DISCUSSION

Relatively little is known about the effects of low concentrations of phenolic compounds on the normal physiological functions of freshwater fishes. Phenolic compounds present in sub-lethal concentrations in water might enter into the blood stream of *Clarias batrachus* through the gills or the mucus epithelium of the mouth and finally be distributed in different organs of the body which in turn affects various metabolic pathways.

In the present study, decreased cortisol level was observed in both phenol and *m*-cresol treated fishes compared to control. There are several studies which provided evidence that the capacity to raise plasma cortisol is impaired in fish exposed to organic pollutants (*Aluru et al.*, 2004) and metals (*Brodeur et al.*, 1997; *Norris*, 2000; *L’évesque et al.*, 2002). As cortisol is involved in the regulation of physiological functions that helps the animal to cope with stress, inhibition of the pituitary-interrenal axis will impair the ability of the animal to cope with stressors (*Vijayan et al.*, 1997). *Hontela et al.* (1992) proposed that prolonged exposure to pollutants may lead to hyperactivity, and as a result in the exhaustion of the pituitary-internal axis. The lack of cortisol response suggests that, similar to other xenobiotics phenol and *m*-cresol can act as an endocrine disruptor and as such impair steroidogenesis. It is not known how phenolics can affect cortisol production. However there are some possible explanations. First, it might be possible that one of the primary steps in the steroid hormone synthesis pathway was compromised. Cholesterol is the substrate for steroid synthesis (*Mommsen et al.*, 1999), particularly the non-esterified cholesterol (*Mukherjee et al.*, 1991). It has been demonstrated in carp (*Cyprinus carpio*) that chronic exposure to water-borne phenol (8 mg l⁻¹) caused significant accumulation of non-esterified cholesterol in both tissues and serum by days 15 and 30 of exposure, respectively. This accumulation was due to the inability of the steroidogenic tissues to synthesize steroids. Second, it has also been shown that xenobiotics can inhibit the transport of cholesterol to the mitochondria (*Hontela, 1997; Walsh et al.*, 2000). Lastly, xenobiotics can affect the action of the adrenocorticotropic hormone (ACTH), which stimulates the synthesis of cortisol by the internal cells in fish (*Wendelaar-Bonga, 1997*). It might be possible that the concentration tested was sufficient to affect steroidogenesis and compromise the cortisol response.

Several studies have corroborated the impairment in the cortisol synthesis and secretion due the action of chemicals. *Gravel and Vijayan* (2006) studied the impacts of three pharmaceuticals
(acetaminophen, ibuprofen and salicylic acid) in rainbow trout and supported the hypothesis that these pharmaceuticals disrupt steroidogenesis in fish inter-renal tissue. These findings were also tested in vitro and observed that salicylic acid produced a depression of ACTH stimulation in cortisol secretion and a lower gene expression of steroidogenic acute regulatory (StAR) protein, which is involved in steroidogenesis of cortisol (Hontela, 2006); the same author also stated that StAR protein may be sensitive target of many environmental pollutants, ranging from pesticides to pharmaceuticals. Also, the expression of StAR and P450scc decreased in fish exposed to xenobiotics because they bind aryl hydrocarbon-receptor (AhR), a cytosolic induced transcription factor, with a consequent depression of steroidogenic enzyme activity and finally altering the cortisol production and secretion (Aluru et al., 2005). Therefore many pollutants halt cortisol secretion and even if the fish is under stress this will probably not be reflected in cortisol response. As cortisol is an important metabolic hormone in fish, any alteration in its dynamics associated with exposure to contaminants could have a significant impact on the ability of the animal to mount a physiological response, thereby attenuating the chances of coping with subsequent natural or anthropogenic stressors. Fish exhibiting an impaired cortisol stress response may be at a disadvantage in coping with environmental stressors. Total carbohydrate content was found to be decreased in liver and muscle of both the treated groups compared to control. Chemical stress causes rapid depletion of stored carbohydrates primarily in liver and other tissues (Jyothi et al., 2000). However, there exist in fish tissues detoxification mechanisms which convert the lipid-soluble compounds into water-soluble metabolites that can be readily excreted (Varanasi and Malins, 1977; Malins and Hodgins, 1981). Such systems are of special significance during continuous exposure, as in the present study, where the process of depuration by simple diffusion cannot be expected to be properly effective due to the constant presence of pollutants in the medium. Hence detoxification mechanisms become active and the hepatic synthesis of detoxifying enzymes requires high energy levels which might be derived from carbohydrate metabolism, for driving the various enzyme-mediated reactions. UDP-glucuronic acid is an important carbohydrate derivative. Phenolics are often excreted as glucuronyl derivatives by conjugating with UDP-glucuronic acid. UDP-glucuronyltransferases (UGTs) are one of the phase II enzymes that catalyse this conjugation. UGTs are induced by a variety of natural and synthetic compounds and play a key role in catalyzing the conjugation and potential excretion of different xenobiotics in fish (Clarke et al., 1992a). On exposure to both the phenolic compounds fishes showed behavioral
changes such as intense and frequent avoidance reactions, consisting chiefly of agitated, erratic and violent swimming bouts. Thus the more extensive breakdown of stored carbohydrates in the muscle may be due to the greater physical activity of the organism.

Inhibition of glucose-6-phosphatase activity was found in the liver of both the treated groups compared to control. Inhibition of glucose-6-phosphatase activity may be a reflection of damage to the microsomal membrane as the enzyme is localized exclusively in the membranes of the endoplasmic reticulum. The blood glucose levels have been used as indicators of stress in fish. In the present investigation a significantly decreased blood glucose level was observed in both the treated groups compared to control. This shows that blood glucose homeostasis was not maintained on exposure to phenolics. This may be due to the lack of cortisol response and decreased glucose-6-phosphatase activity. On exposure to phenolic compounds gills, liver and kidney showed an elevated pyruvate level compared to control. This might be due to the higher glycolysis rate, which is the only energy-producing pathway for the animal when it is under stress conditions. The end product of the glycolytic pathway is pyruvate. Pyruvate occupies an important junction between various metabolic pathways. It may be decarboxylated to acetyl CoA which can enter the TCA cycle or it may be utilized for fatty acid synthesis. Pyruvate may be carboxylated to oxaloacetate which can be used for gluconeogenesis. Muscle of both the treated groups showed a decreased pyruvate level compared to control.

Lactate dehydrogenase is an enzyme recognized as a potential marker for assessing the toxicity of a chemical. LDH is an anaerobic enzyme involved in the conversion of pyruvate to lactate in glycolysis. The LDH in the liver and kidney of fishes treated with phenol showed an elevated activity compared to control. Cohen et al. (2001) have reported a similar increase in LDH activity in juvenile Australian Bass and Macquaria novemaculeata in response to two different crude oil spills. The increase in LDH activity also suggests a significant increase in the conversion of pyruvate to lactic acid, thereby leading to the accumulation of lactic acid. Compared to control a significant decrease in LDH activity in liver and kidney of m-cresol treated fishes and in gills of fishes treated with phenol was observed. This may be due to increased tissue damage. Similar results were obtained when Clarias batrachus were exposed to sub-lethal concentrations of organophosphorus insecticide (Rao, 2006). Stimulation of LDH in muscle of C. batrachus on exposure to phenolic compounds suggests that the final product of glycolysis - pyruvate was preferentially used to produce lactate. Lactate formed is an important gluconeogenic substrate which can be used to cope with the high and rapid demand of energy due
to stress.

Several reports revealed decreased LDH activity in tissues under various toxic conditions (Tripathi et al., 1990; Mishra and Shukla, 2003). LDH is an important glycolytic enzyme in biological systems and is inducible by oxygen stress. Therefore, the activity of several regulatory enzymes may be altered in order to meet the required energy demands under toxic stress (Mayer et al., 1989), including the activity of lactate dehydrogenase (LDH), which sustains the continued process of glycolysis under anaerobic conditions (Diamantino et al., 2001). Several reports revealed decreased LDH activity in tissues under various toxic conditions (Tripathi and Shukla, 1990; Mishra and Shukla, 2003). The level of LDH was found to be increased in the gills and decreased in the liver, kidney and muscles in the monocrotophos exposed fish (Agrahari and Gopal, 2009). Compared to control, ALT and AST activities were found to be highly elevated in all the tissues of fishes treated with phenol and m-cresol compared to control. The highest activity was observed in liver followed by kidney and muscle. In fish, one of the primary energy currencies is amino acids. Transaminases like alanine aminotransferase and aspartate aminotransferase play an important role in the conversion of amino acids to keto acids like pyruvate and oxaloacetate, which could be used as intermediates in Kreb's cycle or directed into the gluconeogenic pathway. ALT is cytosolic whereas AST has both cytosolic and mitochondrial forms. Under normal conditions there is a baseline activity of these enzymes. But when the organism is subjected to stress, the levels of these enzymes are significantly increased in order to meet the increase in ATP demands.

ALT is an enzyme frequently used in the diagnosis of damage caused by pollutants in various tissues such as liver, muscle, and gills (de La Torre et al., 1999, 2000). This enzyme is known to play a key role in mobilizing L-amino acids for gluconeogenesis and function as links between carbohydrate and protein metabolism under altered physiological, pathological and induced environmental conditions (Nichol and Rosen, 1963; Knox and Greengard, 1965; Victor, 1985). Elevation in the levels of AST and ALT in different tissues of C. batrachus can be considered as a response to the stress induced by phenolic compounds to generate keto acids like α-ketoglutarate and oxaloacetate for contributing to gluconeogenesis and/or energy production necessary to meet the excess energy demand. Significant elevations in AST activity was recorded in Cyprinus carpio exposed to copper sulphate (Karan et al., 1998). Elevations in ALT activity were noticed in C. carpio and Oreochromis niloticus exposed to cadmium (de La Torre et al., 2000; De Smet and Blust, 2001; Almeida et al., 2002). ALT activity of Carassius
auratus liver was stimulated by low concentrations of ytterbium (Guo et al., 2002). Similar type of observation was also observed by (Janice et al., 1979), when American oysters and brown shrimps were exposed for a chronic period to crude oil. The exposure to phenol caused an increase of both ALT and AST activities in Notopterus notopterus (Gupta et al., 1983). The amino acids through transamination and deamination reactions might have supplied necessary keto acids to act as precursors for the maintenance of carbohydrate metabolism to meet the energy requirements during pollutant stress.

Alkaline phosphatase and acid phosphatase catalyses the hydrolysis of monophosphate esters and has a wide substrate specificity. The activity of ALP has been significantly elevated in all the tissues (gills, liver, kidney and muscle) treated with phenol and m-cresol compared to control. Increased ALP activity may be due to pathological processes such as liver impairment, kidney dysfunction and bone disease (Barse et al., 2006). Phosphatases play major roles in the moulting physiology of many fishes (Ezhilarasi, 1982). Serum acid phosphatase showed an elevated activity compared to control. An elevation in ACP activity suggests an increase in lysosomal mobilization and cell necrosis due to the toxicity of phenolics. This increase also suggests the supply of phosphate group for energy metabolism. This shows an adverse impact on metabolism, which may lead to negative impact on growth, health and reproduction. Degeneration and necrosis induced in hepatic parenchymatous cells by these toxicants may cause release of acid phosphatase in the serum. Alterations in ALP and ACP activities in tissues and serum have been reported in pesticide treated fish (Palanivelu et al., 2005). Increase in the levels of ALP and AST has been shown to reflect liver damage, whereas an elevation in the ALP activity may be indicative of renal and liver damage (Gill et al., 1990; Bhattacharya et al., 2005).

GDH activity was found to be elevated in almost all tissues treated with phenol compared to control. This increased activity may have helped in funneling more α-ketoglutarate into TCA cycle for more energy generation. Whereas in fishes treated with m-cresol tissues such as liver and gills showed almost constant activity but kidney and muscle showed a decreased activity compared to control. An inhibition of GDH activity in gills, brain, kidney and liver of fishes exposed to toxicants was observed by (Ghosh, 1985).

Fishes exposed to sub-lethal concentrations of different phenolic compounds showed alterations in protein content in different tissues compared to control. Gills and kidneys of both the treated group showed increased protein content compared to control. Liver and muscle of
both the treated groups showed decreased protein content compared to control. The reduction in protein content indicates that under stress conditions the tissue protein may undergo proteolysis, which may have resulted in the production of free amino acids which can be used in the tricarboxylic acid cycle for energy production. This would lead to an increased free amino acid pool (Bayne et al., 1981) which can be used for ATP production by transamination reactions or by gluconeogenic pathway. The tissue protein is metabolised to produce glucose by the process of gluconeogenesis and it is utilized for energy production under stress conditions (Elumalai and Balasubramanian, 1999). The decrease in protein content under stress induced by phenolic compounds may be attributed to the utilization of amino acids in various catabolic reactions. The depletion of protein content may also be due to the rapid utilization of tissue protein as the food utilization decreases when the animals are under stress conditions. Yadav et al. (2007) has reported that the animals exposed to chemicals obtain extra energy requirement from the tissue protein. The depletion of cellular proteins might be caused by one or more of the following factors: inhibition of amino acid incorporation, breakdown of proteins into amino acids and diffusion out of the cells. Badawy et al. (1969) established that the inhibition of RNA synthesis precedes inhibition of protein synthesis and that necrosis occurs later than these two events. The decline in protein content may be related to impaired food intake, the increased energy cost of homeostasis, tissue repair and the detoxification mechanism during stress (Neff, 1985). Another reason that can be attributed for the decrease in proteins under toxic stress may be due to formation of lipoproteins, which are utilized for repair of damaged cell and tissue organelles. Therefore, the sum of these alterations can have a significant effect on energy metabolism. In conclusion, the present work indicates that phenolic compounds causes considerable changes in intermediary metabolism and is likely to induce tissue damage in C. batrachus. The causes for these alterations appear to be the result of high energy demands.

The antioxidant defense mechanism of C. batrachus was responsive to the exposure of different phenolics. Xenobiotics such as phenol are metabolized by the multienzymatic system cytochrome P450 (CYP) (Andersson and Förlin, 1992). Sometimes biotransformation processes lead to increase of toxicity of individual compounds by the formation of electrophilic metabolites that may bind and damage DNA or enzymes. The enzymatic bioactivation of phenolics catalyzed by cytochrome P450 leads to the formation of products such as hydroquinones, catechols and benzoquinones. The metabolites formed can cause increased generation of reactive oxygen species (ROS) or oxidative stress. Aerobic organisms have
developed through evolutionary processes antioxidant defense mechanisms designed to prevent cellular damage from ROS.

In the present study, almost all the tissues treated with phenol and \textit{m}-cresol for 21 days in \textit{C. batrachus} showed significantly elevated SOD and CAT activity compared to control. SOD is the first enzyme to respond against oxygen radicals \textbf{McCord and Fridovich (1969)} and is the one that offers the greatest response to oxidative stress \textbf{(Winston and Di Giulio, 1991)}. The tissue specific increase in SOD activity showed the following trend for fishes treated with phenol: kidney > gills > liver whereas the muscle showed a significantly decreased SOD activity compared to control. On treatment with \textit{m}-cresol, tissues such as liver, kidney and muscle showed a significantly elevated activity whereas gills showed a significantly decreased activity compared to control. Changes in the levels of superoxide dismutase have been detected in fishes exposed to various degrees of oxygen tension \textbf{(Lushchak \textit{et al.}, 2001)} and environmental perturbations \textbf{(Achuba, 2002)}. Superoxide dismutase is inducible in mammals and microorganisms and the level of the enzyme increases with an increased need of protection against toxic oxygen radicals \textbf{(Fridovich 1974; Trostler \textit{et al.}, 1979)}. Mn-containing superoxide dismutase and Cu/Zn dependent superoxide dismutase are involved in the general defense system against natural or chemically induced production of reactive oxygen species \textbf{(Fridovich, 1986)}. Catechol increases the reduction of O$_2$ and this may have resulted in an increased SOD activity. Also catechol reduces the dismutation of O$_2$, and thus leads to the production of larger amounts of H$_2$O$_2$. Thus for the detoxification of increased H$_2$O$_2$ generated a significantly elevated CAT activity was observed in gills, liver and kidney of fishes treated with both the phenolics whereas muscle showed a significantly decreased CAT activity compared to control in both phenol and \textit{m}-cresol treated groups. An increased generation of H$_2$O$_2$ may have occurred due to several reasons such as oxygen depletion \textbf{(Penning \textit{et al.}, 1996)}, dismutation reaction of O$_2$- catalyzed by increased SOD activity.

The elevated CAT activity observed may be for the detoxification of increased H$_2$O$_2$ formed from different reactions. Therefore, the SOD-CAT system provides the first defense against oxygen toxicity. Perhaps a paroxysmal proliferation may have also occurred as they are cell organelles that play key roles in multiple cell functions \textbf{(Mannaerts and Van Veldhoven, 1993)} especially in the metabolism of ROS \textbf{(Singh, 1996)}. The most abundant peroxisomal enzyme is CAT and the proliferation may have resulted in elevated CAT activity. Increase of SOD and CAT in liver is reported in some fish species under oxidative stress \textbf{(Bainy \textit{et al.}, 1996; Sayeed \textit{et al.}, 1996; Bainy \textit{et al.}, 1996; Bainy \textit{et al.}, 1996; Sayeed \textit{et al.}, 1996).
Considering the results for each tissue in both treated groups, it was found that liver showed the highest SOD and CAT antioxidant activity, both enzymes appearing to have an important role in to be highly elevated in liver on exposure to phenolics, since liver plays an important role in the detoxification of xenobiotics and in elimination by conjugating them with glutathione. GST-mediated conjugation may be an important mechanism for detoxifying peroxidised lipid breakdown products, which have a number of adverse biological effects when present in high amounts. Induced GST activity indicates the role of this enzyme in protection against the toxicity of xenobiotic-induced lipid peroxidation (Leaver and George, 1998). Many studies analyzing GST in liver of fish exposed to different insecticides showed an enzymatic induction (Andersson et al., 1985; Rodriguez et al., 1991; Leaver et al., 1992; Scott et al., 1992). However, inhibition of GST activity has also been reported in gills of mosquito fish exposed to carbofuran (Rondon et al., 2005). Thus, it is possible that the enzyme is regulated in vivo by, for instance, thiol-disulphide interchange and proteolysis or by some other mechanism. Since reactive metabolites of foreign compounds are substrates for glutathione transferase, an attractive idea would be that these metabolites modify the microsomal glutathione transferase covalently, thereby increasing the enzyme activity by which these reactive metabolites are eliminated through conjugation. This would allow the cell to adjust rapidly to exposure to reactive compounds. The microsomal metabolism of phenol to species which will bind to proteins is most likely catalyzed by P450 monooxygenases (Sawahata et al., 1983; Wallin et al., 1985). These enzymes are probably the major targets for the covalent binding of phenol. It is likely that the electrophilic metabolites benzoquinone and 2-hydroxybenzoquinone conjugate with the sulphydryl group of the enzyme, thereby activating the enzyme (Irons, 1981). In summary, microsomal glutathione transferase can be activated by reactive metabolites of phenol and m-cresol, and is caused by covalent binding of the metabolites to the enzyme.

GSH is the major cytosolic low molecular weight sulphhydril compound that acts as a cellular reducing and a protective reagent against numerous toxic substances including most inorganic pollutants, through the -SH group (Stryer, 1988). Gills, combating the sequential generation of superoxide radical (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) from the intense metabolic activity characteristic of this tissue. The significant increase in catalase and superoxide dismutase activities in gills, liver and kidney examined may represent an adaptive response to protect the fish from free radical toxicity induced by phenolic compounds.
GPx glutathione peroxidase activity, a seleno-enzyme that neutralizes ROS such as organic and hydrogen peroxides (Matés, 2000) activity in gills, liver and kidney of fishes treated with phenol and m-cresol showed a significantly decreased activity compared to control. Whereas muscle in both treated groups showed a significantly enhanced activity compared to control. CAT and GPx activities are fundamental to remove hydrogen peroxide from cytoplasm, however, only the GPx activity was decreased in C. batrachus exposed to both the phenolics. In theory, reduced enzymatic activity implies that some ROS are not being quenched, thus predisposing cells to oxidative stress. The low GPx activity might be due to a direct phenol inhibition of enzyme synthesis or due to increased generation of hydroperoxide which may have inhibited the enzyme activity. Also catechol toxicity is mainly associated with damage to the protein and generation of hydrogen peroxide, which is capable of causing further damage (Barreto et al., 2009). Significantly elevated GPx activity in muscle shows that an induction in glutathione peroxidase activity has occurred in this tissue.

GST is a multicomponent enzyme involved in the detoxification of many xenobiotics, which plays an important role in protecting tissues from oxidative stress (Fournier et al., 1992). GST was found to be strongly inhibited in kidney and muscle on exposure to different phenolic compounds. GST activity was found liver and muscle showed elevated GSH level when treated with phenolics. Among the tissues, GSH level was found to be highest in liver compared to other tissues which may be due to an adaptive mechanism to slight oxidative stress through an increase in its synthesis which can be provided for the increased GST activity. However, a depletion of GSH was observed in kidney which shows that severe oxidative stress may suppress GSH levels due to loss of adaptive mechanisms and the oxidation of GSH to GSSG. During scavenging the ROS, GSH is oxidized and forms glutathione-protein mixed disulphides; hence, the cell's ability to reduce or synthesize GSH is the key to how effectively the cell can manage the oxidative stress. Total glutathione will be a prospective biological index to indicate exposure to contaminants (Stein et al., 1992). Due to its function in resisting the reactive oxygen toxicity, the changing degree for total glutathione can serve as markers of exposure to pollutants which disturb the piscine oxyradicals.

The conjugated diene level was found to be elevated in liver, kidney and muscle of both the treated groups and also in gills treated with phenol. CD is the initial peroxidative product and is an accurate indicator of lipid peroxidation and its elevated level indicated that lipid peroxidation has been initiated. An increased hydroperoxide level was observed in liver, kidney
and muscle of both the treated groups which may be due to decreased GPx activity observed in these tissues. This maybe because GPx catalyzes the reduction of $H_2O_2$ derived from oxidative metabolism as well as peroxides from oxidation of lipids and is considered the most effective enzyme against lipid peroxidation (Winston and Di Giulio, 1991). Being more polar than parent lipids, hydroperoxides perturb membrane structure/function and can be deleterious to cells (Girotti, 1998). An increased MDA level was observed in both gills and liver on exposure to different phenolics indicating that elevated antioxidant enzyme activities were not efficient enough to prevent lipid peroxidation in these tissues. Significant oxidative damage and lipid peroxidation should theoretically occur if antioxidant defenses were overwhelmed by ROS production (Kappus, 1987; Halliwell and Gutteridge, 1989; Winston and Di Giulio, 1991).

In addition to changes in the antioxidant defense system, one of the hallmarks of oxidative stress is damage to biological macromolecules such as the phospholipids of cell membranes (Shi et al., 2004). MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation. Taken as a whole, our data seems to implicate phenolic compounds as a potent mediator of free radical generation in fish.

In the present study, on exposure to sub-lethal concentration of both phenol and $m$-cresol, branchial ATPases which are important membrane bound proteins showed decreased activity compared to control. Also the serum ion levels showed significant variations compared to control thus affecting the ionic homeostasis. Branchial Na$^+$K$^+$-ATPase activity in both the phenolic compound dosed groups showed a decreased activity compared to control. Phenol treated fishes showed decreased activity compared to $m$-cresol treated group. There are several findings which show that Na$^+$K$^+$-ATPase plays a central role in the whole body ion regulation. Thus, any toxicant that interferes with ionic homeostasis may be reflected as altered Na$^+$K$^+$-ATPase activity which was found to be decreased in the present investigation. Xenobiotics can also alter Na$^+$K$^+$-ATPase activity by disrupting energy-producing metabolic pathways or interacting directly with the enzyme (Alam et al., 2006).

As the primary link between environmental change and physiological response, the neuroendocrine system is a critical part of osmoregulatory adaptations (McCormick, 2001). Corticosteroids are believed to regulate Na$^+$K$^+$-ATPase in the teleost gill (Butler and Carmichael 1972, Forrest et al., 1973, Madsen 1990) both through increase in the numbers of chloride cells and the level of enzyme in each cell. Also glucocorticoids generally enhance the expression of enzymes such as...
ATPases, ion pumps and chloride cells (Thomas et al., 1999). Cortisol can increase the cellular differentiation of chloride cells and stimulate branchial Na⁺K⁺-ATPase activity (McCormick, 1995). But in the present study, a decreased cortisol level was observed when treated with phenolics which may have resulted in decreased ATPase activities. Glucocorticoid receptor gene expression in chum salmon (Oncorhynchus keta) chloride cells (Uchida et al., 1998), support a direct effect of cortisol on chloride cell function in fish.

Decreased Ca²⁺-ATPase and Mg²⁺-ATPase activities were observed in both the treated groups compared to control. Inhibition of Mg²⁺-ATPase activity by phenolic compounds may reduce ATP production as this enzyme has been reported to be involved in oxidative phosphorylation (Racker et al., 1975). A reduction in the activity of Ca²⁺-ATPases indicated the interaction of phenolic compounds with the microsomal and basolateral Ca²⁺ transporting ATPases (Chris and Wong, 2000). The decreased Ca²⁺-ATPase activity may have affected the ability to maintain the calcium homeostasis (Milhaud et al., 1977). Lipid peroxidation which is indicated by high malondialdehyde level in gills of both the treated groups was also found to be higher compared to control. As both these ATPases play an important role in integrity of cellular membrane and stabilisation of branchial permeability, lipid peroxidation maybe one of the reasons for decreased ATPase activity observed. A change in the permeability characteristics of gills may also have resulted in the decreased activity of ATPases. The inhibition of Na⁺ K⁺-ATPase in gills probably disturbs Na⁺, K⁺ pump, resulting in an erratic entry of Na⁺ into the cell along the concentration gradient and the water molecule follows along the osmotic gradient. This process may cause swelling of the cell and finally membrane ruptures (Ozcan et al., 2002). Also in the present investigation, histopathological studies showed pathological abnormalities in gills such as disruption of the secondary lamellae, epithelial desquamation and necrosis and fusion of secondary lamellae in exposed animals on exposure to both the phenolics which may also have affected the branchial ATPase activity.

The freshwater animals compensate their renal and surface loss of ions, mainly sodium and chloride, by absorbing these ions from the external medium through specialized surface structures. Gills of freshwater fish contain the machinery for the active transport of the electrolytes. Toxic substances may cause damage to gill tissues, thereby reducing the oxygen consumption and disturbing the osmoregulatory function of aquatic organisms. Potassium is the main cation of the intracellular fluid and it is also an important constituent of the extracellular
fluid. Ion uptake from water is required to maintain internal acid-base balance and ionic equilibrium between blood and tissues for those ions that are continuously lost by diffusion across permeable parts of the external body surface.

In the present study, a decrease in serum sodium ion levels and an increase in potassium ion levels in the groups exposed to low dose suggest that phenolic compounds can affect osmoregulation. A reduction in the major electrolyte sodium may be due to histological alterations of gills or disturbances in the membrane permeability due to toxicity of phenolics. On exposure to phenolics, altered gill permeability was observed which could have impaired the flux of ions. Freshwater fish tends to have a passive efflux of ions (loss) and a passive influx of water through the gill epithelium (McDonald and Milligan, 1997). To cope with the change in blood osmolarity, they have two main strategies: active uptake of ions through the gill using the Na+K+-ATPase and production of large volumes of diluted urine in the kidney, which can also actively uptake ions (Eddy, 1981; Marshall and Grosell, 2005; Iwama et al., 2005). But in the present investigation branchial Na+K+-ATPase activity was impaired which may have affected the ionic homeostasis. de la Torre et al. (1999) have shown that the inhibition of this enzyme by monocrotophos prevents the buildup of high ion concentrations in the extracellular spaces resulting in a blockage of the movement of internal harmful extra ions towards the external medium via the leakage junctions.

Shifts in the hydromineral balance may be a consequence of the action of pollutants on organs involved in osmoregulation, on the endocrine system, on metabolism or on active transport processes. Usually, after exposure to a single stressor, freshwater fish respond by increasing the efflux of ions through the gills (McDonald and Milligan, 1997). Since freshwater fish take up most of the ions necessary for homeostasis from the water via their gills, the drop of plasma electrolytes is apparently caused by an increased efflux of ions across these organs and an impairment of active ion uptake by the chloride cells of the gill (Wendelaar- Bonga and Lock, 1992). A reduction in the plasma electrolyte level has two important causes. First, there is an elevated passive efflux of ions across the gills due to more or less non-selective branchial permeability to water and ions. This may lead to haemodilution by enhanced osmotic uptake of water across the gills and to passive diffusional ion loss. Second, the inhibition of active ion uptake by the chloride cells of the gills which may further contribute to the negative ion balance of the blood. Many toxic substances including petroleum hydrocarbons cause osmoregulatory disturbances in teleosts (Englehardt et al., 1981). Elevated plasma cortisol may be necessary
to compensate for the hydromineral imbalance caused by phenolic compounds which was also found to be decreased in the present investigation on exposure to phenolics. Thus, ATPases are very sensitive to chemical interaction and can be used as reliable biomarker for the toxicity studies of phenolic compounds. In the present study, it has been found that exposure to phenolic compounds caused decreased activity of Na\(^+\) K\(^+\)-ATPase in gills. This could be due to the effect of phenolic compounds on cell membrane because of their strong affinity for interaction with membrane lipids causing inhibition of membrane-bound ATPases activity by affecting enzyme complex (Mishra et al., 1998). For maintenance of water and ion homeostasis, a strict control of membrane permeability to water and ions, the maintenance of appropriate transepithelial electrical potentials, and the presence of efficient ion-transport mechanisms are essential. Decreased gill ATPase activities in the freshwater fish *Channa punctata* (Bloch) exposed to a diluted paper mill effluent. Chromium compounds were reported to inhibit ATPases, bringing about a failure of osmoregulatory mechanisms (Thaker et al., 1996). In addition to osmoregulatory mechanisms, active transport mechanisms for the absorption of nutrients and essential ions may also have been affected by the inhibition of ATPases. However, it is not clear how the gill ATPases compensate for ionic regulation in the face of exposures to environmental pollutants. The foregoing results showed that the branchial functioning was impaired and hence the ionic homeostasis in *C. batrachus* was affected on exposure to sub-lethal concentrations of both phenol and *m*-cresol.

In the present study, exposure of *C. batrachus* to sub-lethal concentrations of phenol and *m*-cresol resulted in significant haematological alterations. Blood is a pathophysiological reflector of the whole body and therefore, blood parameters are important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari et al., 2004). On exposure to both the phenolic compounds, an increased red blood cell count and haematocrit was observed. The increased erythrocyte number and haematocrit value have been shown in many species exposed to chronic hypoxia. In these conditions, it appears that the spleen and maybe the liver may reactivate the erythropoiesis to compensate the demand due to the increased oxygen transport to peripheral tissues (Rifkind, 1980). Erythropoiesis, whereby the number of red blood cells in the circulation is increased is in fact a mechanism through which fish might compensate for poor oxygen uptake in prevailing hypoxic conditions (Wepener et al., 1992). Another mechanism by which fish might compensate for poor oxygen uptake during hypoxic conditions is via the release of a large number of mature red blood cells in the general circulation. This is
thought to be stimulated by β-adrenergic action on the haemopoietic tissues, which contract and release stored mature red cells. This mechanism might, however, compensate for short-term variations in oxygen concentration in blood or water (Nespolo and Rosenmann, 2002). Similar results were found in reports of acute intoxication by dichlorvos on Clarias batrachus (Benarji, 1990), by trichlorfon on Piaractus mesopotamus (Tavares, 2004) and by quinalphos on O. mossambicus (Sampath, 1993). It has been shown that the erythrocyte number and haemoglobin levels may vary with oxygen requirement (Hubrec et al., 2000; Tavares et al., 2004). Therefore, the increase of packed cell volume in C. batrachus is likely to be due to either increased metabolic demand or gill damages resulting in impairment of oxygen transport, or both. In fact, we also have observed drastic changes of the gill ultra-structure in the present investigation on exposure to phenolics. An increased level of haemoglobin was observed in both the groups exposed to phenolic compounds when compared to control. Two reasons can be attributed to the increased level of haemoglobin. One is, the presence of phenolic compounds in water creates a high oxygen demand and the compensation by the organism for low dissolved oxygen content is by synthesizing more haemoglobin for binding more oxygen. Another reason that can be attributed is; phenolic compounds or their toxic metabolites are oxidized to free radicals within erythrocytes and induce haemolysis of the erythrocyte membrane. As a consequence, haemoglobin is released which was shown as increased haemoglobin level. The released haemoglobin induces a multitude of toxic effects, summarized by Everse and Hsia (1997). The effects include nephrotoxicity through the formation of Hb dimers, formation of cross-linked haemoglobin, which induce haem oxygenase and liberation of the haem moiety. Other effects such as, cell damage, peroxidation, induction of phagocytosis and liberation of iron may also occur. The iron liberated itself can serve as a prooxidant and increases the risk of bacterial infections. Eyer et al. (1975); Riley (1984) reported that xenobiotics such as phenolic compounds are able to oxidize Oxy-Hb to Met-Hb in a so-called co-oxidation reaction in which the haem oxygen serves as the active oxidant that oxidizes both the ferrous haem centre of haemoglobin and the reducing xenobiotic (R-H):

\[ \text{Hb}^{2+} + \text{O}_2 + \text{R-H} \rightarrow [\text{Met-Hb}^{3+} + \text{O}_2^-] + \text{H}^+ + \text{R}^* \]

The highly reactive free radical intermediates were found to attack -SH groups on the haemoglobin molecule, at position β-93 (Maples et al., 1990) or on the constituents of the erythrocyte membrane (Feix and Butterfield, 1980; Wyse et al., 1989a, 1989b; Hensley et al., 1993; Butterfield et al., 1994). Apart from the decrease in oxygen binding capacity, the Met-Hb
generators irreversibly destroy the haem proteins with which they interact, thereby releasing metabolites that may affect thiol-dependent bioactivities and functional membrane processes. The blood responses seemingly indicate adaptation to hypoxic conditions arising from prooxidants, gill degradation and perhaps oxygen-level fluctuations. The results also show that homeostatic mechanisms were in motion to favour adaptation. Iron liberation from oxidatively modified haemoglobin or myoglobin when drastic oxidizing conditions such as hydrogen peroxide or lipid hydroperoxides were applied was reported by Gutteridge (1986); Harel et al. (1988) Rice-Evans et al. (1993). In the present investigation also an increased level of lipid hydroperoxides was observed on exposure to phenolic compounds. Since iron ions play an important role as redox catalysts (Fenton reaction, Haber-Weiss), iron liberation from erythrocytes will increase the total prooxidant effect of the xenobiotic. The physiological function of haemoglobin is to transport oxygen to the tissues; this process depends on the ability of the ferrous form (Hb$^{2+}$) to reversibly bind molecular oxygen. However in the presence of xenobiotics, oxyhaemoglobin (Oxy-Hb) is able to turn to methaemoglobin (Met-Hb) (the Hb$^{3+}$ form), which is unable to transport oxygen. This conversion is associated with superoxide anion production (Mishra and Fridovich, 1972) and products such as hydrogen peroxide or hydroxyl radicals, which may be derived from superoxide anion itself. The red blood cell membrane is the most popular model membrane system due to both its availability and the large amount of information available. In vitro studies carried out in the present investigation showed a direct effect of the phenolic compounds on the erythrocyte membrane which has resulted in strong, membrane-destabilising effect. The compact structure of biological membrane derives from weak non-covalent bonds between lipids, proteins and water, determined by hydrophobic, electrostatic and Vander Waals interactions and by hydrogen bonding. Any chemical compound which interferes with these interactions can alter the membrane structure and, consequently, slow down or completely inhibit the membrane processes. Thus the haemolytic effect has been attributed to different phenomena. These include: blockage of sulphhydryl groups present on the outside of the membrane while those inside the cells are relatively unaffected (Jacolyn, 1971; MacGregor and Clarkson, 1974); or disruption of the linkage between band 3 and bands 2.1 and 4.2 in the membrane proteins (Ralston and Crisp, 1981). The activity of a chemical compound to an organism depends on several physical, chemical and biological factors, among which interactions are possible (Bradbury, 1994). McFarland (1970) expressed chemical toxicity as the result of two preceding events. The first event is the penetration of a chemical compound from the
environment to the site of action in the organism. The second event is the interaction between the chemical compound and the site of action. Phenols are of interest to environmental toxicologists, which has led to the development of quantitative structure-activity relationships (QSAR) models. Several molecular descriptors are widely used in toxicology and among these the important ones are: the negative logarithm of the acid dissociation constant (pKa) and the logarithm of the octanol/water partition coefficient (log Kow). The increase of hydrophobicity and the value of log Kow, and the decrease of pKa value result in more effective membrane penetration by xenobiotics and, thus, enhance their toxicity (Dani et al., 2004). Some phenolic compounds have high pKa (where Ka is the first dissociation constant) values and others have relatively small pKa values. Schultz et al. (1998) suggested that phenolic compounds can be categorized either as polar narcotics (which have high pKa values) or uncoupling agents by their pKa values. Phenolic compounds taken for the present investigation were phenol, m-cresol and 4- nonylphenol which have pKa values 9.89, 10.99 and 10.28 respectively. Phenols with pKa values > 8.0 exhibit polar narcosis, whereas compounds with pKa values < 6.5 are uncoupling agents. Narcosis can be defined as the reversible state of arrested activity of protoplasmic structures resulting from exposure to the xenobiotic (Schultz, 1989) and narcotic compounds are deemed electrophilically unreactive. The mode of action of polar narcosis is not well characterized, but it is assumed to be a non-specific disruption of the functions of the biological membranes causing progressive lethargy, unconsciousness and death (Veith and Broderius, 1987; Oberg, 2004). Compounds with a narcosis mechanism exhibit baseline toxicity or toxicity associated with hydrophobicity, and compounds with other mechanisms have toxicity higher than the baseline toxicity (Verhaar et al., 1992).

In fact, log Kow (octanol-water partition coefficient) is found to be a significant descriptor of toxicity for the whole group of phenols (Ren and Schultz, 2002). In the present work, the hydrophobicity (log Kow=5.44) of the 4- nonylphenol showed a positive and significant relationship with loss of membrane integrity. Steric effects of the alkylated ring structure have been proposed to affect the in vitro cytotoxicity of mono- and di-alkylated phenols (Selassie et al., 2002). Alkylphenols and other chemicals with acidic hydrogen donating functional groups such as anilines and halogenated phenols have previously been reported to cause higher toxicity than that could be predicted by non-polar narcosis in fish and consequently being grouped as polar narcosis (Schultz et al., 1986; Veith and Broderius, 1990).

The findings in the present study are consistent with the assumption that alkylphenols cause...
toxicity through a polar narcosis mode of action. Reactive oxygen species formation in erythrocytes on exposure to xenobiotics initiates oxidative processes. The consequence of oxidative stress is enhanced lipid peroxidation of cell membrane, aggregation of membrane proteins, an increase of its permeability and outflow of potassium from cell and enhanced binding of their own immunoglobulin G (Bartosz, 2003). These changes cause accelerated removal of erythrocytes from blood and thus reduction of their life, which leads to anaemia (Bradshaw et al., 1995; Giardina et al., 1995; Bartosz, 2000). Lipid peroxidation, resulting from the binding of phenolic compounds to polyunsaturated fatty acids in the erythrocyte membrane may have resulted in haemolysis. In carp, lipid peroxidation resultant from sub-lethal effects of phenol is also found in phospholipid composition of erythrocyte membranes with subsequent alterations in membrane fluidity and permeability (Kotkat et al., 1999). A direct action of a phenolic compound butylated hydroxyanisole (BHA) on the integrity of the erythrocyte membrane was observed by (Nohl and Stolze, 1998) leading to haemolysis independent of the formation of prooxidant species. The effects of the investigated xenobiotics on the erythrocyte membrane can be summarized as a clear effect of all xenobiotics on the lipid phase of the erythrocyte membrane affecting the membrane fluidity that may have resulted in strong membrane-destabilising effect, eventually leading to haemolysis.

Lysosomal perturbations observed during this study reflected a clear gradient from the control. The results obtained in the present investigation showed that on exposure to different phenolic compounds leakage of lysosomal marker enzyme acid phosphatase occurred. Injury of the lysosomal membrane by the phenolic compounds may have led to leakage of the hydrolytic lysosomal enzymes into the cytoplasm leading to disturbance of cell functions and resulting in degeneration and possibly in neoplasia. All these changes reflect overloading or damage of the lysosomal digestive and detoxifying system. Lysosomal response indicated as the injury of this central cell compartment, resulted in severe liver lesions. Lysosomal enzymes released into the cytosol presumably cause changes in the membrane fluidity resulting in increased fusion rates. Lowe et al. (1981) found that an increase in lysosomal volume accompanied by the formation of pathologically enlarged lysosomes was directly associated with membrane destabilisation in the digestive gland of mussels exposed to oil-derived contaminants. The significant negative correlation between the lysosomal stability and the extension of liver lesion indicates that the lysosomal stability test clearly reflects the overcharge and breakdown of the detoxifying capacity of liver (Kohler, 1989b). The assessment of lysosomal membrane stability in the
digestive gland of marine mussels and snails proved to be a highly sensitive measure for the functional state of the cell (Moore, 1985). As in mammals, the fish liver is the central organ for the accumulation and detoxification of organic and inorganic contaminants. Earlier ultra structural studies in flounder liver evidenced severe alterations of the lysosomal system in relation to the contaminant burden (Kohler et al., 1986; Kohler, 1989a, 1990). In vitro studies were carried out by taking labilisation measurements at intervals from 0, 15, and 30 up to 45 minutes. With increase in time and concentration of phenolic compounds, the rate of release of the lysosomal marker enzyme acid phosphatase increased, this showed a decrease in membrane stability. The lysosomal membrane stability has been proved to be a useful index of cellular conditions and correlates significantly with physiological conditions of organisms. Lysosomal damage is well established as a biomarker of stress in a wide range of vertebrates (Tabata et al., 1990) and many agents such as various disease conditions, stress, hormones and drugs can induce destabilising alterations in lysosomes. The depletion of dissolved oxygen concentration of waters due to the presence of phenolic compounds leads to formation of free radicals, especially superoxide (O$_2^-$), which acts by oxidizing various cellular substrates, especially unsaturated fatty acids in phospholipids of biological membranes, which are very susceptible to free radical damage. Malondialdehyde, the major oxidation product of peroxidised polyunsaturated fatty acids was found to be higher in liver in all phenolic compounds treated groups compared to control. Peroxidised membranes become rigid and lose permeability and integrity.

Cumulative effects of lipid peroxidation have been implicated as underlying mechanisms in numerous pathological conditions in humans (atherosclerosis, haemolytic anaemia, ischemia etc.) and other organisms (Steinberg, 1997). In general, the overall effects of lipid peroxidation are decrease in membrane fluidity and increase in the leakiness of the membrane. Also in the present investigation the histopathological examination showed changes in the liver on exposure to both phenol and m-cresol. The most evident change observed in the hepatocytes was necrosis. Cortisol, the principal glucocorticoid hormone which plays an important role in maintaining the stability of biological membranes was found to be decreased on treatment with different phenolic compounds. Thus it can be inferred that necrosis, lipid peroxidation and decreased cortisol response may have resulted in the labialization of the hepatic lysosomal membrane under in vivo conditions. However, it was also noted that the concentration of phenolic compounds tested evoked adverse effects on cellular functions resulting in metabolic alterations. This suggests that
effect on cellular metabolic functions was one of the causes of cytotoxicity of these chemicals and that the disruption of membrane integrity may be a secondary effect. The lysosomal tests clearly reflect the breakdown of the adaptive capacity of the fish liver to toxic injury. All the results obtained in both in vivo and in vitro studies showed that the tested compounds affected the lysosomal membrane stability and resulted in the disruption of cellular homeostasis to the point where membrane integrity was compromised. To conclude, lysosomal membrane stability is a predictive indicator for cell injury and pathology and supporting evidence indicates that this parameter is generic in animals.

The results from the present study suggest that the histopathological lesions observed in the organism are due to exposure to phenolic compounds. Histopathological characteristics of specific organs express condition and represent time-integrated endogenous and exogenous impacts on the organism stemming from alterations at lower levels of biological organization (Chavin, 1973). Therefore, histological changes occur earlier than reproductive changes and are more sensitive than growth or reproductive parameters and, as an integrative parameter, provide a better evaluation for the health of the organism than a single biochemical parameter (Segner and Braunbeck, 1988).

The damage of gills of fish exposed to the sub-lethal concentrations of phenolic compounds was severe. Extensive architectural loss was observed in the gills of phenol treated group. Richmonds and Dutta (1989) divided the commonly reported gill lesions into two groups: (1) the direct deleterious effects of the irritants and (2) the defense responses of the fish. The observed lamellar necrosis and complete desquamation of the gill epithelium are direct responses induced by the action of phenolic compounds. Another important histopathological change observed in the phenol treated group was hyperplasia. Morphologically, hyperplasia refers to an increase in the number of normal cells that constitute a given tissue.

Gill alterations such as hyperplasia of the epithelial cells can be considered adaptive, since they increase the distance between the external environment and blood, serving as a barrier to the entrance of contaminants. Hyperplasia observed maybe the fish's response (1) to ward off or block something that irritates its tissues, whether externally or internally, or (2) to quickly heal an injured or irritated site. Hyperplasia, however, may play a role in the early stages of neoplasia. Gill hyperplasia might serve as a defensive mechanism leading to a decrease in the respiratory surface and an increase in the toxicant-blood diffusion distance. Increased mucus production and fusion of lamellae were obvious on exposure to both the phenolic compounds. Mucus cells
contain mucins, polyanions composed of glycoproteins that can be effective in trapping toxicants and aid in the prevention of toxicant entry into the gill epithelium (Perry and Laurent, 1993). Extensive epithelial desquamation was also observed in the phenol treated group. It is well known that changes in fish gill are among the most commonly recognized responses to environmental pollutants (Mallatt, 1985; Laurent and Perry, 1991; Au, 2004). After acute exposure to hexavalent chromium, *Channa punctatus* exhibited marked degenerative changes in the histology of gills, kidney and liver tissues (Mishra and Mohanty, 2008). The gills of both phenol and *m*-cresol treated group exhibited lamellar telangiectesis (localised dilation of blood vessel). This appearance of the secondary lamellae results from the collapse of the pillar cell system and breakdown of vascular integrity with a release of large quantities of blood that push the lamellar epithelium outward (Alazemi et al., 1996). Shortening and clubbing of ends of the secondary gill lamellae and clubbing of adjacent lamellae were well marked in the *m*-cresol treated group. Complete lamellar fusion may have reduced the total surface area for gas exchange. Otherwise, they increase the distance of the water-blood barrier, which together with epithelial lifting and the increase in mucus secretion may drastically reduce the oxygen uptake.

As fish gills are critical organs for their respiratory and osmoregulatory functions, the injuries in gill tissues observed as a result of exposure to phenolic compounds may have reduced the oxygen consumption and resulted in the disruption of the osmoregulatory functions of the fish. As gills are the major site of osmotic and ionic regulation in fish, any change in gill morphology may result in perturbed osmotic and ionic status which was observed as decreased branchial ATPases activity in the present investigation.

Also the histopathological alterations could be attributed to increased peroxidative damage to gill membrane in fishes exposed to phenolic compounds. It is important to stress that lamellar fusion and disappearance of secondary lamellae can lead to a notable reduction in the respiratory surface, which consequently can hinder gas exchanges (Rajabanshi and Gupta 1988; Poleksic and Mitrovic-Tutundzic, 1994). The defense responses will take place at the expense of the respiratory efficiency of the gills and eventually, the respiratory impairment must outweigh any protective effect against pollutant uptake. Significant deformations were observed in liver on exposure to both the phenolic compounds. Liver being the main organ of various key metabolic pathways, toxic effects of chemicals usually appear primarily in the liver. This, in turn, provides important data on the chemical’s toxicity and mode of action. Also it is a principal site of detoxification based on the fact that in teleosts it is the major site of cytochrome P450 which
inactivates some chemicals and activates others. Furthermore, nutrients derived from gastrointestinal absorption are stored in hepatocytes and released for further metabolism by other tissues (Moon et al., 1985), bile synthesized by hepatocytes aids in the digestion of fatty acids (Boyers et al., 1976) and carries conjugated metabolites of toxicants (Gingerich, 1982) into the intestine for excretion or enterohepatic recirculation, and the yolk protein vitellogenin is synthesized within the liver (Vaillant et al., 1988). Many organic compounds induce toxicopathic lesions in the liver of fish species. Stressor- associated alterations of hepatocytes may be found in the nucleus or cytoplasm or both. An important observation in the current study on exposure to phenol was clear cell foci which exhibited an altered staining pattern. Focal lesions are precursors to the development of hepatocellular neoplasm indicating a reduced capacity to metabolize xenobiotics. Myers et al. (1990) suggest that there are strong and consistent associations among all of the putatively preneoplastic foci of cellular alteration (basophilic, eosinophilic, and clear cell foci), between focal lesions and the different types of neoplasms, and among the various neoplasm types. Hepatocellular foci of altered hepatocytes have been suggested as an early stage in the stepwise formation of hepatic neoplasia and as such provide an excellent example of a histopathological biomarker for contaminant exposure (Hinton et al., 1992). Histologic examination of mummichog (Fundulus heteroclitus) from a creosote-contaminated site in the Elizabeth River, Virginia, revealed high incidences of hepatic neoplastic lesions (Vogelbein et al., 1990). Stehr et al. (2003) observed that on chemical contaminant exposure English sole (Pleuronectes vetulus) in Vancouver Harbour, Canada showed toxicopathic liver lesions such as neoplasms, preneoplasms, specific degeneration/necrosis and non- neoplastic proliferative lesions.

Another important change observed in the liver of treated groups was necrosis. Necrosis, which is a passive mode of cell death shows that the capacity to maintain homeostasis was affected. Thus occurrence of necrosis may be one of the important reasons for decreased lysosomal membrane stability observed leading to the leakage of lysosomal marker enzyme acid phosphatase to the soluble fraction. Also the increased level of the important marker enzyme ALT in liver indicates the stress induced by the phenolic compounds in this tissue. In both the phenolic compounds treated groups shrunk and pyknotic nuclei were observed in liver. Pyknotic nuclei observed indicate that the cells became hypofunctional. Pyknosis results in irreversible condensation of chromatin in the nucleus of a cell. Acute toxic injury usually includes cloudy swelling or hydropic degenerations and pyknosis, karyorrhexis and karyolysis of nuclei (Hawkes,

Cloudy swelling, bile stagnation, focal necrosis, atrophy and vacuolization have been reported in the Corydoras paleatus exposed to methyl parathion (Fanta et al., 2003). Cengiz and Unlu (2006) reported hypertrophy of hepatocytes, increase of kupffer cells, circulatory disturbance, narrowing of sinusoids, pyknotic nuclei, fatty degeneration and focal necrosis in the liver of Gambusia affinis exposed to deltamethrin. The cellular degeneration in the liver may be also due to oxygen deficiency as a result of gill degeneration and/or to the vascular dilation and intravascular haemolysis with subsequent stasis of blood (Mohamed, 2001).

The kidney is a highly dynamic organ in most of the vertebrates. Kidney receives about 20% of the cardiac output. Any chemical substances in the systemic circulation are delivered in relatively high amounts to this organ. Thus a nontoxic concentration of a chemical in plasma could become toxic in the kidney. The kidney of the fish receives largest proportion of postbranchial blood, and therefore renal lesions might be expected to be good indicators of environmental pollution (Ortiz et al., 2003). In the present study the most evident changes observed in the kidney of phenol treated groups were glomerular congestion, pyknotic nuclei and renal tubular architectural loss. m-cresol treated group showed histopathological alterations such as necrosis and vacuolation of tubular epithelial cells. It was also observed that in both the treated groups epithelial cells have become swollen and basophilic.

Heavy metal-induced alterations of interrenal cells were demonstrated in several other species (Norris et al., 1997; Hontela, 1998; Levesque et al., 2003) which may be due to the stress impact of metals in this endocrine component. Elsan treatment in Channa punctatus resulted in a significant decrease in the dimension of Bowman's capsule and glomerulus, and the tubules lost their regular shape due to precipitation of cytoplasm and karyolysis (Banerjee and Bhattacharya, 1994). Hypertrophy of renal cells, changes in the nuclear structure, formation of vacuoles, necrosis and degeneration of renal components were noticed on the renal cells of Cyprinus carpio exposed to malathion and sevin (Dhanapakiam and Premlatha, 1994). Dass and Mukherjee (2000) reported dilation of tubules, necrotic changes characterized by karyorrhexis and karyolysis at the nuclei of affected cells of Labeo rohita exposed to hexachlorocyclohexane. The exposure of fish to toxic agents such as pesticides and heavy metals induces histological alterations in several components of the trunk kidney (Kendall, 1975; Kirubagar and Joy, 1988; Ortiz et al., 2003; Velmurugan et al., 2007). Cengiz (2006) observed lesions in the kidney tissues of fish exposed to deltamethrin, characterized by
degeneration in the epithelial cells of renal tubule, pyknotic nuclei in the hematopoietic tissue, dilatation of glomerular capillaries, degeneration of glomerulus, intracytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen. Ayas et al. (2007) observed histopathological changes in liver and kidney of three different fish species having different feeding habits in Sariyar Reservoir, Turkey, contaminated with organochlorine pesticide residues. They noticed characteristic changes such as mononuclear cell infiltration, congestion and nuclear pyknosis in liver and kidney.

As a conclusion, the findings of the present histological investigations demonstrate a direct correlation between exposure to phenolic compounds and histopathological disorders observed in several tissues. All the histopathological observations indicated that exposure to sub-lethal concentrations of phenolic compounds caused destructive effect in the gills, liver and kidney tissues of Clarias batrachus. It is important to stress that phenolic compounds are biotransformed in the liver of fish by phase I and phase II reactions. In phase I, reactions of oxidation, reduction and hydrolysis catalysed by CYP 450 system occur, whereas phase II involves the conjugation of the phase I products with the endogenous molecules, such as glutathione, sulphate or glucuronic acid (Andersson and Forlin, 1992; Siroka and Drastichova, 2004). The activation in fishes frequently depends on oxidative metabolism catalyzed mostly by microsomal cytochrome P-450-dependent mixed-function oxidases. However, CYP-catalyzed biotransformation may also activate nontoxic procarcinogens to potent carcinogens or even to toxic metabolites (Yan and Caldwell, 2001). The metabolites get distributed throughout the organism by the bloodstream, causing even greater damage. The observed abnormal behaviour and altered histopathology of vital organs demonstrate the severe adverse effects to exposure of phenolic compounds in C. batrachus.

The current study reinforces the application of histopathology as a powerful tool for monitoring anthropogenic contamination within aquatic environments. Whilst links between such pathologies and contaminants are not definitive, such surveillance provides a useful insight into individual, population and overall ecosystem quality. When these pathological endpoints are assessed in conjunction with other parameters such as parasite community structure, sediment and water chemistry, enzyme responses, bile metabolite levels and molecular damage indices, a clearer picture of the complex interactions between anthropogenic and natural environmental modifiers will emerge.