Background: Biofuels are renewable and alternative energy sources which are recognized with the highest potential to meet the global energy demand and microalgae are promising biofuel feedstock. Microalgae have distinguishable features when compared to first and second generation biofuel feedstock such as high photosynthetic efficiency, rapid growth, high lipid content, high mitigation efficiency, noncompetition with food crops for farmland and less water demand than land crops. To generate large amount of microalgal biomass and meet the energy consumption demand, large scale microalgal cultivation is a plausible solution in the future. At the same time, improvement of biomass and lipid content in microalgal cells also presents a direction towards the sustainable development of microalgal biodiesel. Thus, it is extremely important to apply feasible strategies to induce microalgal lipid accumulation.

The use of nanoparticles in commercial applications is rapidly increasing. Nanoparticles display higher surface-to-volume ratio which are widely used in catalytic reactivity, thermal conductivity, antimicrobial activity and chemical steadiness. However there is little known of the fate and behavior of the nanoparticles on microalgae. This research focuses on chemical synthesis of nanometer sized heavy metals and a fundamental study on its effect on microalgae and heavy metal adsorption. Firstly, laboratory synthesis procedures of heavy metal nanoparticles are described. Firstly, the effect of heavy metal nanoparticles on the growth rate, biomass concentration, biochemical composition and lipid production in microalgae are examined to determine the metal tolerance. Secondly, the metal tolerant microalgae were grown in media added with heavy metals and the productivity of biomass and lipid were investigated.

Objectives
In this study, a two-phase scenario was studied with following objectives.
(1) To improve metal tolerance of microalgae using metal nanoparticles and
(2) To enhance the microalgal biomass and lipid accumulation using metal polluted water.

Methodology
Isolation and Identification of Algae
Algal samples were collected from heavy metal industrial effluents and identified by standard protocols described by Anderson (2005) and Stanier (1971).

Nanoparticle synthesis
Copper sulphate, magnesium nitrate, lead acetate and zinc chloride were used to prepare the metal nanoparticles by chemical method. Various concentrations of metal nanoparticles were added to Bristol's medium and the microalgae were cultivated for a period of 20 days. The effect of nano particles on the growth of microalgae were examined and the highest concentration which increased the growth parameters in terms of specific growth rate, biomass productivity, cellular pigments, protein content, carbohydrate content and lipid production was selected for further experiments. The metal resistant strain was further cultivated in heavy metal containing media to determine the heavy metal removal efficiency, biomass and lipid productivity.

Specific Growth Rate
Specific growth rate ($\mu$) of the microalgae was calculated according to the following formula.

$$\mu = \frac{\ln\left(\frac{N_t}{N_0}\right)}{T_t - T_0}$$

Where, $N_t$ and $N_0$ are the dry cell weight concentration (g L$^{-1}$) at the end ($T_t$) and start ($T_0$) of log phase respectively.

Biomass Concentration
Biomass (g L$^{-1}$) of microalgae grown in the presence of metal nanoparticles was determined by measuring the optical density of samples at 600 nm (OD$\text{OD}_{600}$) using UV-Vis spectrophotometer.

Biomass concentration = OD$\text{OD}_{600} \times 0.6$ ………….. Eq. (1)

Chlorophyll Estimation
Chlorophyll contents of the microalgae were estimated according to Becker (1994) and then calculated using the Eq.(2).

$\text{Chl (mg/L)} = 8.02 \times \text{OD}_{663} + 20.21 \times \text{OD}_{645}$ ………….. Eq. (2)

Carotenoids Estimation
Carotenoids were determined by following the procedure of Whyte (1987) and total carotenoids were calculated using the Eq.(3).

$\text{Ct (mg/L)} = 4.32 \times \text{OD}_{444} - 0.0439$………….. Eq. (3)
Protein Assay
The extraction of proteins from microalgae was performed using alkali method and the protein content was estimated using Bovine Serum Albumin (BSA) as standard (Lowry et al., 1951).

Carbohydrate Assay
Cellular carbohydrates were estimated using the anthrone method described by Gerhardt et al., (1994) after hot alkaline extraction (Levya et al., 2008).

Lipid Productivity
Lipid extraction from algal cells were carried out by chloroform:methanol extraction method (Folch et al., 1956) and the lipid productivity was determined using the following Equation (4).

\[ \text{Lipid productivity (g L}^{-1} \text{d}^{-1}) = \text{Biomass productivity} \times \text{Lipid content} \]

Eq. (4)

Results and Conclusion:
Microalgal samples collected from heavy metal processing industrial effluents were identified and found that *Chlorella vulgaris* as the predominant species. To investigate the behavior of nanoparticles on microalgal growth and metal adsorption, microalgae were initially exposed to metal nanoparticles. Four metal nanoparticles namely CuNP, PbNP, MgNP and ZnNP of varying concentrations were used at initial screening to determine heavy metal tolerance and the concentrations were different for each metal nanoparticle.

Initial growth in the presence of metal nanoparticles was varying with different days and the growth rate was increasing at the beginning followed by decline phase. Highest specific growth rate was observed in 20 mg L\(^{-1}\), 100 mg L\(^{-1}\), 50 mg L\(^{-1}\), and 100 mg L\(^{-1}\) for CuNP, PbNP, MgNP and ZnNP respectively. Biomass concentration was highest at 20 mg L\(^{-1}\), 100 mg L\(^{-1}\), 50 mg L\(^{-1}\), and 50 mg L\(^{-1}\) for CuNP, PbNP, MgNP and ZnNP respectively.

Comparative analysis of effect of metal nanoparticles and heavy metals on native strain and metal resistant induced strain is mentioned below. The data represents only the highest values obtained from a metal nanoparticles concentration. In general, there was a difference among the growth parameters studied. The amount of chlorophyll content was increased between 21.3% and 79.8% in metal resistant strains in the presence of CuNP, MgNP and ZnNP. However, there was a decrease in the carotenoid content observed in the study.

Protein content of the microalgae was decreased from 45 mg L\(^{-1}\) to 17 g L\(^{-1}\) and 87.5 mg L\(^{-1}\) to 65.3 mg L\(^{-1}\) when the cells were grown in the presence of nanoparticles/ salt of copper and lead respectively. At the same time the content was
increased from 20 mg L\(^{-1}\) to 29 g L\(^{-1}\) and 40 mg L\(^{-1}\) to 60 mg L\(^{-1}\) in the presence of nanoparticles/ salt of magnesium and zinc. Carbohydrates content was decreased in the metal salt treated cells except the Zn salt where there was a 72.4% increase.

The lipid productivity was increased significantly in cells grown in the presence of ZnNP in which 65% increase was observed. The other metal salts have influenced the lipid production in the range of 68.7% - 75% increase. In general, the lipid productivity was lesser in native cells and was increased in nanoparticle induced metal resistant strains.

Heavy metal removal efficiency of the microalgae was studied by growing nanomaterial induced metal resistant strain in heavy metal containing growth medium. Heavy metal analysis was performed through ICP-MS and the removal rates were recorded as 74.87% (copper), 91.32% (magnesium), 99.8% (lead) and 82.4% (zinc). It is estimated that major portion of world population depends on the energy derived from fossil fuels and development of alternative energy sources is necessary as the existing fossil reserves only for few decades. Biofuels especially from microalgae substitute to fossil
derived fuels due to similar physicochemical properties. Strategies to improve biomass and lipid content in microalgal cells are required with additional benefit. This work claims the increased production of biomass and lipid in microalgae along with removal of heavy metals using nanometals.

Scope for Future Work
In this study, variations among growth parameters and lipid production are observed in microalgal cells cultivated with metal nanoparticles and metal salts in the growth medium. There were both decrease and increase in protein and carbohydrates content but biomass and lipid productivity was increased in the metal salts medium using metal nanoparticle induced metal resistant strains as inoculum. However these results were obtained under controlled laboratory conditions but in order to make this strategy feasible, mass cultivation of microalgae in metal polluted waters in open systems like sewerage treatment plants needs to be performed for higher production of biomass and lipid along with heavy metal removal.