INTRODUCTION

“Water is life’s matter and matrix, mother and medium. There is no life without water. The following quote from Albert Szent-Gyorgyi, illustrates that how important is water for life. Water is not just important for nature or organism, but it is the most precious natural resource upon which the society relies for its quality of life including health and recreation. But now a days due to the presence of various toxic pollutants ranging from plastic bags to chemical wastes our water bodies have turned to pools of poisons. Major cause of ill health in India is polluted water and 80% of stomach diseases are caused by it. According to an estimate, 580 people in India die due to illness caused by polluted water every day.

In the world market thousands of chemicals are entering annually, maximum of them are hazardous to the aquatic environment. Pollution of the aquatic ecosystems is worldly recognized as a potential threat to all animals including humans as well as aquatic organisms which interact with aquatic environment (Biney et al., 1987). In the developing countries, 90 to 95% of all sewage and 70% of industrial wastes are dumped untreated into surface water (Obiakor et al., 2012). Industrial effluent is a main source of pollutants for aquatic ecosystems. Wastewater is generated by many industries during their operation and processing. Depending on the type of industry, the wastewater contains suspended solids, both biodegradable and non biodegradable organics, dissolved inorganic and several other compounds. In India almost all the water bodies are found to be contaminated with one or another industry and ample evidences are available regarding the mismanagement of industrial waste (Jadhav and Singare, 2015; Singare and Dhabarde, 2014).

The history of environmental chemical pollutants is dominated by several chemical compounds of different characteristics such as polycyclic aromatic hydrocarbons, polychlorinated insecticides, polychlorinated biphenyls, synthetic surfactants and heavy metals etc. In early 1980s it became evident that alkylphenolic compounds are significant environmental pollutants which are derived from non ionic surfactants of alkylphenol polyethoxylate. After that nonylphenol ethoxylates (NPEOs) and their metabolites became a major focus of environmental research.

Nonylphenol ethoxylates are cost effective surfactants used globally in industrial, institutional, commercial and household applications such as detergents,
antistatic agents, emulsifiers, demulsifiers, wetting and dispersing agents and solubilisers (Fiege et al., 2000; Lorenc et al., 2003; Soares et al., 2008). Furthermore, these chemicals are being used in the products we use in our daily life as shampoos, deodorants and skin care products etc. Due to the extensive use of nonylphenol ethoxylates, they reach sewage treatment works in substantial amounts where they are incompletely degraded to nonylphenol (NP) due to microbial action (Ahel et al., 1994; Johnson et al., 2005; Koh et al., 2005; Nakada et al., 2006; Shao et al., 2003; Vazquez-Duhalat et al., 2005) and then via food chain accumulates in the bodies of biota. Since its discovery, the production of nonylphenol has increased exponentially between 100 to 500 million pounds every year and meet the definition of high production volume chemical (EPA, 2010). Worldwide production of NP is approximately 500,000 tons, out of which 60% of this quantity is finally discharged to water bodies (Ying et al., 2002). European Union has included NP and their derivative in the list of priority hazardous substances (Directive 2000/60/EC, 2000).

![Nonylphenol ethoxylates](image)

NP has attracted the attention due to its pervasiveness in the environment. Concerns have increased recently as it can mimic natural hormones and its copious level in environment can disrupt endocrine, immune, and reproductive systems (Masuno et al., 2003; Ying et al., 2002). Its production rate in different countries are: 154,200 tons in the USA in 2001, 73,500 tons in Europe in 2002, 16,500 tons in Japan in 2001 and 16,000 tons in China in 2004 (Soares et al., 2008). EPA have accepted the risk of nonylphenol so most countries have banned the usage of alkylphenol ethoxylates as surfactants, and its production is decreasing gradually in these countries (Soares et al., 2008). However, in many developing countries, including India, China and South America, no action has been taken to decrease the usage of NP step by step. Rather the use and production of NPEOs in these countries has increased annually (Mao et al., 2012).

Fate of any compound in the environment depends upon the physico-chemical properties and these properties in turn depend upon the structure of the compounds.
Toxicity of a compound is related to its hydrophobicity and electrophilicity. Tissue penetration, adsorption and excretion are related to hydrophobicity and acute toxicity is determined by electrophilicity. Nonylphenol ethoxylates (NPEOs) are aromatic compounds with polyethoxylate chain and a alkyl chain having nine carbon, which are attached to benzene ring. Structure of nonylphenol may vary, as carbon chain may attach at various positions on the phenol group, it may be ortho, para and meta in position and can be either linear or branched representing a different isomer. The para isomer is the typical member of family making up to 90% of the commercial form and is used in experimentation and environmental analysis.

![Image of ortho, meta and para isomer of alkylphenol](image)

NPEOs have different physical and chemical properties and as increase in the ethoxylate chain length occurs decrease in hydrophobicity and solubility has been found. Lower ethoxylates NP compounds are found to be more persistent and dangerous for living organisms. The half life of these compounds is above 60 years in sediments (Shang et al., 1999). Nonylphenol ethoxylates are non ionic in water. Due to this property they are used in variety of applications like detergents, cleaners and emulsifiers etc. Being amphipathic in nature it may surround the substances like oil and grease and can isolate them from water.

### Table 1.1. Physico-chemical properties of 4-Nonylphenol.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C_{15}H_{24}O</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>220.34g/mole</td>
</tr>
<tr>
<td>Appearance</td>
<td>Transparent or light straw-colored, high-viscosity liquid; slight phenolic odor</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>0.95 (20°C)</td>
</tr>
<tr>
<td>Melting point</td>
<td>approx. -8°C</td>
</tr>
<tr>
<td>Vapor pressure</td>
<td>0.3Pa or lower (25°C)</td>
</tr>
<tr>
<td>Aquatic solubility</td>
<td>6mg/L (20°C)</td>
</tr>
<tr>
<td>Log Kow</td>
<td>4.48</td>
</tr>
</tbody>
</table>

Soares et al. (2008)
Introduction

The continuous input of pollutants into environment has led to the development of many different techniques to evaluate and monitor their fate and effect. A sensitive and reliable approach to estimate the potential effect of pollutants is the use of sentinel organism. A wide variety of organisms have been used to monitor areas with different pollution levels. Among these fishes represent excellent experimental model for toxicological studies as they inhabit all zones of aquatic habitat, bioaccumulate the environmental pollutants, respond to mutagens at low concentration and provide early warning for pollution induced environmental changes. Fishes are sensitive for DNA damage induction and show slow DNA repair than mammals. These are able to accumulate and metabolize the pollutant in a similar manner as higher vertebrates and may be used to evaluate the potential indicator of some contaminants to produce teratogenic and carcinogenic effects in human.

The investigation of the genotoxic potential of various aquatic pollutants has become a major task in the monitoring of environmental pollution. Genotoxicity biomonitoring is important in aquatic organisms due to several reasons. Firstly, the genetic diversity in natural environment can be preserved by preventing the pollution induced mutations. Secondly, the genotoxicity biomonitoring helps to assess the health status of aquatic organisms by evaluating the mutagenic/ carcinogenic effects of xenobiotics in aquatic organisms. DNA damage initially occurs at the cellular level, then at organism and finally at community and population level. Due to all these reasons genotoxicity studies are gaining importance. A number of techniques have been developed to test DNA alterations in aquatic organisms. These include mainly chromosomal aberrations, sister chromatid exchange, comet assay and micronucleus assay. Comet assay and micronucleus assay have advantage over other tests as these are technically easier and less time and resource consuming.

To identify genomic alterations in animals the micronucleus test is one of the most widely used technique. The assay was developed first for mammalian species but also found its wide applications for other aquatic as well as wild and transplanted animals. Micronuclei are the small chromatin mass or lagging chromosomes which are not incorporated in daughter nuclei during mitosis and remain in cytoplasm. MN formation may result into change in chromosome number or can affect the gene activity. Such changes lead to genetic damage or imbalance to the organism and its progeny.
(Hartwell et al., 2000). On the other hand comet assay is another method being used worldwide for detecting DNA damage in prokaryotic and eukaryotic cells. The advantages of using comet assay for biomonitoring are that it can detect very low level of damage, require very less sample for processing, flexible to use any cell, easy to perform, low in cost and require less time to complete the study (Gedik et al., 1992).

Alkaline version of comet assay was introduced by Singh and coworkers in 1988. Further Olive et al. (1990) gave another version in which lysing in alkaline followed by electrophoresis at either neutral or mild alkaline conditions was proposed. Klaude et al. (1996) introduced further improvements in the test procedure. As DNA is a highly organized structure with matrix proteins in the nucleus, its organization gets disrupted when there is damage in the DNA; this is the concept underlying the single cell gel electrophoresis. The damage leads to relaxing of DNA strands and loss of compact structure which results into expanding the DNA strand out of the agarose cavity when an electric field is applied. The image of DNA migration obtained looks like a comet in the sky having head and tail. The circular head corresponds to undamaged DNA and tail for damaged DNA. Brighter and longer the tail, higher will be the damage.

To determine the effects of external stressors the haematological variables are also being extensively used. Large number of pollutants present in water bodies exert their toxic effects by generating reactive oxygen species and causing oxidative stress which leads to alteration in blood composition and immune mechanisms. Sampath et al. (1993) stated that the study of haematological parameters in fish reveal the conditions within the body of fish long before any external manifestation of effects of unfavorable environmental contaminants is ascertained. So fish haematology is an important tool to study the status of nutrition, health, diseases and stress in response to changing environmental conditions. Variations in haematological parameters of fish in response to different pollutants have been noted by David et al. (2015); Ghaffar et al. (2015); Mallesh et al. (2015).

Water pollution also leads to induce the pathological changes in fish. As an indicator of exposure to contaminants, histology represents a useful tool to assess the degree of pollution. It provides a rapid and inexpensive method for detection of various
pollutants in different tissues and organs so that target site can be identified. Histopathological alterations caused by different environmental contaminants in liver, gill and kidney of different fish species have been studied (Sachar et al., 2015; Ubong et al., 2015).

Concentration of the contaminant and duration of its exposure to the organism are the two important determinant of toxicity caused by a contaminant. Depending upon the duration, contaminant exposure is of two types i.e. Acute and chronic. Acute exposure are routinely from 24 to 96 hours and relatively of short duration, while chronic exposure are for a significant part of life of test organism and are for longer duration. Survival and other responses like growth and reproduction, fitness and well being of populations are routinely being studied in chronic exposure studies. Recovery after exposure to a chemical is a revitalizing phenomenon as every organism strives to overcome the stress to prove its existence (David and Kartheek, 2015). Fish is having a homeostatic mechanism which allows it to stabilize and overcome the stress.

So keeping all these things in mind, this thesis aimed at increasing the knowledge and understanding the toxic effects of 4-NP towards an aquatic vertebrate model *Channa punctatus*. *C. punctatus* is distributed throughout India. Such a species is of commercial importance due to its easy maintenance, high food value and availability throughout the year. Moreover *C. punctatus* has been used in fundamental research and considered as an excellent model for toxicological studies. Focus is on intertissue differences with respect to the toxicity following acute and sub chronic exposure. Along with this the recovery pattern is studied after sub chronic exposure. After acute and sub chronic exposure of 4-nonylphenol the emphasis is mainly on behavioural response, cytogenotoxicity, haematological changes and histological alterations in *C. punctatus*. 