SUMMARY

The most important source of pollution is industrial effluents. The improper waste disposal practices create the new pollution problems. In India, rapid industrial development has resulted in the significant increase in the liquid waste that discharged into nearby water bodies or into the open land which causes a number of environmental problems (Ramana et al., 2002). For easy access of quality water and disposal of waste material, the industries in India are located on the river banks. The adverse input of diverse industrial wastes has elevated the pollution. Sewage and industrial disposal have greatly enhanced the addition of heavy metals, pesticides, hydrocarbon into the aquatic ecosystem. Industrial effluents contain so many pollutants (Kumar, 1996). In many cases, it is reported that, during analysis of liquid wastes from industries, the toxicant level after treatment of toxic chemicals, is in considerable amount indicating the lack of efforts and perfect technology. Water quality is particularly associated with water use and to the state of monetary change (Chennakrishnan et al., 2008). The careless disposal of industrial effluents and other wastes contaminates the water (Mathuthu et al., 1997). Thus, environmental pollution is one of the most formidable dangers that confront mankind today, and it is necessary for man to use his intellectual power not only at technological development but also towards the protection of natural life.

In South Gujarat region various industrial zones developed by Gujarat Industrial Development Corporation (GIDC), Sachin industrial area is one of them. Sachin has a large industrial area managed by Diamond SEZ, SurSEZ (Surat Special Economic Zone), Gujarat Industrial Development Corporation (GIDC), and many other private SEZs. It is a second largest industrial settlement of Asia in terms of area. Secondly, Sachin GIDC has various manufacturing units. Sachin industrial zone is one of the areas zoned for industrial development in and around Sachin. Currently, there is a limited information about the types and abundance of pollutants discharged by industries into the water system. To fill the gap and due to the present-day tremendous industrial, pollution has prompted us to carry the systematic and detail study of ecotoxicological and genotoxicological characterization of industrial effluent from Sachin industrial zone, Surat, South Gujarat, India.
In order to achieve the aim, the following objectives were identified and that led to a logical progression through the present research work:

a) Sampling of industrial effluent and sample preparation.

b) Physico-chemical and heavy metal analysis of different industrial effluent from Sachin industrial zone, Surat, south Gujarat, India.

c) Identification and characterization of organic compounds in the industrial effluents by using GC-MS.

d) Ecotoxicological evaluation of industrial effluent by following method
   - Alga, *Pseudokirchneriella subcapitata* growth inhibition test
   - Acute immobilization test by using *Daphnia magna*
   - *Daphnia magna* reproduction test
   - Fish, acute toxicity test by using Zebrafish, *Danio rerio*
   - Fish, embryo toxicity test by using Zebrafish, *Danio rerio*

e) Genotoxicological evaluation of industrial effluent by following method
   - Bacterial reverse mutation (AMES) test using different strains of *Salmonella typhimurium*

Industrial effluents were collected from following area such as Site 1: It is the main industrial belt of Sachin Gujarat Industrial Development Corporation (GIDC), Site 2: It is situated in the south of Sachin GIDC about 10 km from Sachin city. The distance between Site 1 and Site 2 is about 5 km. Site 3: It is located on the bank of Mindhola River near the Arabian Sea.
PHYSICO-CHEMICAL ANALYSIS OF DIFFERENT INDUSTRIAL

Effluent Water samples collected were analyzed for physico-chemical parameters viz., water temperature, turbidity, conductivity, total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), pH, dissolved oxygen (DO), bio-chemical oxygen demand (BOD), chemical oxygen demand (COD), nitrite, nitrate, phosphate, chloride and oil & grease following the standard methods described by Trivedy and Goel (1986).

Physicochemical parameter of collected samples from 3 different sites of Sachin were recorded. Temperature- May 2014 (30-31°C), September 2014 (22-23°C) and January 2015 (21-22°C), maximum temperature was recorded in May 2014 from site 2 and minimum from site 2 and 3 in January 2015. Maximum Turbidity of 186 NTU and minimum of 144 NTU was recorded from the samples collected from site 1 and site 2 on January 2015 and September 2014 respectively.

The conductivity was high (78.5 mS/cm) at site 1 on September 2014 and minimum at site 3 on September 2014. Maximum total solid of 72090 mg/L was recorded at site 1 on September 2014 and minimum in site 2 on January 2015. Maximum total dissolved solids of 71400 mg/L was recorded in site 1 on September 2014 and minimum in site 2 on January 2015.

During all the months (May 2014, September 2014 and January 2015) at every site (1, 2 and 3) TSS was higher than standard limit of CPCB (100 mg/L). The maximum concentration of TSS was recorded in site 1 on September 2014.

The pH of effluent was 4.81 which was collected from site 3 on May 2014; it was lower than CPCB standard (pH 5.5- 9.0). All other sample pH were within the limit.

Maximum chloride concentration of 35780 mg/L was recorded in site 1 on September 2014 and minimum in site 2 on May 2014.

The minimum DO of 1.13 mg/L followed by 1.50 mg/L was recorded in site 1 on January 2015 and May 2014 respectively.

BOD of all the collected samples were not in the limit when compared to CPCB (30 mg/L), maximum concentration of 2896 mg/L was recorded in site 1 on September 2014.
Summary

- COD of all the collected sample were not within the limit when compared to CPCB standard (250 mg/L). Sample collected from site 2 on January 2015 showed maximum concentration of COD.
- Nitrate (mg/L) concentration of all the collected samples were not within limit when compared to CPCB standard (10 mg/L).
- Phosphate concentration of all the collected sample were within the limit when compared to CPCB standard (5 mg/L), except sample collected from site 1 on May 2014.
- Oil & grease concentration of all the collected sample were higher than CPCB standard concentration (10 mg/L), maximum concentration of 26.80 mg/L was recorded in site 1 on September 2014.

HEAVY METAL CONCENTRATION

- Heavy metals of industrial wastewater was estimated using Atomic Absorbance Spectrophotometer (AAS) after pre-concentration by evaporation of unfiltered samples in the presence of HNO₃. Heavy metals were analyzed following the standard methods of (APHA., 1995; Trivedy and Goel., AOAC 1995; EPA., 1991; EPA., 1996; SANCO., 2009; and Elmer, 1996).
- All the collected sample were subjected to heavy metal analyzed (Copper, Chromium, Lead, Cadmium, Zinc and Nickel), the results showed that concentration of metals were within the limit when compared to CPCB standard. But chromium concentration of sample collected from site 1 on September was slightly increased when compared to standard.

The results of water quality indicated that BOD, COD, oil & grease, and nutrients like nitrate and phosphate from industrial effluent of three different months were higher in concentration and above the permissible limits of CPCB. It is urgently required for continuously monitoring of effluents and to take necessary actions for proper treatment of the wastewater prior their disposal to water bodies and to save natural water resources in Sachin industrial zone.
GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) STUDY OF INDUSTRIAL EFFLUENT

All the collected samples were subjected GC-MS analysis.

May 2014: Site 1

- The GC-MS analysis of collected sample exhibited 10 major peaks in chromatogram.
- Only 6 peak was coincide with library data and identify similar possible compounds.
- Peak 1 and 2 exhibited compounds were aromatic while 3, 4, 5 and 8 peak derived compounds were belongs to aliphatic.

May 2014: Site 2

- The GC-MS analysis of collected sample exhibited 10 major peaks in chromatogram.
- Only 8 peak was coincide with library data and identify similar possible compounds.
- Peak 9 & 10 exhibited compounds were aliphatic while peak 1, 4, 5, 6, 7 and 9 derived compounds were belongs to aliphatic and aromatic.

May 2014: Site 2

- The GC-MS analysis of collected sample exhibited 10 major peaks in chromatogram.
- Only 4 peak was coincide with library data and identify similar possible compounds.
- Peak 2, 3, 6 exhibited compounds were belongs to aromatic while peak 10 derived compounds were belongs to heterocyclic and aromatic.

September 2014: Site 1

- The GC-MS analysis of collected sample exhibited 10 major peaks in chromatogram.
- Only 7 peak was coincide with library data and identify similar possible compounds.
- Peak 3, 5, 7 & 9 exhibited compounds were aromatic while 1, 4 and 6 peak derived compounds were belongs to both aromatic and aliphatic group.

September 2014: Site 2

- The GC-MS analysis of collected sample exhibited 10 major peaks in chromatogram.
- Only 6 peak was coincide with library data and identify similar possible compounds.
- All the peak derived compound were belongs to aromatic.
September 2014: Site 3

- The GC-MS analysis of collected sample exhibited 10 major peaks in chromatogram.
- Only 7 peak was coincide with library data and identify similar possible compounds.
- Peaks 2, 3, 5, 7 and 8 derived compound were belongs to aromatic compounds while peak 1 exhibited aliphatic and aromatic compounds.

January 2015: Site 1

- The GC-MS analysis of collected sample exhibited 52 major peaks in chromatogram.
- Only 14 peaks were coincide with library data and identify similar possible compounds.
- Peak 1, 7, 11, 12, 13, 16, 24, 26 exhibited compounds were aliphatic while peak 2, 21, 38, 48 and 50 derived compounds were belongs to aromatic.

January 2015: Site 2

- The GC-MS analysis of collected sample exhibited 6 major peaks in chromatogram.
- All peaks were coincide with library data and identify similar possible compounds.
- Peak 1, 3, 4, 5 and 6 exhibited compounds were aromatic, while peak 2 derived compound were belongs to aromatic and aliphatic group.

January 2015: Site 3

- The GC-MS analysis of collected sample exhibited 10 major peaks in chromatogram.
- All peaks were coincide with library data and identify similar possible compounds.
- Peak 6 and 9 exhibited compounds were aromatic.
- Peak 1 exhibited compounds were aromatic and cyclic.
- Peak 2 exhibited compounds were aromatic, aliphatic and cyclic.
- Peak 3, 4 and exhibited compounds were aromatic and aliphatic.
- Peak 5 exhibited aromatic, cyclic and cyclic aromatic compounds.
- Peak 7 exhibited aliphatic cyclic and aromatic compounds.
- Peak 8 exhibited compounds were aromatic, aliphatic cyclic and cyclic.
- Peak 10 exhibited compounds were aromatic and cyclic aromatic.
Summary

Based on the analysis and comparison of the data obtained with the results allowed the determination of wastewater constituents. GC-MS spectral studies of nine samples of three selected sites of Sachin industrial area shows that the presence of various aromatic, aliphatic, heterocyclic, cyclic, aromatic cyclic and aliphatic cyclic compounds. These organic pollutants can cause several diseases like hypoglycemia, skin irritation, headache, reproductive disorders and even cancer. Thus, the investigation clearly indicated that treatment of industrial wastewater should be practiced before throwing it out.

ECOTOXICOLOGICAL EFFECTS OF INDUSTRIAL EFFLUENT

Toxic effect of effluent were subjected to ecotoxicological studies such as growth inhibition effects in Alga (*P. subcapitata*), acute immobilization in *Daphnia magna*, acute and embryo toxicity test in Zebrafish (*Danio rerio*).

**ALGA (Pseudokirchneriella subcapitata), GROWTH INHIBITION TEST**

The method was followed as per OECD 201, 2006.

**Alga, Growth Inhibition Test (May 2014: Site 1)**

- The collected effluent was tested at different concentrations of 0.0, 1.56, 3.13, 6.25, 12.50 and 25.0% v/v.
- The treatment inhibit the average specific growth rate of alga, when concentration was increased. The highest concentration of the effluent showed maximum effects on average specific growth rate. From 3.13 - 25.0% v/v concentrations were statistically differ from control.
- At 25.0% v/v concentration of effluent inhibit more than 90% biomass and yield of alga; growth also inhibited upto 61.80%.
- The 1.56% and 3.13% v/v concentrations were considered as NOEC and LOEc, respectively based on average specific growth rate.
Alga, Growth Inhibition Test (May 2014: Site 2)

- The collected effluent were tested at different concentrations of 0.0, 1.07, 2.35, 5.17, 11.36 and 25.0% v/v.
- Increasing concentration of effluent the average specific growth rate was decreased when compared to control.
- More than 90% biomass and yield reduction was recorded at 25% concentration and also treatment inhibit 59.16% growth rate.
- The 1.07% and 2.35% v/v concentrations were consider as NOEC and LOEC, respectively based on average specific growth rate.

Alga, Growth Inhibition Test (May 2014: Site 3)

- The collected effluent was tested at different concentrations of 0.0, 0.19, 0.38, 0.75, 1.50 and 3.0% v/v.
- Increasing concentration of effluent the average specific growth rate was decreased when compared to control.
- More than 88% biomass and yield reduction were recorded at 3% concentration and also treatment inhibit 58.53% growth rate.
- The 0.19% v/v concentration considered as NOEL and 0.75% v/v concentration consider as LOEC based on average specific growth rate.

Effective Concentrations (May 2014: Sites 1, 2 and 3)

- The EC50 values of 9.04, 7.47 & 1.29% v/v (Biomass), 20.37, 20.85 & 2.70% v/v (Growth rate) and 8.67, 7.18 & 1.21% v/v (Yield) by the treatment of site 1, 2 and 3, respectively for inhibition of algal growth.
- Based on the EC50 values, site 3 of May 2014 was highly toxic to alga followed by site 2.
Alga, Growth Inhibition Test (September 2014: Site 1)
- The collected effluent was tested at different concentrations of 20.82, 29.15, 40.82, 57.14 and 80.0% v/v with control.
- The average specific growth rate of control was 1.60/day, there was no statistical difference was found between control and treatments (20.82 and 29.15% v/v). At 29.15% v/v consider as NOEC and 40.82% v/v considered as LOEC based on average specific growth rate.
- At 80% v/v concentration inhibit more than 90% biomass and yield on 0-72 h. it also inhibited the growth rate 66.72%.

Alga, Growth Inhibition Test (September 2014: Site 2)
- The collected effluent was tested at different concentrations of 17.51, 22.76, 29.59, 38.46 and 50.0% v/v with control.
- The average specific growth rate of control was 1.55/day, there was no statistical difference found between control and treatments (17.51 and 22.76% v/v). At 22.76% v/v concentration consider as NOEC and 29.59% v/v considered as LOEC based on average specific growth rate.
- At 50% v/v concentration inhibit more than 90% biomass and yield; it also inhibit the growth rate (64.24%).

Alga, Growth Inhibition Test (September 2014: Site 3)
- The collected effluent was tested at different concentrations of 10.41, 14.58, 20.41, 28.57 and 40% v/v with control.
- The average specific growth rate of control was 1.60/day, there was no statistical difference was found between control and treatments (10.41 and 14.58% v/v). At 14.58% v/v concentration consider as NOEC and 20.41% v/v considered as LOEC, based on average specific growth rate.
- At 40% v/v concentration inhibit more than 85% biomass and yield; it also inhibited the growth rate of 53.23%.
Summary

Effective Concentrations (September 2014: Sites 1, 2 and 3)

- The EC$_{50}$ values of 52.16, 35.64 & 26.69% v/v (Biomass), 72.68, 49.76 & 42.99% v/v (Growth rate) and 50.10, 35.35 & 26.66% v/v (Yield) by the treatment of site 1, 2 and 3, respectively for inhibition of algal growth.
- Based on the EC$_{50}$ values, site 3 of September 2014 was highly toxic to alga followed by site 2.

Alga, Growth Inhibition Test (January 2015: Site 1)

- The collected effluent was tested at different concentrations of 13.02, 18.22, 25.51, 35.71 and 50% v/v with control.
- The average specific growth rate of control was 1.65/day, there was no statistical difference found between control and treatments (13.02 and 18.22% v/v). At 18.22% v/v concentration consider as NOEC and 25.51% considered as LOEC, based on average specific growth rate.
- At 50% v/v concentration inhibited the biomass (91.00%), yield (94.72%) and growth rate (57.14%) of alga.

Alga, Growth Inhibition Test (January 2015: Site 2)

- The collected effluent was tested at different concentrations of 0.26, 0.54, 1.13, 2.38 and 5.0% v/v with control.
- The average specific growth rate of control was 1.61/day, there was no statistical difference found between treatment 0.26% v/v and control. At 0.26% v/v concentration consider as NOEC and 0.54% v/v considered as LOEC, based on average specific growth rate.
- At 5.0% v/v concentration inhibited the biomass (92.42%), yield (96.70%) and growth rate (66.42%) of alga.
Summary

Alga, Growth Inhibition Test (January 2015: Site 3)

- The collected effluent was tested at different concentrations of 0.20, 0.30, 0.44, 0.67 and 1.0% with control.
- The average specific growth rate of control was 1.65/day, there was no statistical difference found between treatment 0.20% v/v and control. At 0.20% v/v concentration consider as NOEC and 0.30% V/V considered as LOEC, based on average specific growth rate.
- At 1.0% v/v concentration inhibit the biomass (91.62%), yield (94.88%) and growth rate (57.47%) of alga.

Effective Concentrations (January 2015: Sites 1, 2 and 3)

- The EC$_{50}$ values of 32.94, 1.77 & 0.59% v/v (Biomass), 53.63, 4.79 & 1.03% v/v (Growth rate) and 32.93, 1.55 & 0.55% v/v (Yield) by the treatment of site 1, 2 and 3, respectively for inhibition of alga.
- Based on the EC$_{50}$ values, site 3of January 2015 was highly toxic to alga followed by site 2.
- Site 3 was 55 times more toxic than site 1.

Algae, a primary producer, are ecologically important organism in the aquatic food chain and are an abundant bio-resource in aquatic systems. The current research revealed that the effluents prevented algal growth even at very higher dilutions. Among the effluents collected from different sites, the effluent obtained from site 3 of January 2015 had very toxic effect on *P. subcapitata*. This finding can deepen our understanding of the usefulness and effectiveness of toxicity assessment using green alga *P. subcapitata*.

**ACUTE IMMOBILISATION AND REPRODUCTION TEST STUDY OF *Daphnia magna***

The method was followed as per OECD 202, 2004 for acute immobilization and OECD 211, 2012 for reproduction study.
Summary

Acute Immobilization Study of *Daphnia magna* (May 2014: Site 1)

- The collected effluent was tested at different concentrations of 1.25, 2.50, 5.00, 10.00 and 20.00% v/v with control.
- There was no immobilization recorded in control and 1.25% v/v concentration, it consider as NOEC.
- At 2.5% v/v concentration consider as LOEC.
- At 100% immobility was recorded at 20% v/v concentration.
- Also treatment exhibited clinical sign of toxicity (bottom, Lethargy and Floating).

Acute Immobilization Study of *Daphnia magna* (May 2014: Site 2)

- The collected effluent was tested at different concentrations of 0.63, 1.25, 2.50, 5.0 and 10.00% with control.
- There was no immobilization recorded in control and 0.63% v/v concentration, it consider as NOEL.
- At 1.25% v/v concentration consider as LOEC.
- At 100% immobility was recorded at 10% v/v concentration.
- Also treatment exhibited clinical sign of toxicity (bottom, lethargy and floating).

Acute Immobilization Study of *Daphnia magna* (May 2014: Site 3)

- The collected effluent was tested at different concentrations of 0.19, 0.38, 0.75, 1.50 and 3.00% v/v with control.
- There was no immobilization recorded in control and 0.19% v/v concentration, it consider as NOEC.
- The 0.38% v/v concentration consider as LOEC.
- At 100% immobility was recorded in 3% v/v concentration and also 1.5% v/v exhibited 95% immobility.
- The treatment exhibited clinical sign of toxicity (bottom, lethargy and floating).
Effective Concentrations (May 2014: Sites 1, 2 and 3)

- The EC$_{50}$ values of 5.07, 3.22 and 0.55% v/v were recorded for immobilization effect of treatment, sites 1, 2 and 3, respectively.
- Based on the EC$_{50}$ values, site 3 of May 2014 was highly toxic to *Daphnia magna*.
- Site 3 was 55 times more toxic than site 1.

**Acute Immobilization Study of Daphnia magna (September 2014: Site 1)**

- The collected effluent was tested at different concentrations of 9.88, 14.81, 22.22, 33.33 and 50.0% v/v with control.
- There was no immobilization recorded in control and 9.88% v/v concentration, it considered as NOEL.
- At 14.81% v/v concentration consider as LOEC to *Daphnia magna*.
- The 100% immobility was recorded in 50.0% v/v concentration and also 33.33% v/v exhibited 80% immobility.
- The treatment exhibited clinical sign of toxicity (bottom, lethargy and floating).

**Acute Immobilization Study of Daphnia magna (September 2014: Site 2)**

- The collected effluent was tested at different concentrations of 10.41, 14.58, 20.41, 28.57 and 40.0% v/v with control.
- There was no immobilization recorded in control and 10.41% v/v concentration, it considered as NOEC.
- Lowest Observed Effect Level Concentration was 14.58% v/v to *Daphnia magna*.
- At 100% immobility was recorded in 40.0% v/v concentration.
- The treatment exhibited clinical sign of toxicity (bottom, lethargy and floating).

**Acute Immobilization Study of Daphnia magna (September 2014: Site 3)**

- The collected effluent was tested at different concentrations of 1.92, 3.64, 6.93, 13.16 and 25.0% v/v with control.
- There was no immobilization recorded in control and 1.92% v/v concentration, it considered as NOEC.
- Lowest Observed Effect Concentration was 3.64% v/v to *Daphnia magna*.
- At 100% immobility was recorded in 25.0% v/v concentration.
- The treatment exhibited clinical sign of toxicity (bottom, lethargy and floating).
Effective concentrations (September 2014: Sites 1, 2 and 3)

- The EC$_{50}$ values of 24.58, 22.70 and 9.38% v/v were recorded for immobilization effect of treatments, site 1, 2 and 3, respectively.
- Based on the EC$_{50}$ values, site 3 of September 2014 was highly toxic to *Daphnia magna*.

Acute Immobilization Study of *Daphnia magna* (January 2015: Site 1)

- The collected effluent was tested at different concentrations of 2.99, 5.09, 8.65, 14.71 and 25.0% v/v with control.
- There was no immobilization recorded in control and 2.99% v/v concentration, it consider as NOEC.
- Lowest Observed Effect Concentration was 5.09% v/v to *Daphnia magna*.
- At 100% immobility was recorded in 25.0% v/v concentration.
- The treatment exhibited clinical sign of toxicity (bottom, lethargy and floating).

Acute Immobilization Study of *Daphnia magna* (January 2015: Site 2)

- The collected effluent was tested at different concentrations of 0.26, 0.36, 0.51, 0.71 and 1.0% v/v with control.
- There was no immobilization recorded in control and 0.26% concentration, it consider as NOEC.
- Lowest Observed Effect Concentration was 0.36% v/v to *Daphnia magna*.
- At 100% immobility was recorded in 1.0% v/v concentration.
- The treatment exhibited clinical sign of toxicity (bottom, lethargy and floating).

Acute Immobilization Study of *Daphnia magna* (January 2015: Site 3)

- The collected effluent was tested at different concentrations of 0.01, 0.02, 0.04, 0.06 and 0.10% v/v with control.
- There was no immobilization recorded in control and 0.01% v/v concentration, it consider as NOEC.
- Lowest Observed Effect Concentration was 0.02% v/v to *Daphnia magna*.
- At 100% immobility was recorded in 0.1% v/v concentration.
- The treatment exhibited clinical sign of toxicity (bottom and lethargy).
Effective concentrations (January 2015: Sites 1, 2 and 3)

- The EC$_{50}$ values of 9.70, 0.58 and 0.04% v/v were recorded for immobilization effect of treatment, site 1, 2 and 3, respectively.
- Based on the EC$_{50}$ values, site 3: January 2015 was highly toxic to *Daphnia magna*.

Reproduction Study of *Daphnia magna* (May 2014: Site 1)

- The reproduction test was conducted at different concentrations of 0.26, 0.36, 0.51, 0.71 and 1.0% v/v effluent with control.
- There was no statistical difference between control and 0.26% v/v concentrations for offspring production, this minimum concentration considered as NOEC.
- Lowest Observed Effect Concentration was 0.36% v/v to daphnids offspring production.
- Increasing concentrations of effluent the offspring production was reduced when compared to control.
- At 1.0% v/v concentration reduced 55% offspring production of daphnids.

Reproduction Study of *Daphnia magna* (May 2014: Site 2)

- The reproduction test was conducted at different concentrations of 0.25, 0.32, 0.41, 0.54 and 0.70% v/v effluent with control.
- There was no statistical difference between control and 0.25% v/v concentrations for offspring production, which is considered a NOEC.
- Lowest Observed Effect Level was 0.32% v/v to offspring production.
- Increasing concentrations of effluent the offspring production was reduced when compared to control.
- At 0.7% v/v concentration reduced 60.41% offspring production.

Reproduction Study of *Daphnia magna* (May 2014: Site 3)

- The reproduction test was conducted at different concentrations of 0.01, 0.03, 0.05, 0.1 and 0.2% v/v effluent with control.
- There was no statistical difference between control and 0.01% v/v concentrations for offspring production, this minimum concentration considered as NOEL.
- Lowest Observed Effect Concentration was 0.03% v/v to offspring production.
Increasing concentrations of effluent the offspring production was reduced when compared to control.
At 0.7% v/v concentration reduced 65.96% offspring production

Effective concentrations (May 2014: Sites 1, 2 and 3)
- The EC$_{50}$ values of 0.85, 0.58 and 0.1% v/v were recorded for inhibition reproduction by the effect of treatment, site 1, 2 and 3, respectively.
- Based on the EC$_{50}$ values, site 3 of May 2014 was highly toxic to *Daphnia* reproduction.

Reproduction Study of *Daphnia magna* (September 2014: Site 1)
- The reproduction test was conducted at different concentrations of 0.51, 1.08, 2.27, 4.76 and 10.0% v/v effluent with control.
- There was no statistical difference between control and 0.51% v/v concentrations for offspring production, this minimum concentration was consider as NOEC.
- Lowest Observed Effect Concentration was 1.08% v/v to offspring production.
- Increasing concentrations of effluent the offspring production was reduce, when compared to control.
- At 10.0% v/v concentration was reduced 58.11% offspring production.

Reproduction Study of *Daphnia magna* (September 2014: Site 2)
- The reproduction test was conducted at different concentrations of 2.17, 3.26, 4.89, 7.33 and 11.00% v/v with control.
- There was no statistical difference between control and 2.17% v/v concentrations for offspring production, this minimum concentration was consider as NOEC.
- Lowest Observed Effect Concentration was 3.26% v/v to offspring production.
- Increasing concentrations of effluent the offspring production was reduced when compared to control.
- At 11.0% v/v concentration reduced 65.79% daphnids offspring production.
Reproduction Study of *Daphnia magna* (September 2014: Site 3)

- The reproduction test was conducted at different concentrations of 0.10, 0.22, 0.45, 0.95 and 2.0% v/v effluent with control.
- Control and 0.10% v/v concentration produced more than 77%, this minimum concentration consider as NOEC.
- Lowest Observed Effect Concentration was 0.22% v/v to offspring production.
- Increasing concentrations of effluent the offspring production was reduced when compared to control.
- At 2.0% v/v concentration reduced 71.43% daphnids offspring production.

Effective concentrations (September 2014: Sites 1, 2 and 3)

- The EC$_{50}$ values of 6.27, 8.23 and 0.94% v/v were recorded for inhibition 50% reproduction by the treatments of site 1, 2 and 3, respectively.
- Based on the EC$_{50}$ values, site 3 of September 2014 was highly toxic to *Daphnia* reproduction.

Reproduction Study of *Daphnia magna* (January 2015: Site 1)

- The reproduction test was conducted at different concentrations of 0.32, 0.56, 0.98, 1.71 and 3.0% v/v effluent with control.
- There was no statistical difference between control and 0.32% v/v concentration of effluent during offspring production. This minimum concentration consider as NOEC.
- Lowest Observed Effect Concentration was 0.56% v/v to offspring production.
- Increasing concentrations of effluent the offspring production was reduced when compared to control.
- At 3.0% v/v concentration reduced 65.04% daphnids offspring production.
Reproduction Study of *Daphnia magna* (January 2015: Site 2)

- The reproduction test was conducted at different concentrations of 0.01, 0.02, 0.05, 0.11 and 0.25% v/v effluent with control.
- There was no statistical difference between control and 0.01% v/v concentration for offspring production, this minimum concentration consider as NOEC.
- Lowest Observed Effect Concentration was 0.02% v/v to daphnids offspring production.
- Increasing concentrations of effluent the offspring production was reduced when compared to control.
- At 0.25% v/v concentration reduced 69.76% for daphnids offspring production.

Reproduction Study of *Daphnia magna* (January 2015: Site 3)

- The reproduction test was conducted at different concentrations of effluent at 0.001, 0.002, 0.003, 0.006 and 0.010% v/v with control.
- Control and 0.001% v/v concentration was statistically similar offspring produced, this minimum concentration consider as NOEC.
- Lowest Observed Effect Concentration was 0.002% v/v to daphnid offspring production.
- Increasing concentrations of effluent the offspring production was reduced when compared to control.
- At 0.01% v/v concentration reduced 63.70% offspring production.

Effective concentrations (January 2015: Sites 1, 2 and 3)

- The EC$_{50}$ values of 1.79, 0.11 and 0.01% v/v were recorded for inhibition of reproduction by the treatment, site 1, 2 and 3 respectively.
- Based on the EC$_{50}$ values, site 3 of January 2015 was highly toxic to *Daphnia* reproduction.
Findings indicate that toxicity observed on mobility and reproductive output may be attributed by the synergistic effects of physico-chemical properties and range of chemicals present in the effluents. The properties of organic substances and type, physico-chemical parameters and concentration of heavy metals in comparison with their individual toxic levels are important factors that affect the overall toxicity of the industrial effluents. In most cases, it is difficult to detect individual toxic substances in effluent. Nonetheless, a comprehensive acute and chronic tests could also assess the impact of pollutants in the wastewater on aquatic ecosystems.

**ACUTE TOXICITY STUDY OF ZEBRAFISH, *Danio rerio***

The method followed as per OECD 203, 1992 for fish acute toxicity test and OECD 236, 2013 for fish embryo toxicity test.

**Acute Toxicity Study of Zebrafish (May 2014: Site 1)**

- The acute toxicity was conducted at 6.03, 7.23, 8.68, 10.42 and 12.50% concentrations of effluent with control.
- There was no mortality in control and 6.03% concentration, this minimum concentration was considered as NOEL.
- At 7.23% was consider as LOEL to fish.
- Cent percent mortality was recorded at 12.50% concentration.
- Clinical sing (Loss of equilibrium) was recorded at 8.68, 10.42 and 12.50% concentrations.

**Acute Toxicity Study of Zebrafish (May 2014: Site 2)**

- The acute toxicity was conducted at 48.23, 57.87, 69.44, 83.33 and 100% concentrations of effluent with control.
- There was no mortality in control and 48.23% concentration, this minimum concentration considered as NOEL.
- At 57.87% was consider as LOEL for fish.
- Cent percent mortality was recorded at 100% concentration.
- Clinical sing (Loss of equilibrium) was recorded at 57.87, 69.44, 83.33 and 100% concentrations.
Acute Toxicity Study of Zebrafish (May 2014: Site 3)

- The acute toxicity was conducted at 0.38, 0.75, 1.50, 3.0, and 6.0% concentrations with control.
- There was no mortality in control and 0.38% concentration, this minimum concentration consider as NOEL to fish.
- At 0.75% was consider as LOEL to fish.
- Cent percent mortality was recorded at 6.0% concentration of effluent.
- Clinical sings (Swimming at surface, Lying on bottom, Lethargy and Loss of equilibrium) were recorded during the exposure period.

Lethal Concentration (May 2014: Sites 1, 2 and 3)

- The $LC_{50}$ values of 8.59, 66.74 and 2.21% were recorded for fish mortality by the effect of treatment, site 1, 2 and 3 respectively.
- Based on the $LC_{50}$ values, site 3: May 2014 was highly toxic to Zebra fish.

Acute Toxicity Study of Zebrafish (September 2014: Site 1)

- The acute toxicity was conducted at 26.03, 36.44, 51.02, 71.43 and 100.0% concentrations of effluent with control.
- There was no mortality in control and 26.03% concentration, this minimum concentration considered as NOEL.
- At 36.44% concentration was consider as LOEL to fish.
- Cent percent mortality was recorded at 100.0% concentration of effluent.
- Clinical sing (Lying on bottom) was recorded at 71.43 and 100.0% concentration of effluent.

Acute Toxicity Study of Zebrafish (September 2014: Site 2)

- The acute toxicity was conducted at 15.80, 23.70, 35.56, 53.33 and 80.0% concentrations of effluent with control.
- There was no mortality in control and 15.80% concentration of effluent which is considered as NOEL to fish.
- At 23.70% concentration was consider as LOEL to fish.
- Cent percent mortality was recorded at 80% concentration of effluent.
- Clinical sing (Loss of equilibrium) was recorded at 23.70, 35.56, 53.33 and 80.0% concentration of effluent.
Summary

Acute Toxicity Study of Zebrafish (September 2014: Site 3)

- The acute toxicity was conducted at 18.22, 25.51, 35.71, 50.0 and 70.0% concentrations of effluent with control.
- There was no mortality in control, 18.22 and 25.51% concentration, based on no mortality 25.51% was consider as NOEL to fish.
- At 35.71% of effluent was consider as LOEL to fish.
- Cent percent mortality was recorded at 70% concentration of effluent.
- Clinical sings (Lethargy and Lying on bottom) were recorded during the exposure period.

Lethal Concentration (September 2014: Sties 1, 2 and 3)

- The LC_{50} values of 54.92, 41.36 and 46.25% were recorded for fish mortality by the effect of treatment, site 1, 2 and 3 respectively.
- Based on the LC_{50} values, site 2: September 2014 was highly toxic to Zebra fish.

Acute Toxicity Study of Zebrafish (January 2015: Site 1)

- The acute toxicity was conducted at 48.23, 57.87, 69.44, 83.33 and 100.0% concentration of effluent with control.
- There was no mortality in control and 26.03% concentration of effluent based on no mortality 26.03% concentration was consider as NOEL to fish.
- At 36.44% concentration of effluent was consider as LOEL to fish.
- Cent percent mortality was recorded at 100.0% concentration of effluent.
- Clinical sings (Loss of equilibrium, swimming on surface, Lying on bottom) were recorded at 71.43 and 100.0% concentration of effluent.

Acute Toxicity Study of Zebrafish (January 2015: Site 2)

- The acute toxicity was conducted at 15.80, 23.70, 35.56, 53.33 and 80.0% concentration of effluent with control.
- There was no mortality in control and 48.23% concentration of effluent, based on no mortality 48.23% concentration was consider as NOEL.
- Based on less mortality of fish at 57.87% concentration consider as LOEL.
- Percent mortality was recorded at 100% concentration of effluent.
- Clinical sings (loss of equilibrium, swimming on surface, lying on bottom) were recorded at 57.87, 69.44, 83.33 and 100.0% concentration of effluent.
Summary

Acute Toxicity Study of Zebrafish (January 2015: Site 3)

- The acute toxicity was conducted at 2.60, 3.64, 5.10, 7.14 and 10.0% concentrations with control.
- There was no mortality in control, 2.60 and 3.64% concentration.
- At 3.64% was consider as NOEL
- At 5.10% was consider as LOEL
- Cent percent mortality was recorded at 10% concentration
- Clinical sing (swimming at surface, lying on bottom, loss of equilibrium, Lethargy) were recorded at 3.64, 5.10, 7.14 and 10.0%.

Lethal Concentration (January 2015: Site 1, 2 and 3)

- The LC$_{50}$ values of 74.33, 5.85 and 6.40% were recorded for fish mortality by the effect of treatment, site 1, 2 and 3 respectively.
- Based on the LC$_{50}$ values, site 2: January 2015 was highly toxic to Zebra fish.

Fish Embryo Toxicity Test (May 2014: Site 1, 2 and 3)

Hatchability

- All the collected effluent toxic to fish embryo.
- Lethal concentration of 3.18, 40.52 and 1.74% v/v for hatchability of fish were recorded after treatment of site 1, 2 and 3 respectively, on May 2014.
- Based on the LC$_{50}$ value site 2 considered as highly toxic for embryo.

Mortality

- At 3.44 and 2.60% v/v concentration of effluent from site 1 and 3, respectively for 50% mortality.
- Site 2 was less toxic than other 2 sites.
Summary

Fish Embryo Toxicity Test (September 2014: Sites 1, 2 and 3)

Hatchability

➢ All the collected effluent toxic to fish embryo.
➢ Lethal concentration of 37.46, 31.18 and 27.08% v/v for hatchability of fish were recorded after treatment of effluent collected from site 1, 2 and 3, respectively.
➢ Based on the LC\textsubscript{50} value site 3 considered as highly toxic for embryo.

Mortality

➢ At 43.72, 36.65 and 33.07% v/v concentration of effluent collected from site 1, 2 and 3 respectively for 50% mortality.
➢ Site 3 was toxic than other 2 sites

Fish Embryo Toxicity Test (January 2015: Sites 1, 2 and 3)

Hatchability

➢ All the collected effluent exhibited toxic to fish embryo
➢ Lethal concentration of 35.01, 2.43 and 0.80% v/v were recorded for hatchability of fish after treatment of effluent collected from site 1, 2 and 3 respectively.
➢ Based on the LC\textsubscript{50} value site 3 considered as highly toxic for embryo.

Mortality

➢ At 32.94, 3.08 and 1.07% v/v concentration of effluent collected from site 1, 2 and 3 respectively for 50% fish mortality.
➢ Site 3 was toxic than other 2 sites.

The fish is secondary consumer and play ecological significant role in the in the aquatic food chain. The current research revealed that all of the industrial effluent from three different sites of Sachin industrial area from May 2014, September 2014 and January 2015 tested had acute toxicity effect on zebrafish and its embryos. The effluents also prevented the hatching of the embryos at a very higher dilutions. Among the effluents collected from different sites, the effluent obtained from site 2 and 3 of January 2015 had a very toxic effect on Zebrafish, \textit{Danio rerio}.
GENOTOXICOLOGICAL EVALUATION OF INDUSTRIAL EFFLUENT BY AMES TEST

The present study was carried out to evaluate the genotoxicity of industrial effluent from Sachin industrial area, Surat, South Gujarat, India employing the Ames plate incorporation test. The method was followed as per OECD 471, 1997.

AMES Test (May 2014: Site 1)

- Reduction in number of revertant colonies was observed 37% to 78% at the concentration of 25% v/v in the absence of metabolic activation, while reduction in number of revertant colonies was observed between 40% to 74% at the concentration of 25% v/v in the presence of metabolic activation.
- Results revealed that there was no positive mutagenic effect was observed in tester strains TA1537, TA1535, TA98, TA100 and TA102 at tested concentrations 3.13, 6.25, 12.5, 25, 50 and 100% v/v in the absence and presence (5% v/v S9 mix) of metabolic activation, when compared with the concurrent negative control.

AMES Test (May 2014: Site 2)

- Reduction in number of revertant colonies was observed 39% to 81% at the concentration of 25% v/v in the absence of metabolic activation, while reduction in number of revertant colonies was observed between 40% to 77% at the concentration of 25% v/v in the presence of metabolic activation.
- Results revealed that there was no positive mutagenic effect was observed in tester strains TA1537, TA1535, TA98, TA100 and TA102 at tested concentrations 3.13, 6.25, 12.5, 25, 50 and 100% v/v in the absence and presence (5% v/v S9 mix) of metabolic activation, when compared with the concurrent negative control.

AMES Test (May 2014: Site 3)

- Reduction in number of revertant colonies was observed 40% to 67% at the concentration of 3.13% v/v and 54% to 90% at the concentration of 6.25% v/v in the absence of metabolic activation, while reduction in number of revertant colonies was observed between 44% to 65% at the concentration of 3.13% v/v and 58% to 86% at the concentration of 6.25% v/v in the presence of metabolic activation.
Results revealed that number of revertant colonies was observed 1.1 to 1.3 times higher than the negative control in the tester strains TA98, TA100 and TA102 at the concentrations of 0.39, 0.78 and 1.56% v/v, while there was no increase in number of revertant colonies was observed in tester strains TA1537 and TA1535 at concentrations of 0.39, 0.78 and 1.56% v/v both in the absence and presence (5% v/v S9 mix) of metabolic activation, when compared with the concurrent negative control.

AMES Test (September 2014: Site 1)

Reduction in number of revertant colonies was observed 12% to 37% at the concentration of 50% v/v and 49% to 83% at the concentration of 100% v/v in the absence of metabolic activation in the tester strains TA1537, TA1535, TA98, TA100 and TA102, while reduction in number of revertant colonies was observed between 13% to 41% at the concentration of 50% v/v and 42% to 68% at the concentration of 100% v/v in the presence of metabolic activation in the tester strains TA1537, TA1535, TA98, TA100 and TA102.

Results revealed that there was no positive mutagenic effect was observed in tester strains TA1537, TA1535, TA98, TA100 and TA102 at tested concentrations 3.13, 6.25, 12.5, 25, 50 and 100% v/v in the absence and presence (5% v/v S9 mix) of metabolic activation, when compared with the concurrent negative control.

AMES Test (September 2014: Site 2)

Reduction in number of revertant colonies was observed 41% to 77% at the concentration of 50% v/v in the absence of metabolic activation in the tester strains TA1537, TA1535, TA98, TA100 and TA102, while reduction in number of revertant colonies was observed between 39% to 65% at the concentration of 50% v/v in the presence of metabolic activation in the tester strains TA1537, TA1535, TA98, TA100 and TA102.

Results revealed that there was no positive mutagenic effect was observed in tester strains TA1537, TA1535, TA98, TA100 and TA102 at tested concentrations 3.13, 6.25, 12.5, 25, 50 and 100% v/v in the absence and presence (5% v/v S9 mix) of metabolic activation, when compared with the concurrent negative control.
Summary

AMES Test (September 2014: Site 3)

- Reduction in number of revertant colonies was observed 39% to 73% at the concentration of 50% v/v in the absence of metabolic activation in the tester strains TA1537, TA1535, TA98, TA100 and TA102, while reduction in number of revertant colonies was observed between 35% to 65% at the concentration of 50% v/v in the presence of metabolic activation in the tester strains TA1537, TA1535, TA98, TA100 and TA102.

- Results revealed that there was no positive mutagenic effect was observed in tester strains TA1537, TA1535, TA98, TA100 and TA102 at tested concentrations 1.56, 3.13, 6.25, 12.5, 25 and 50% v/v in the absence and presence (5% v/v S9 mix) of metabolic activation, when compared with the concurrent negative control.

AMES Test (January 2015: Site 1)

- Reduction in number of revertant colonies was observed 61% to 85% at the concentration of 50% v/v in the absence of metabolic activation in the tester strains TA1537, TA1535, TA98, TA100 and TA102, while reduction in number of revertant colonies was observed between 49% to 90% at the concentration of 50% v/v in the presence of metabolic activation in the tester strains TA1537, TA1535, TA98, TA100 and TA102.

- Results revealed that there was no positive mutagenic effect was observed in tester strains TA1537, TA1535, TA98, TA100 and TA102 at tested concentrations 3.13, 6.25, 12.5, 25, 50 and 100% v/v in the absence and presence (5% v/v S9 mix) of metabolic activation, when compared with the concurrent negative control.

AMES Test (January 2015: Site 2)

- Reduction in number of revertant colonies was observed 39% to 84% at the concentration of 3.13% v/v in the absence of metabolic activation in the tester strains TA1537, TA1535, TA98, TA100 and TA102, while reduction in number of revertant colonies was observed between 44% to 77% at the concentration of 3.13% v/v in the presence of metabolic activation in the tester strains TA1537, TA1535, TA98, TA100 and TA102.
Results revealed that number of revertant colonies was observed 1.1 to 1.3 times higher than the negative control in the tester strains TA98, TA100 and TA102 at the concentrations of 0.39, 0.78 and 1.56% v/v, while there was no increase in number of revertant colonies was observed in tester strains TA1537 and TA1535 at concentrations of 0.39, 0.78 and 1.56% v/v both in the absence and presence (5% v/v S9 mix) of metabolic activation, when compared with the concurrent negative control.

**AMES Test (January 2015: Site 3)**

- Reduction in number of revertant colonies was observed 47% to 90% at the concentration of 0.78% v/v in the absence of metabolic activation in the tester strains TA1537, TA1535, TA98, TA100 and TA102, while reduction in number of revertant colonies was observed between 41% to 79% at the concentration 0.78% v/v in the presence of metabolic activation in the tester strains TA1537, TA1535, TA98, TA100 and TA102.

- Results revealed that number of revertant colonies was observed 1.1 to 1.4 times higher than the negative control in the tester strains TA98 and TA100 at the concentrations of 0.1, 0.2 and 0.39% v/v, while there was no increase in number of revertant colonies was observed in tester strains TA1537 and TA1535 at concentrations of 0.1, 0.2 and 0.39% v/v both in the absence and presence (5% v/v S9 mix) of metabolic activation, when compared with the concurrent negative control. There was clear mutagenic effect was observed (2.0 to 2.1 times higher than control) in the tester strain TA102 at the concentrations of 0.1, 0.2 and 0.39% v/v, while there was no increase in number of revertant colonies was observed in tester strains TA1537 and TA1535 at concentrations of 0.1, 0.2 and 0.39% v/v both in the absence and presence (5% v/v S9 mix) of metabolic activation, when compared with the concurrent negative control.
Summary

The present research builds up a basic framework to acquire more information about the prevalence and levels of mutagenic agents in industrial effluents. Furthermore, bacterial reverse mutation test (Ames test) being simple, quick and relatively easy to perform can be used as an initial screening test to assess the suitability of industrial effluents to be released into the environment. Although the industrial effluents from site 1 and 2 of May 2014, site 1, 2 and 3 of September 2014 and site 1 of January 2015 did not produce any genotoxic response, there was some indication of higher toxicity while there was clear indication of mutagenic effect was observed in the industrial effluents from site 3 of May 2014 and site 1 and 2 of January 2015 in the tester strains TA98, TA100 and TA102 both in the absence and presence of metabolic activation system. Our findings suggest that TA98, TA100 and TA102 were sensitive towards the industrial effluents and showed considerable mutagenicity. This is however, a warning indication and if no measures are taken to rectify this ever increasing mutagenic contamination in the environment it would lead to dire consequences.