SUMMARY
Introduction-

Fishes are aquatic and poikilothermic animals. Hence, their existence and performance is dominated by the quality of their environment. Conditions in large bodies of water although are relatively stable, a greater magnitude of environmental change exists in smaller bodies of water, particularly in the presence of man-made stressors. Thus, in the aquatic environment, life goes on under dynamic and unstable circumstances, forcing fishes to acclimatize to various factors, such as, changes in population density, pressure, temperature, dissolved gases, light, pH, etc, which impose a considerable amount of stress on their lives and predispose them to diseases. A fish’s survival in the face of environmental stress thus depends up on its ability to adjust its physiological processes so as to maintain relatively constant internal body chemistry. Any stress requiring an adjustment in excess of ability to accommodate will be lethal or will result in disease (Wedemeyer, 1970, Wedemeyer and Wood, 1974). Fish diseases do not occur as single caused events but are the end result of interactions of the etiologic agent, the fish and the environment. The environmental factors which can adversely affect fish and cause stress are –chemical, physical, biological and procedural factors. (Wedemeyer et al, 1999)

Stress is a state produced by an environmental or other factor which extends the adaptive response of an animal beyond the normal range, or which disturbs the normal functioning to such an extent that the chances of survival are significantly reduced (Brett, 1958). Or in other words, ‘Stress’ means the sum of all the physiological responses by which an animal tries to maintain or reestablish a normal metabolism in the face of physical and chemical forces (Selye, 1950). A series of morphological, biochemical and physiological changes occur as a result of stress and constitute the General Adaptation Syndrome
(Wedemeyer et al,1999). Hence, studying such changes in affected tissues are important in determining the nature and extent of toxicants effects on organisms and help in providing an early warning that pollution is reaching harmful levels. With their ability to detect sudden changes in environment and monitor short or long term changes in water quality, fishes thus make efficient biomarkers.

Phenols are hydroxy-derivatives of aromatic hydrocarbons and under natural conditions are formed during the decomposition of organic materials. They are an important constituent of coal tar. The major portion of the phenol present in the environment, however, is of anthropogenic origin. Production and use of phenol and its products (wood, iron and steel industry), exhaust gases, residential wood burning, cigarette smoke and smoked food are potential sources of phenols. Besides, atmospheric degradation of benzene under the influence of light is another potential source. While the main emissions of phenol occur to air, a minor portion disappears from air to soil and water bodies by wet deposition or rain. Although no data are available on the atmospheric levels of phenol, background levels are expected to be less than 1 µg / cubic meter. Urban and sub urban levels vary between 0.1 to 8.0 µg / cubic meter. Industrial pollution, particularly due to the iron and steel industry, leads to elevated levels of phenol in sediments and ground water (WHO task group, IPCS, A report, 1993) and needs to be taken care of.

The Bhilai Steel Plant is an integrated steel plant situated 30 kilometers (west) of Raipur, the capital of the state of Chhattisgarh (INDIA). Besides the major marketable product which is good quality steel, it also produces important by products, such as, Coal tar, Naphthalene and Benzol. Coke which is used as a fuel and reducing agent in the blast furnace is converted from coal in the coke ovens. The effluent thus generated, has a strong phenolic
odour and contains a high amount of phenol, besides the presence of other toxic substances.

Phenol and phenolic compounds are stressful environmental factors which because of their lipophilic properties present a threat against natural environment and also to human health (Hori et al., 2006). They have been found to induce genotoxic (Jagetia and Aruna, 1997), carcinogenic (Tsutsui et al., 1997) and immunotoxic effects (Taysse et al., 1995) on fish health, besides being responsible for reduction in fish weight and fertility (Saha et al., 1999). Some phenolic compounds, such as, Polycyclic Aromatic Hydrocarbons and Polychlorinated biphenyls are Endocrine disrupters (Kashiwada et al., 2002; Pait and Nelson, 2003; Tollefsen, 2006; Barse et al., 2006; Martin-Skilton et al., 2006) and adversely affect fish metabolism (Gupta et al., 1983; Abdel-Hameid, 1994). Most importantly, phenol has been listed in the NRWQC as a priority pollutant with an organoleptic effect criterion of 300µg/l (USEPA, 2009). Hence, it is very toxic to fish and has a unique quality of tainting the taste of fish if present in marine environments at 0.1-1.0 ppm (Kirk and Othmer, 1982; Neff, 2002). Thus, the importance of taking into consideration phenol intoxication in natural aquatic habitats becomes evident.

Levels of Phenols with means of 0.01 to 5.7 mg/l (max. 53 mg/l) have been reported in effluents from various industrial sources (Howard, 1989). Highest levels were found to be associated with the Iron and Steel industry. The ground water at hazardous sites contains phenol up to the level of 2.48 to 85000µg/l (VIEW database, ATSDR, 1989). A major portion of the work on the effect of Phenol has been conducted on humans and experimental mammals, such as- Mice (VonOettingen&Sharples, 1946; Kostovetskii&Zholdakova, 1971); Rats (Deichman & Witherup, 1944; Conning & Hayes, 1970; Thomson & Gibson, 1984; Schlicht et al, 1992) and Rabbids (Flickinger, 1976; Vernot et al, 1977).

Gupta (1988) made observations on the size related toxicity of industrial effluents from the Sangam Dyeing Factory (Ludhiana), the Sterling Steel and ABC Paper Mills (Hoshiarpur) to Cyprinus carpio (Ham.) and Channa punctatus (Bl.) and found the effluent from the Sterling Steel Mill to be highly toxic to fish than that emerging from the Dyeing Factory.

The present work on the effect of phenolic industrial wastes on food fishes is a preliminary one. Sinha (PhD Thesis, 1999) studied the physico-chemical characteristics of the Bhilai steel plant effluent and reported it to be rusty brown in color and with a pungent phenolic odour. Besides other parameters, such as, total alkalinity, chloride ions, total dissolved solids (TDS), chloride ions and sulphates etc. were also found to be far above normal levels. Although the Bhilai Steel Plant happens to be an important industry of Chhattisgarh, no studies however, have yet been done on the effect of its phenolic effluents on...
food fishes. Hence the present work is an attempt to understand the impact of a stressful phenolic environment on the food fish *Channa punctatus*.

An industrial effluent is a complex of stressors and studies on their effect must be rather complicated. Thus, a reductionistic approach has been employed taking into consideration only one important constituent for comparison. The coke oven effluent arises from a place where important byproducts are generated in the form of coal tar and benzene. Hence, Phenol has also been taken as the environmental stressor along with the phenolic effluent.

The present dissertation consists of studies on the following major aspects –

I. **The effluent channel**

II. **Water Analysis**

III. **Toxic effects of Phenol and Phenolic effluent**

Short and Long term exposures to high and low concentrations of Phenol and Effluent have been done and toxic effects studied on the basis of -

(a) *% Mortality and Lethal Concentration*

(b) *Behavioral effects.*

(c) *Hematological effects*

(d) *Histopathological effects.*

The methodology employed, results and discussion have been described separately in the following chapters.

I. **The effluent channel**

A preliminary survey was conducted to gather useful information regarding the source and course of the coke oven effluent channel, its catchment area and survey of fish
species living therein. The channel starts from its source at Purena (Bhilai 3) and ends up in the river Kharoon, a tributary of the river Mahanadi which caters to the needs of water and fish for Raipur, the capital of Chhattisgarh and adjoining cities. The soil texture of the embankment near Purena appeared to be oily black with intense phenolic odour in the wastewater. A survey of the villages (Nardhi, Sirsa and Aundhi) in the vicinity of the channel was also conducted in the form of a questionnaire to understand the effect of the effluent on the humans and animals dwelling there and using the water for their daily chores. About 30 villagers were interviewed in the survey. Accordingly, fishes were not reported to be present in the channel near Nardhi village, although in few instances Turtles could be found. Presence of fishes like Carps and Tilapia were reported to be present within the channel near Sirsa. They were ill grown, blackish, with heavy mucus secretion and reddish papules on their surface. The fish were complained of being unpalatable, indigestible with intense phenolic odour in their flesh. The embankment of the channel in this place consisted of only members of the grass family. In Aoundhi, although fishes were absent, the phenolic wastewater was being used for irrigation of Paddy by the local villagers. Use of this wastewater by the villagers for bathing their Cattle was reported to cause an itching and burning sensation in the skin of both humans and animals and accompanied by dermatitis.

Inevitably, all these factors raise questions on the toxic effects of the effluent on the aquatic life dwelling in the river. On the basis of the reports made by the villagers about its prior existence in the effluent channel far off from the source, *Channa punctatus* was taken as the experimental model.

II. Water Analysis-
Effluents discharged by the Bhilai Steel Plant, namely, through Kosanala, Khursipar and Purenanala were collected from their origin points and analyzed for their physicochemical characteristics such as, pH, temperature, odour, DO, BOD, Alkalinity, Chloride, Nitrate, Nitrite, Sulphates, Phenol, Ca, Mg, Hardness, Total Dissolved and suspended Solids, by Standard Methods (APHA, 1985). The effluent discharged by the Coke ovens through the Purena Channel (Bhilai 3) contained the highest amount of phenol in the range of 5-6 mg/l. Three regions of Purena Channel (Purena, Somni and Kumhari) were selected for water analysis. Collection of water samples was also done both in upstream and downstream direction, from the region where the effluent channel happens to meet the river Kharoon. The analyses were performed before exposing fishes, both in summer and winter for two consecutive years (2004 and 2005). In all cases, the amount of phenol was found to exceed the Natural recommended water quality criteria values for freshwater fish according to USEPA, 2010. The coke oven effluent taken from the source at Purena channel was used as the environmental stressor to expose fishes for long and short term durations. Commercially available Phenol of the same concentration was taken as a reference for comparison.

III. Toxic effects of Phenol and Phenolic effluent

Live, healthy fishes belonging to the same age group were collected from local ponds and acclimatized under normal laboratory conditions for 15 days. They were then separated into 7 groups of 16 fishes each. Exposure to various concentrations of Phenol and phenolic effluent along with normal tap water as control was done in 20 liters glass aquaria. Two ranges of concentrations, viz., low (0.01%, 0.015%, 0.02% & 0.025%) and high (10%, 20% and 30%) of both phenol and phenolic effluent, were taken after dilution with tap water.
Short term exposure was given for a period of 7 days and collection of samples done at 7
time points ie.24,48,72,96,120,.144 &168 hours. Long term exposure to fishes was given for
duration of 4 weeks and collection of samples done at the end of each week. The following
aspects were studied-

(a) **% Mortality and Lethal Concentration**

No mortality was caused during short term exposures. However,% mortality after
long term exposure was recorded to be between 15 to 25% in the low concentrations and
between 25-35% in high concentrations of the effluent. Mortality of fishes started after 48
hours of exposure .The lethal concentration of phenol was calculated to be 5.6 g/l.

(b) **Behavioral effects**-

Behavioral effects in response to the above mentioned concentrations of the effluent
were also recorded at different time points, both in case of short and long term exposures.
Although, no obvious changes were visible in all low concentrations (0.01 %, 0.15 %, 0.2
% ,0.25 %) of the effluent in the first 24 hours, during short term exposures, however, after
48 hours, fishes exhibited a rapid and erratic swimming behavior, accompanied with rapid
operculum movements and heavy mucus secretion. In response to higher concentrations
(10% ,20% ,30 %) of effluent, additionally, exposed fishes made desperate attempts to
escape out of the toxic environment. The behavioral features mentioned during short term
exposures intensified further during long term exposures to high concentration of effluent. A
loss of swimming balance accompanied by an erect body supported by the caudal fin was
evident in 30% effluent. The fishes appeared pale and restless often dashing against the wall
of the aquarium. All this shows the presence of a large amount of environmental stress
among the fishes and reflects the toxic nature of the phenolic effluent. The various aspects of behavioral response of the fishes have been discussed in this chapter.

(c) **Hematological effects**

Phenols are reported to have cytotoxic effects on fish (Boge and Roche, 1996) and human (Bukowska and Kowalska, 2004) erythrocytes. Large uptake of phenol above lethal concentrations by fish erythrocytes (Swift, 2006) and severe changes in erythrocyte membrane properties in humans due to Chlorophenol exposure (Bukowska et al, 2009) have also been reported.

The blood samples collected at the aforementioned time points after short and long term exposures, were analyzed for various hematological parameters, such as, Total Erythrocyte count (TEC) and Total Leukocyte count (TLC) in Cells/ cu.mm., Hemoglobin (gm/dl) and Differential leucocyte count according to the method of Dacie & Lewis, 1984. The results obtained were statistically analyzed by ANOVA using MS Excel. The experiment was repeated consequently for two years, both in summer and winter and the results reproduced each time. Interpretation of results have been done on the basis of seasonal and overall effects of short and long term exposures of different concentrations of phenol and phenolic effluent on the hematology of *C. punctatus*.

In case of short term exposure to phenol and phenolic effluent a marked % elevation above normal up to 120 hours, followed by a decline thereafter in TEC, TLC and Gm% Hb was visible. Percent rise was found proportional to increasing concentration. The fishes also exhibited a tendency of resuming back augmented TEC levels as a consequence of adaptive response to low concentration of effluent. This property was however lost at higher concentrations. The time required for adaptive response increased with increasing
concentrations. Besides, an augmentation in TLC up to 120 hours followed by decline thereafter was also noticed. Percent rise was found to be proportional to increasing concentrations of Phenol. In case of effluent, on the contrary, TLC went on increasing gradually up to 168 hours in all concentrations. Similar disturbances were also seen in case of gm % hemoglobin. Short term exposure to low concentration of phenolic effluent possibly leads the fish to a stage of resistance when adaptation to stress has occurred while higher concentrations lead to a stage of exhaustion, when severity of stress leads to loss of adaptive response.

Long term exposure to phenol and effluent also showed a % hike in TEC,TLC and Hb% upto 3 weeks followed by a tendency to come down in the 4th week. Effect of season on the above mentioned parameters showed greater augmentation in hematological parameters during summer season. Morphological deformations and clumping in erythrocytes of effluent and phenol exposed fishes reflect membrane changes and are worth taking concern.

(d) Histopathological effects-

Health is a condition in which an organism is in complete accord with the environment and there is an exquisite coordination between the different body functions. Disease is the reaction of an individual to harmful irritation by an irritant and leads to disorders in the normal vital functions, a decrease in the adaptability and mobilization of the defense forces of the body of the organism. Histopathological alterations can also be used as indicators to understand the effect of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem. In the presence of predisposing factors, stress leads to onset of diseases. The disease process starts with biochemical changes and is followed by structural changes, such as degeneration and
necrosis. Histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular changes in the affected organism.

The gills are the most vulnerable site of the Teleost body and are the first organs to encounter a toxicant attack. Besides, gills the liver is the major organ of xenobiotic metabolism and liver lesions are often associated with aquatic pollution. Histopathological studies on fishes due to pollutants have been studied by several workers, namely (Haniffa and Sundaravadanam, 1984; Razani et al, 1986; Gupta and Dalela, 1987; Banerjee and Bhattacharya, 1994; Mercy et al, 1996; Singh and Mehrotra, 1999; Nanda, 2000; and Das and Mukherjee, 2000). The present study was aimed to investigate the histopathological changes in the Gills, Liver, Intestine and Kidney of *C. punctatus* in response to phenolic effluent exposure.

Two fishes each, from the experimental and control groups were sacrificed after short term exposure to effluent, by decapitation at 24 hours intervals for 168 hours and dissected open by mid-ventral incision. In case of long term exposure, collection of samples was made at weekly intervals for 4 weeks. Fresh samples of target organs, viz., liver, gills intestine and kidney were examined individually and washed in normal saline, fixed and processed for histopathological studies by Microtomy. Intestine of control fishes showed tightly packed circular muscle fibres, submucosal tissue and longitudinal muscle fibres. No changes were found to occur during short term exposure to low and high concentration of effluent. However, remarkable changes were visible in intestine of fishes exposed for long term to high concentration of effluent particularly after second week of exposure. The pathological findings in the intestine included- atrophy in the muscularis, severe degenerative and necrotic
changes in the intestinal mucosa and submucosa with necrotized cells aggregated in the intestinal lumen, haemorrhage in the submucosa and aggregations of inflammatory cells in the mucosa and submucosa with edema between them and atrophy in the submucosa. Dilation was observed in the blood vessels of serosa. Similar changes were seen in other organs also.

**CONCLUSIONS**

It is strongly opined that although fishes generally try to adapt to stressful conditions of a phenolic environment in short term exposures, long term exposures could be harmful to these aquatic beings even in very low concentrations. According to the National Recommended Water Quality Criteria phenol has been listed as a priority pollutant with an organoleptic effect criterion of 300µg/l (USEPA,2009). It is very toxic to fish and has a unique quality of tainting the taste of fish, if present in marine environments at 0.1-1.0 ppm (Kirk and Othmer,1982; Neff,2002). Hence, dumping of this phenolic wastewater of the Purena Channel into the river Kharooin may have deleterious consequences and must be taken care off.