore space in root zone. This in turn may reduce the nutrient uptake and thus suppress plant growth performance (Gupta, 2007).

Although potassium is not a constituent of important organic compounds in the cells, it is essential for the process of respiration and photosynthesis. It is strongly fixed in soils and readily available to plants. The application of nickel electroplating effluent in soil might have increased the concentration of soil nutrients such as sodium and potassium ions. Sweeney and Graetzm (1991) also reported a high level of these nutrients in digested distillery effluent. The increase in available potassium ions in the present study is in accordance with the findings of Dhankhar and Singh (2007).

Various researchers have reported an increase of macronutrients in the soil treated with distillery (Devarajan *et al.*, 1994) and paper factory (Chhonkar *et al.*, 2000) effluents which confirm the results of the present study.

High amount of magnesium often causes soil infiltration problems, reduces the productivity, weakens the soil structure and aggregates the surface soil. Poor development of seedlings and nutritional disorders in the crops may occur when the plants are grown using untreated nickel electroplating effluent (Gupta, 2007).

The findings obtained from the soil irrigated with untreated effluent of the present study is in alignment with the results of Bachewar and Mehta (2000) who reported that the soil treated with the toxic zinc electroplating industry effluent resulted in colour change and raised the pH, calcium, magnesium, potassium and iron contents.

Higher concentration of macronutrients in the soil contaminated with untreated nickel electroplating effluent might harden the soil which results in the closure of the pores, causing less aeration and this retard the plant growth (Singh *et al.*, 2005).

4.4.4.1. e. Micronutrients

Table XI represents the amount of micronutrients present in the soil samples tested. The amount of zinc, iron, manganese and copper was found to be high in the soil treated with T₂ which was 2.13 ppm, 7.07 ppm, 3.1 ppm and 0.95 ppm respectively. These values are above the tolerance limits which are presented in Table X. In T₃, the amount estimated was 1.07 ppm of zinc, 5.23 ppm of iron, 1.81 ppm of manganese and 0.59 ppm of copper. Control soil recorded the least value when compared with the other two treatments which was 0.95 ppm, 3.33 ppm, 1.04 ppm and 0.42 ppm of zinc, iron, manganese and copper. The results showed a significant increase in the micronutrient contents in T₂ and T₃ when compared with control (T₁).

Micronutrients are critical for crop production, as the completion of the life cycle of plant could be limited if their deficiency or toxicity occurs (Sharma *et al.*, 2003).

Several workers have reported an increase of micronutrients in the soil treated with copper ore tailings (Anilkumar, 2000), pesticides (Sharma *et al.*, 2001) and gold ore tailings (Venugopal, 2002). The increase in the availability of micronutrients might be due to direct contribution from the effluent as well as solubilization and chelation effect of organic matter supplied by the effluent (Devarajan *et al.*, 1996).

The nickel content in the soil treated with different treatments was presented in Table XI. The amount of nickel was found to be 2.3 mg/kg in T₁, 4.27 mg/kg in T₃ and a maximum of 5.6 mg/kg in T₂. The level of nickel was found to be higher in T₂ treated soil which exceeded the toxic limit (2 - 5 mg/kg) specified.

The findings of the present work confirm the observations of Zafar *et al.* (2007) who reported higher accumulation of nickel in the soil contaminated with the industrial effluent.

Presence of heavy metal in the soil may affect the normal soil chemistry and reduce the nutrient release and uptake of water (Nwoko *et al.*, 2007). Increased amount of nickel might have attributed to the loss of soil fertility, destruction of inherent mechanism such as cation exchange capacity (Nagaraju *et al.*, 2003). This could have reduced the growth of the plants.

The fungal isolates present in the treated effluent may modify the root system of the plant so as to tolerate adverse soil conditions such as high temperature, pH, metal toxicity and drought (Das *et al.*, 2009). The microbially treated effluent can be safely used for irrigating crop fields encountering the problems of high cost of fertilizers and the presence of microbes in the effluent may also help in the degradation of the heavy metals.

PHASE 5

5.1. IMPACT OF NICKEL ELECTROPLATING EFFLUENT (UNTREATED AND TREATED) ON THE GROWTH OF *Clarias gariepinus*

In the present study an attempt was made to assess the impact of tap water (T₁), untreated nickel electroplating effluent (T₂) and treated nickel electroplating effluent (T₃) on the growth and biochemical constituents of the fish, *Clarias gariepinus*.

5.1.1 Growth (Length and weight gain) characteristics of *Clarias gariepinus* in different treatments

The growth characteristics (length and weight) of the fish *Clarias gariepinus* grown in tap water (T₁), untreated nickel electroplating effluent (T₂) and treated nickel electroplating effluent (T₃) are presented in Table XII.

The length gain recorded was 1.8, 1.17 and 0.04 cm respectively in the fishes grown in treated effluent (T₃), tap water (T₁) and untreated effluent (T₂). A maximum weight gain of 0.85 g was observed in the fishes grown in treated effluent (T₃). The weight gain was 0.41g in the fishes grown in tap water (T₁). A minimum weight gain of 0.04 g was noticed in the fishes grown in untreated effluent (T₂). The growth rate was found to be 0.014 in fishes grown in treated effluent whereas it was negligible in fishes grown in tap water (0.007) and untreated effluent (0.0006). The weight gain recorded was a maximum of 27.41 per cent in T₃ fishes, which is followed by 13.22 per cent in T₁ fishes. A minimum of 1.29 per cent weight gain was observed in T₂ fishes.

TABLE XII
GROWTH CHARACTERISTICS OF *CLARIAS GARIEPINUS* GROWN IN
DIFFERENT TREATMENTS

Treatments	Mean Length gain (cm)	Mean weight gain (g)	Growth rate	Percentage weight gain
T ₁	1.17	0.41	0.007	13.22
T ₂	0.04	0.04	0.0006	1.29
T ₃	1.8	0.85	0.014	27.41

The values are mean of triplicates

T₁ - Control, T₂ - Untreated Effluent, T₃ - Treated Effluent

Clarias gariepinus can respire rapidly and can tolerate both oxygenated and poorly oxygenated waters. The air breathing apparatus in this fish could have enabled it to develop tolerance and was well acclimatized to laboratory conditions and therefore could survived in untreated and treated nickel electroplating effluent successfully. It is also used as a biological indicator in ecotoxicological studies.

Growth is the manifestation of various physiological functions which is dependent on food, physiology, environment and biological achievement in living organisms. If an organism is to survive successfully in a given environment its various organs must function in a coherent manner. The net energy is partitioned between metabolism, growth and reproduction. If metabolism is elevated, the growth will be limited unless the intake of food is increased (Hatikakoty, 2002).

The growth rate of the fishes was maximum in treated effluent when compared to other treatments. The nutritive nature of the treated effluent might be the reason for this growth enhancement and weight gain. Fishes are known to convert efficiently the biowastes into edible protein (Yalcin *et al.*, 2002).

An increase in the growth of fishes grown in treated effluent is in conformity with the findings of several workers who used biologically treated sewage to grow various fishes like *Carassius auratus* (Kakuta and Murachi, 1998), *Cyprinus carpio* (Bharadwaj and Sharma, 1997) and *Channa orientalis* (Rao and Hymavathi, 2001).

Minimum growth recorded in the fishes grown in untreated effluent (T₂) is in agreement with the findings of Chattopadhyay *et al.* (1987) who reported similar such reduction in growth of the fishes treated with higher amount of zinc. The growth rate of *C. gariepinus* was comparatively less in T₂ when compared with T₁ and T₃ which might be due to the inhibitory effect of nickel on food intake and food conversion efficiency as stated by James *et al.* (1994).

5.1.2 Biochemical constituents of *C. gariepinus* grown in different treatments

In any diseased condition, pathological changes are preceded by biochemical changes. Thus, the health of an animal depends on the harmonious equilibrium of all the biochemical reactions occurring in the body whereas the diseased condition reflects the alterations in the normal biochemical reactions. Biochemical tests are one of the valuable tools in discerning the damages caused to the organism and the results are useful in assessing the toxicity of the metal caused in the organism.

Fish is highly nutritious, easily digestible and much sought after food. The nutritional value of fish depends on biochemical constituents such as proteins, carbohydrates and lipids. They play an important role in body construction and energy metabolism. Alterations in these biochemical constituents may be due to adverse effect of heavy metals present in the effluent at higher concentrations (Vincent *et al.*, 1995).

5.1.2. a. Total protein content

The amount of total protein content was analysed in different organs (muscles, liver, gills and kidney) of the selected fish grown in tap water (T_1), untreated nickel electroplating effluent (T_2) and treated nickel electroplating effluent (T_3) and the values are recorded in Table XIII.

TABLE XIII
TOTAL PROTEIN CONTENT IN *CLARIAS GARIEPINUS* EXPOSED TO DIFFERENT TREATMENTS

Treatments	Total protein content (mg / g) in different organs			
	Muscle	Liver	Gills	Kidney
T_1	41.79	37.14	33.54	30.48
T_2	30.23 (-27.6%)	28.83 (-22.3%)	25.15 (-25%)	24.55 (-19.4 %)
T_3	63.35 (51.5%)	54.67 (47.1%)	47.49 (41.5%)	41.59 (36.4%)
CD (5%)	1.6025			

The values are mean of triplicates

Figures in parentheses indicates the percentage decrease / increase over control

T_1 - Control, T_2 - Untreated Effluent, T_3 - Treated Effluent

Proteins are the basic building nutrients of any growing cells which usually accounts for 65-68% of the dry matter and confer the biological specificity among various types of cells. They are related with all physiological processes which maintain a simple biochemical system in living condition. Any alteration in the protein turnover may cause adverse effects on the biological functions (Prasanth, 2007).

The total protein content recorded was a maximum of 63.35 mg/g in the muscle, 54.67 mg/g in liver, 47.49 mg/g in gills and a minimum of 41.59 mg/g in kidney of the fishes grown in treated effluent (T₃). Total protein content estimated in the fishes grown in tap water (T₁) was 41.79 mg/g, 37.14 mg/g, 33.54 mg/g and 30.48 mg/g respectively in the muscle, liver, gills and kidney tissues. Lower levels of total protein content were recorded in the fishes grown in untreated effluent (T₂) which was 30.23 mg/g, 28.83 mg/g, 25.15 mg/g and 24.55 mg/g respectively in muscle, liver, gills and kidney. The total protein content estimated in different organs (muscle, liver, gills and kidney) of the selected fish showed statistically a significant difference between the treatments and their control.

Percentage increase of total protein content observed in the fishes grown in microbially treated effluent was a maximum of 51.5, 47.1, 41.5 and 36.4 respectively in the muscle, liver, gills and kidney tissues when compared with that of control. A decrease of 27.6, 22.3, 25 and 19.4 percentage of total protein was observed respectively in the muscle, liver, gills and kidney of fishes grown in untreated nickel electroplating effluent when compared with control.

5.1.2. b. Total carbohydrate content

Table XIV presents the amount of total carbohydrates estimated in different organs of fishes grown in tap water (T₁), untreated nickel electroplating effluent (T₂) and treated nickel electroplating effluent (T₃).

TABLE XIV
TOTAL CARBOHYDRATE CONTENT IN *CLARIAS GARIEPINUS*
EXPOSED TO DIFFERENT TREATMENTS

Treatments	Total carbohydrate content (mg / g) in different organs			
	Muscle	Liver	Gills	Kidney
T ₁	3.05	4.19	2.45	1.4
T ₂	2.73 (-10.4%)	3.47 (-17.1%)	1.62 (-33.8 %)	0.97 (-30.7 %)
T ₃	3.94 (29.1%)	5.6 (33.6%)	2.8 (14.2%)	1.52 (8.5%)
CD (5%)	0.0316			

Values are mean of triplicates

Figures in parentheses indicates the percentage decrease / increase over control

T₁ - Control, T₂ - untreated effluent ,T₃ - treated effluent

Carbohydrates are the cheapest sources of energy in the animal food and play an important role in metabolism. They are stored in the form of glycogen, sugar and their derivatives in the liver of any fish. Hence, liver is found to be the richest source of carbohydrates when compared to other organs studied.

Maximum amount of total carbohydrates was estimated in the selected organs of the fishes (muscle, liver, gills and kidney) grown in treated effluent. The values recorded were 3.94 mg/g in muscle, 5.6 mg/g in liver, 2.8 mg/g in gills and 1.52 mg/g in kidney tissues. The total carbohydrates recorded in the fishes grown in tap water were 3.05 mg/g, 4.19 mg/g, 2.45 mg/g and 1.4 mg/g respectively in muscle, liver, gills and kidney. A minimum level of total carbohydrate content was estimated in the fishes grown in untreated effluent which was recorded as 2.73 mg/g in muscle, 3.47 mg/g in liver, 1.62 mg/g in gills and 0.97 mg/g in kidney. The values recorded for the total carbohydrate content showed a significant variation in muscle, liver, gills and kidney tissues of the fishes grown in different treatments.

Amount of total carbohydrates showed an increase of 29.1, 33.6, 14.2 and 8.5 per cent respectively in muscle, liver, gills and kidney tissues of the fishes grown in treated effluent and a decrease of 10.4, 17.1, 33.8 and 30.7 per cent was recorded in muscle, liver, gills and kidney tissues of the fishes grown in untreated nickel electroplating effluent over control.

5.1.2. c. Lipid content

The amount of lipid content present in the selected organs of the fishes grown in tap water (T_1), untreated nickel electroplating effluent (T_2) and treated nickel electroplating effluent (T_3) is shown in Table XV.

TABLE XV
LIPID CONTENT IN CLARIAS GARIEPINUS EXPOSED TO
DIFFERENT TREATMENTS

Treatments	Lipid content (mg/g) in different organs			
	Muscle	Liver	Gills	Kidney
T ₁	8.85	7.01	6.24	5.88
T ₂	5.84 (-34%)	3.4 (-51.4%)	2.84 (-54.4 %)	1.54 (-73.8 %)
T ₃	11.73 (32.5 %)	9.12 (30%)	7.6 (21.7%)	6.68 (13.6 %)
CD (5%)	0.0776			

The values are mean of triplicates

Figures in parentheses indicates the percentage decrease / increase over control

T₁ - Control, T₂ - untreated effluent , T₃ - treated effluent

Lipids are important sources of energy and fatty acids are essential for growth and survival of fishes. Lipids play a vital role in the structure of biological membranes at both cellular and subcellular levels. They are also important in flavour and textural properties of fishes. The fatty acid compositions of fishes are affected by environmental factors such as temperature, salinity, seasonal variations, maturity and also diet composition (Kulkarni and Dharwadkar, 1998).

Lipid content recorded in T₃ fishes was a maximum of 11.73 mg/g in the muscle, 9.12 mg/g in liver, 7.6 mg/g in gills and 6.68 mg/g in kidney. The lipid content recorded in T₁ fishes

was 8.85 mg/g, 7.01 mg/g, 6.24 mg/g and 5.88 mg/g respectively in muscle, liver, gills and kidney tissues. The level of lipid was found to be 5.84 mg/g in muscle, 3.4 mg/g in liver, 2.84 mg/g in gills and 1.54 mg/g in kidney tissues of T₂ fishes. A significant difference in lipid content was noted in the selected organs of the fishes grown in different treatments.

Percentage increase of lipid content in the fishes exposed to treated effluent was 32.5, 30, 21.7 and 13.6 respectively in the muscle, liver, gills and kidney tissues whereas a decline of 34, 51.4, 54.4 and 73.8 per cent of lipid content was observed respectively in the muscle, liver, gills and kidney tissues of T₂ fishes over control.

5.1.2. d. Nickel content

The nickel content estimated was maximum in the liver with 1.05 mg/kg followed by 0.92 mg/kg in kidney, 0.72 mg/kg in gills and 0.65 mg/kg in muscle tissues of the fishes exposed to untreated effluent (T₂). The levels of nickel content recorded in the fishes grown in treated effluent (T₃) were 0.34 mg/kg, 0.25 mg/kg, 0.17 mg/kg and 0.08 mg/kg respectively in liver, kidney, gills and muscle tissues whereas a minimum level of 0.09 mg/kg in liver, 0.07 mg/kg in kidney, 0.05 mg/kg in gills and 0.02 mg/kg in muscle tissues were recorded in the fishes grown in tap water (Table XVI). The amount of nickel content in the tissues of T₁ and T₃ fishes were within the tolerance limits (0.5 – 0.6 mg/kg) prescribed by WHO (1985).

TABLE XVI
NICKEL CONTENT IN *CLARIAS GARIEPINUS* EXPOSED TO
DIFFERENT TREATMENTS

Treatments	Nickel content (mg / g) in different organs			
	Muscle	Liver	Gills	Kidney
T ₁	0.02	0.09	0.05	0.07
T ₂	0.65	1.05	0.72	0.92
T ₃	0.08	0.34	0.17	0.25
CD (5%)	0.0295			

The values are mean of triplicates

T₁ - Control, T₂ - untreated effluent ,T₃ - treated effluent

From the above results it is clearly evident that all the biochemical constituents (total proteins, total carbohydrates and lipid) estimated in different organs (muscle, liver, gills and kidney) of the fishes grown in untreated nickel electroplating effluent showed a decrease in their contents over their control.

The decrease in the amount of protein content in different organs of the present study is supported by the findings of various researchers in *Channa punctatus* exposed to monocotrophos (Ramamurthy *et al.*, 2009), mercury, arsenic, lead, copper, cadmium and chromium (Jana and Bandyopadhyay, 1987), *Heteropneustes fossilis* treated with vegetable oil factory effluent (Kondal *et al.*, 1998), *Clarias batrachus* exposed to malathion (Arunkhare and Singh, 2002) and pesticides (Jha and Verma, 2002 and Shukla *et al.*, 2005),

Anabas scandans exposed to lead nitrate (Chandravathy *et al.*, 1994) and *Labeo rohita* exposed to pesticides (Radha and Rajendran, 2009).

This reduction in protein content of the fishes grown in untreated effluent may be due to the toxic effect of nickel present in the effluent. The decrease may also be due to lack of oxygen availability and energy demand under heavy metal stress (Ahmed, 1979), mobilization of tissues to produce glucose through gluconeogenesis to meet energy demand (Elankumaran *et al.*, 1992), utilization of lipoproteins for the repair of damaged cell organelles, and increased lipolysis (Ghosh and Chatterjee, 1989) and decrease in the level of isocitrate dehydrogenase and malate dehydrogenase (Kondal *et al.*, 1998). Block in the protein or amino acid synthesis or protein denaturation may also be other reasons for the decrease in protein content (Jha, 1988). Due to proteolytic activity the fishes utilize the products of degradation for metabolic purposes and these may be fed into TCA cycle through aminotransferase system to meet excess demand of energy during toxic conditions (Chandravathy *et al.*, 1994).

From the results obtained, it is clear that the amount of total carbohydrates estimated showed a reduction in different organs of the fishes grown in untreated nickel electroplating effluent when compared with control. This reduction in total carbohydrate content was also reported by many researchers in *Labeo rohita* exposed to tannic acid (Somenath, 1991), mercury (Rajasubramanian *et al.*, 2006), heavy metals (Reddy and Srikar, 1991) and quinalphos (Das and Mukherjee, 2005), *Oreochromis mossambicus* to titanium dioxide factory effluent (Mohanan and Nair, 2000), dairy effluent (Noorjahan *et al.*, 2003) and zinc (Govindarajan, 2007), *Tilapia mossambicus* to chromium trioxide (Thangam and Sivakumar, 2004)

and *Cirrhina mrigala* to phosphamidan (Nirmala and Eliza, 2005). The results of the present study were found to be in accordance with these reports.

Reduction in total carbohydrate content may be due to decrease in glycogenesis or gluconeogenesis (Szincz and Forth, 1988) or it may be due to increased secretions of catecholamine which might have depleted the glycogen reserve under stress conditions (Pickering, 1981). The low amount of carbohydrates in the gills of the fishes may suggest that it is highly vascularised and may possess low glycogen synthetic potential which may be the reason for decreased carbohydrate content (Karakoc and Dincer, 2003).

The biochemical analysis of lipid content in different organs of the fishes grown in untreated nickel electroplating effluent (T₂) showed a reduction when compared with control. Several authors have conducted experiments and reported a decline in lipid content of *Channa punctatus* and *Fundulus heteroclitus* exposed to copper (Weis *et al.*, 1986) and heavy metals (Radhakrishnaiah *et al.*, 1991), *Heteropneustes fossilis* to nickel chrome electroplating effluent (Gupta, 1991), *Cyprinus carpio* to zinc electroplating effluent (Kaur, 1992), *Oreochromis mossambicus* exposed to cadmium (Hammed and Kumaravel, 2006), *Ophiocephalus* to dairy effluent (Sabarinath *et al.*, 2006) and *Catla catla* to sevin, an organophosphochloro pesticide (Nagaraj and Sarma, 2008). These findings may support the results of the present study.

The decrease in the lipid content of T₂ fishes in the present investigation may be due to a decline in the lipid synthesizing capacity or an increase in the hydrolysis of hepatic lipids to combat stress condition (Virk and Sharma, 1999) or its mobilization to meet the energy requirements and increased lipase activity (Jha, 1988).

Heavy metals are mainly stored in the liver which is the major storage and regulatory organ for metal homeostasis. They enter the body of fish by gills or by diffusion through body surface or by food and drinking. Liver, kidney, gills and intestine are the main site for metallothionein formation in fishes. Metals bound to metallothionein are either stored or excreted from the body and they tend to be toxic to living organisms at relative concentrations. They possess the intrinsic capacity to cause injury, including carcinogenic, mutagenic and teratogenic effects. The preliminary mode of action by which metals exert toxicity is by reacting with sulfur-donor atoms of proteins resulting in enzyme inactivation and destabilization of biomolecules, thus interfering with cell metabolism (Pelgrom *et al.*, 1994).

Maximum amount of nickel was found to accumulate in the liver of T₂ fishes which might be due to the fact that liver is the vital organ for detoxification of heavy metals followed by kidney which eliminates them through various routes. The results of the present study confirms with the findings of Mohammed and Al-Mohanna (1994) who reported a maximum accumulation of zinc in liver and kidney of *Rita rita*, while minimum accumulation was found in the muscle and gill tissues.

Bioaccumulation of heavy metals in fishes have been reported by various authors in *Oreochromis mossambicus* exposed to metals (James *et al.*, 1991), *Catla catla* to cadmium and chromium (Vincent *et al.*, 1995 and Vincent *et al.*, 1996), *Channa punctatus* to heavy metals (Sornaraj *et al.*, 1995), *Micropogon undulates* to zinc (Chipman *et al.*, 1998), *Cyprinus carpio* to nickel and chromium (Virk and Sharma, 1999) and *Rita rita* to copper (Paul and Mukhopadyay, 2001). These studies may support the results of the present investigation.

From the results obtained it is clearly evident that the maximum growth (length and weight gain) and biochemical constituents (total proteins, total carbohydrates, lipids and nickel) were observed in the fishes grown in microbially treated nickel electroplating effluent. This might be due to the sorption of nickel from the effluent by the fungal isolate, *A. niger*. Increase in the growth and biochemical constituents of T₃ fishes is in accordance with the studies conducted in *Ophiocephalus striatus* grown in heavy metal polluted effluent treated with water hyacinth (Devi and Gopal, 1986), zebra and gold fish grown in bleached kraft paper mill effluent treated with *Rhizopus oryzae* (Nagarathnamma and Pratima, 1999 and Diniz *et al.*, 2009), *Lepidocephalichthys thermalis* grown in dye house effluent treated with the species of *Aspergillus*, *Rhizopus* and *Geotrichum* (Anandapandian *et al.*, 2003), *Tilapia mossambica* in microbially treated effluent (Samyuktha *et al.*, 2006), *Chanos chanos* in fish farm effluent treated with carrageenophytes (Rodrigueza and Montano, 2007) and *Liptopenaeus vannamei* grown in microbially treated Tilapia fish farm effluent (Kuhn *et al.*, 2009).

Reduction in nickel content of the fishes grown in treated effluent may be due to the capacity of the *A. niger* which can adsorb organic and inorganic chemicals. Since the nutrients present in the treated effluent were within the tolerance limits, they might have enhanced the growth of the fishes.

5.2. Histological changes in the selected organs (gills, liver and kidney) of *Clarias gariepinus* exposed to nickel electroplating effluent

The histological studies in the organs of fishes provide ample information regarding the impact of pollutants in the environment (Stebbing, 1985). No mortality was recorded in the fishes which were

treated in tap water (T₁), untreated nickel electroplating effluent (T₂) and treated nickel electroplating effluent (T₃).

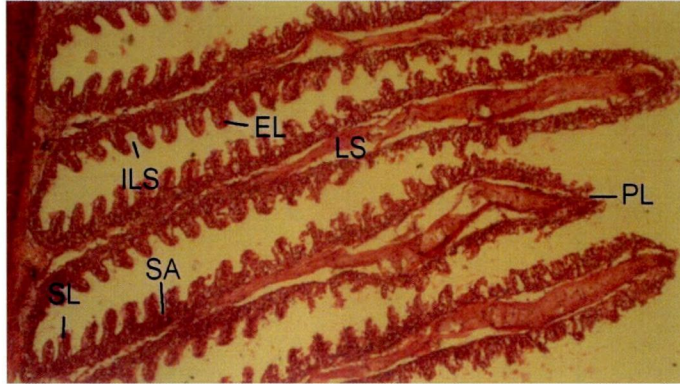
5.2. a. Histological changes in the gills of *C. gariepinus*

The section of the gills of fishes grown in tap water showed intact nature of primary and secondary gill lamellae. Each primary gill lamella was flat and leaf like in structure. It consisted of double rows of secondary (respiratory) lamellae with a central supporting axis. They are situated laterally on either side of the interbranchial septum. The secondary lamellar surface is covered with simple squamous epithelial cells and capillaries. The secondary lamellae on both sides were highly vascularised and covered by a layer of epithelial cells with uniform interlamellar spaces (Plate XIII A). In the present study, no recognizable changes were observed in the gills of fishes grown in treated effluent. The sections off these fishes also showed similarity in structure with those of control gills (Plate XIII C).

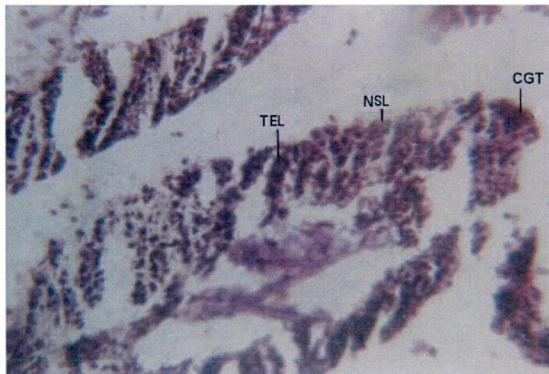
Histological studies conducted in the gills of fishes exposed to untreated nickel electroplating effluent (T₂) showed degenerative changes, necrosis and odema in the secondary lamella. Clubbing and fusion of gill tips along with epithelial thickening and reduction in the interlamellar space and lamellar fusion were also observed (Plate XIII B). The findings of the present study are also supported with the studies conducted in *Lebistes reticulates* treated with cyphenothrin (Erkmen *et al.*, 2000), *Ctenopharyngodon idellus* with fenvalerate (Tilak *et al.*, 2001), *Labeo rohita* with copper (Manisha and Dhande, 2005), *Channa punctatus* with cartap (Mishra *et al.*, 2005), *Poecilia reticulata* with methyl red (Shweta *et al.*, 2006), *Cirrhinus mrigala* to mercury (Kumar and Ashwani, 2006), *Oreochromis mossambicus* with copper (Venketesan, 2007), *Mystus vittatus* with pesticides (Sornaraj *et al.*, 2008) and *Clarias gariepinus* with soap and detergents (Ogundiran *et al.*, 2009).

PLATE XIII

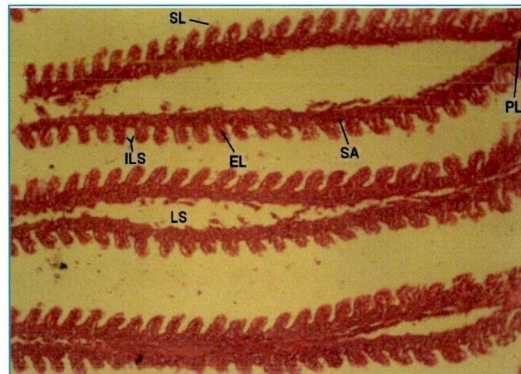
HISTOLOGY OF GILLS OF *CLARIAS GARIEPINUS*



A - Gill Section of *C. gariepinus* - Control



B - Section of gills in *C. gariepinus* exposed to untreated nickel electroplating effluent



C - Section of gills in *C. gariepinus* exposed to treated nickel electroplating effluent

- PL - Primary Lamellae
- SL - Secondary Lamellae
- LS - Lamellar Space
- ILS - Inter Lamellar Space
- EL - Epithelial Lining
- SA - Supporting Axis
- NSL - Necrosis of Secondary Lamellae
- TEL - Thickening of Epithelial Lining
- CGT - Clubbing of Gill Tips

The damages caused in the gills of the fishes grown in untreated nickel electroplating effluent may be due to the toxic effect of the heavy metal in the effluent. This toxicity might impair the efficiency of gaseous exchange in the gills of the fishes (Ayoola, 2008). The formation of edema in the gills of fishes grown in untreated effluent may also be due to the higher count of WBC which might have caused inflammatory reaction to the fishes under heavy metal stress or it may be due to the failure of sodium and potassium pump (Tao *et al.*, 2000). The clubbing and fusion of the secondary lamella in the fishes grown in untreated effluent may be due to the entry of heavy metals which have reduced the surface area for respiratory process in gills (Mitchell and Corton, 2004).

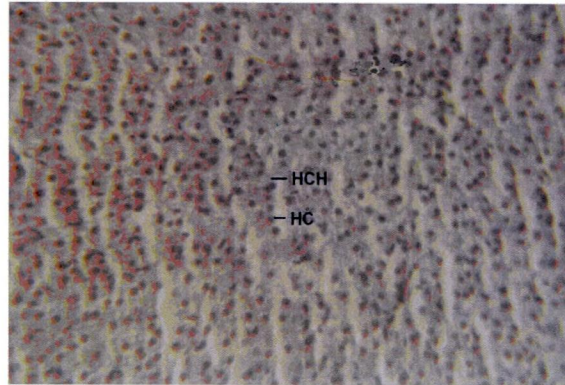
5.2. b. Histological changes in the liver of *C. gariepinus*

The stained sections of the liver of control fish were observed under the microscope. The histology of the liver showed hepatic cells supported with connective tissues (lattice fiber). Hepatocytes were located among the sinusoids and they formed cord like structures, the hepatic cell which had large nuclei cords (Plate XIV A). No remarkable changes were observed in the liver histology of fishes exposed to treated effluent. The structural aspects were found to be similar with that observed in control fishes (Plate XIV C).

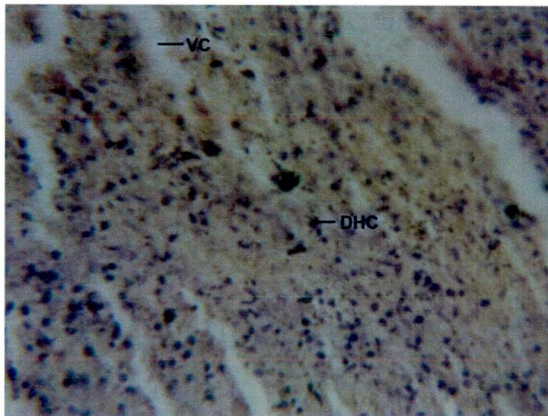
The sections of the liver exposed to untreated nickel electroplating effluent showed symptoms of necrosis, degeneration of hepatocyte cells and formation of vacuoles (Plate XIV B). These histological changes revealed the fact that the liver damage was severe in the fishes grown in untreated effluent.

PLATE XIV

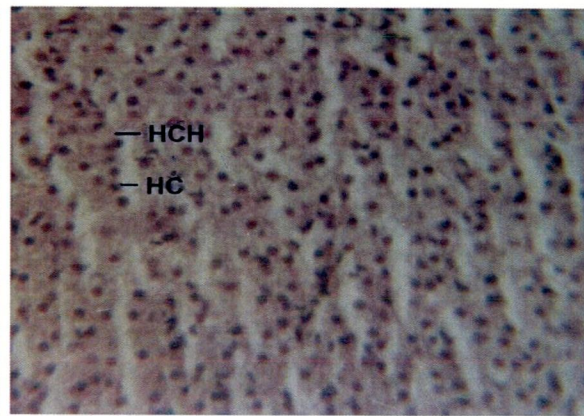
HISTOLOGY OF LIVER OF *CLARIAS GARIEPINUS*



A - Section of liver in *C. gariepinus* (Control)



B - Section of liver in *C. gariepinus* exposed to untreated nickel electroplating effluent



C - Section of liver in *C. gariepinus* exposed to treated nickel electroplating effluent

- HC – Vacuoles
- HCH – Degenerated Hepatocyte Cells
- VC – Hepatocyte Cells
- DHC – Hepatic Chords

The present observations are in confirmity with the findings of *Etroplus suratensis* exposed to polluted waters of Uppanar estuary (Iyyappan *et al.*, 1998), *Cirrhinus mrigala* to myxobolus (Das *et al.*, 2000), *Ctenopharyngodon idellus* to fenvalerate (Tilak *et al.*, 2001), *Hypophthalmichthys molitrix* to nickel (Athikesavan *et al.*, 2006), *Labeo rohita* to zinc (Loganathan *et al.*, 2006) and cadmium (Kumar *et al.*, 2006), *Channa punctatus* to pesticides (Mishra *et al.*, 2006) and *Oreochromis mossambicus* to zinc and cadmium (Dyk *et al.*, 2007).

5.2. c. Histological changes in the kidney of *C. gariepinus*

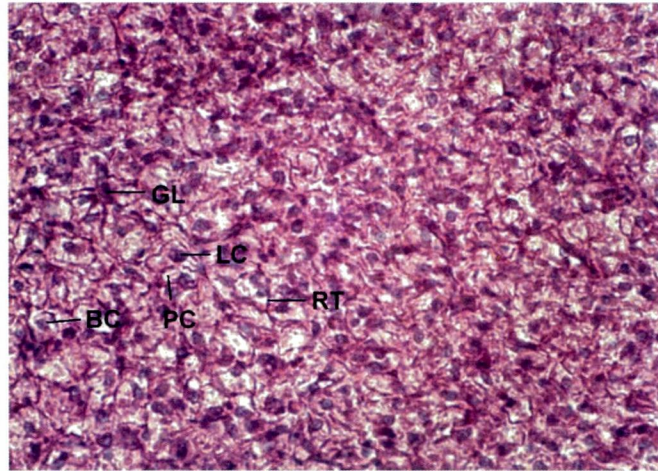
The histology of the kidney of control fishes showed the lymphoid tissues, Bowman's capsule with glomeruli and renal tubules (Plate XV A).

The renal tissues of the fishes that were exposed to untreated nickel electroplating effluent showed marked pathological changes. Highly degenerative changes were found in lymphoid tissues which included severe necrosis and dilated renal tubules with infiltration of parenchymal cells. The glomeruli were congested, Bowman's capsule was dilated and necrotic changes were seen (Plate XV B).

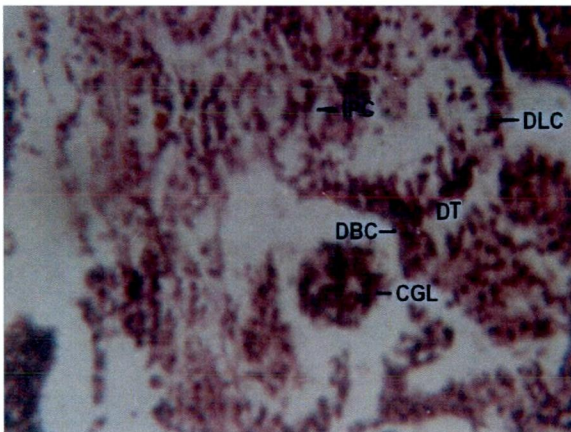
The results of the present study has been supported by many workers who observed various histopathological changes in the kidney of *Cirrhinus mrigala* exposed to myxobolus (Das *et al.*, 2000), mercury (Kumar and Ashwani, 2006), *Ctenopharyngodon idellus* to fenvalerate (Tilak *et al.*, 2001), *Channa punctatus* to zinc (Pallavi and Neera, 2006) and *Oreochromis mossambicus* to carbamate (Rita and Mitton, 2006).

PLATE XV

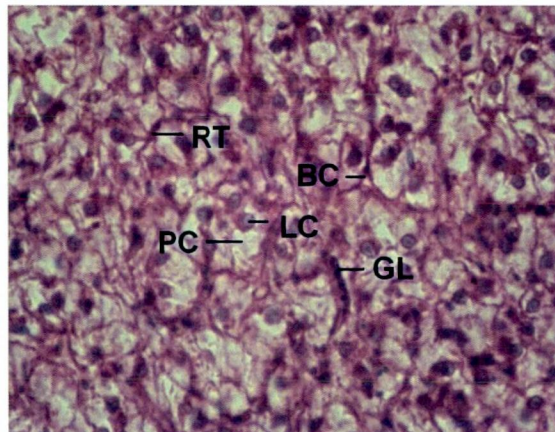
HISTOLOGY OF KIDNEY OF *Clarius gariepinus*



A - Section of kidney in *C. gariepinus* (Control)



B – Section of kidney in *C. gariepinus* exposed to untreated nickel electroplating effluent



C - Section of kidney in *C. gariepinus* exposed to treated nickel electroplating effluent

- | | |
|-----|------------------------------------|
| LC | – Lymphoid Cells |
| BC | – Bowman's Capsule |
| GL | – Glomeruli |
| PC | – Parenchyma Cells |
| RT | – Renal Tubules |
| DLC | – Degeneration of Lymphoid Cells |
| DBC | – Dilation of Bowman's Capsule |
| CGL | – Congested Glomeruli |
| IPC | – Infiltration of Parenchyma Cells |
| DT | – Dilated Tubules |

Renal excretion is one of the ways of eliminating the toxicants which result in severe pathological changes in haemopoietic tissues causing necrosis, cloudy swelling of renal tubules, disintegration of interstitial tissue and pycnotic nuclei (Rao, 1984).

Histologically no changes were observed in the kidney tissues of the fishes exposed to treated nickel electroplating effluent. The structural aspects were found to be similar with those observed in control fishes (Plate XV C).

The literature available on the histological changes of the fishes grown in treated effluent is scarce. Nagarajan and Devi (2006) reported that *Labeo rohita* when exposed to treated distillery effluent showed no marked changes in the histo architecture of the fish, but severe damage was inflicted on the gills and liver of the fishes exposed to untreated effluent. This study may support the findings of the present investigation.