Chapter-IV
Discussion
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The results on the toxicity of Bisphenol A to the crab, *Paratelphusa jacquemontii* shows that there are marked differences in the sensitivity to Bisphenol A. As the information is very scarce and fragmentary with invertebrate models the present investigation is carried out to understand the toxic effects of Bisphenol A on metabolism, biochemical changes and genotoxic effects. Our objective in this investigation is to study biophysiochemical aspects in crustaceans. In the present investigation bisphenol A is administered to the crab, *Paratelphusa jacquemontii* to study its role in alteration in the biochemical, enzymatic and histopathological changes in crusteations. To understand genotoxic effects of Bisphenol A study in onion, *Allium cepa* root meristem is also carried out.

Most of the information about the effects of environmental pollutants on aquatic animals has been obtained from mortality studies. Often very little is known about the damage to different internal organs or about disturbed physiological and biochemical processes within an organism following environmental poisons (Larsson *et al*., 1976). As far as invertebrates are concerned, Bayne, 1973, Gabbott and Bayne, 1973 and Bayne, 1975 investigated stress caused by pollutants in the bivalve, *Mytilus edulis* and found that seasonal and laboratory induced stress due to a pollutant could be detected by studying number of parameters, such as blood sugar levels, disruption of energy balance represented by alterations in glycogen, protein and lipid content of tissues and related enzyme activities. These metabolic parameters have been often utilized to detect lethal and sub-lethal effects of contaminants. The changes induced in the carbohydrate metabolism by severe muscular exercise are due to the stress exerted by heavy metal uptake in vertebrate and invertebrate organisms (Black, 1958; Black *et al*., 1962; Baemish, 1968; Laul, *et al*., 1974).

### 4.1 Biochemistry and metabolism :

In the present investigation exposure of the crabs, *Paratelphusa jacquemontii* to 50 ppm and 300 ppm concentration for 15,30,45 and 60 days to Bisphenol A shows marked fluctuations in the levels of blood and tissue metabolites. Within the living cell metabolites play a very important role in connecting many different pathways as they are the intermediates of biochemical reactions. Many regulatory processes occur within
the cell like regulation of transcription and translation, regulation of protein-protein interactions and allosteric regulation of enzymes through their interaction with metabolites. The levels of the metabolites are complex as they are determined by the concentrations and the properties of the enzymes. Thus, the level of metabolites represents integrative information of the cellular function, hence defines the phenotype of a cell or tissue in response to genetic or environmental changes.

In the present study from table 2 and 5 it can be discerned that in the crabs treated with concentration of Bisphenol A, Glycogen content after administration of Bisphenol A for 15 days was observed to decrease significantly as the doses are increased upto 50 ppm and 300 ppm as compared to control. The same case was also observed in the case of muscle and gill glycogen content. The exceptional observations were found in the case of ovaries and testis. The observation were indicated that the glycogen is required for the process of spermatogenesis hence the concentration of glycogen is decreased in the testis of the crab, exposed to 300 ppm. The Observation of ovaries are also indicates that, gluconeogenesis takes place to meet the energy need during the vitellogenesis. In hepatopancreas and muscles, the decrease in glycogen was more marked. This indicates that perhaps there is impairment in the synthesis of glycogen and at the same time utilization of reserve glycogen for energy needs. A decrease in the glycogen was accompanied by an increase in glucose levels in blood even after small dose of bisphenol A for 15 days, causing hyperglycemia. This hyperglycemia is possibly due to increased glycogenolysis in the hepatopancreas under the stress of Bisphenol A. Hyperglycemia is a typical stress response and is usually accompanied by increased breakdown of liver and muscle glycogen. However, Ranganathan and Ramamurthi,(1979) have reported that fall in muscle glycogen suggests an increased turnover of glycogen and that the muscle glycogen does not contribute towards hyperglycemia. The sole sourse of glucose for activities of ovaries, testis, muscles and gills is the hepatopancreas. The muscle tissue in crustations is adapted to anaerobic respiration by converting glucose in to lactate and this may be the reason to decrease in the glycosen contents in the muscle in 50 ppm and 300 ppm as compared to control. The glycogen content is also deplated in case of gills which use fresh oxygen for oxidation of glucose and hence it is not necessary to convert glycogen into glucose.
Similar results were observed in the sets for 15 days, 30 days, 45 days and 60 days. Decrease in the glycogen concentration of the hepatopancreas and muscle tissue was observed. Glycogen contents in ovary from 30 days onward indicates that during vitellogenesis active yolk synthesis takes place and the carbohydrate moiety is conjugated to yolk in hepatopancreas leading to a decrease in the concentration of glycogen. This decrease in glycogen content may be explained by fact that vitellogenesis may be in the terminal phase leading to depletion of glycogen content in the hepatopancreas and muscles. The glycogen contents were decreased in the hepatopancreas in all the sets, indicates that as the hepatopancreas is the main center for metabolic activities including detoxification of Bisphenol A. Adiyodi, (1969c) has proposed hepatopancreas as an important site of intermediary metabolism in crustaceans and may be the major source of vitellogenic precursors.

A decrease in the glycogen was accompanied by an increase in glucose levels in blood, causing hyperglycemia. This hyperglycemia is possibly due to increased glycogenolysis in the hepatopancreas under the stress of Bisphenol A. In hepatopancreas and muscles, the decrease in glycogen was more marked (Mayekar et al., 2003). This indicates that perhaps there is impairment in the synthesis of glycogen and at the same time utilization of reserve glycogen for energy needs. Hyperglycemia is a typical stress response and is usually accompanied by increased breakdown of liver and muscle glycogen. However, Ranganathan and Ramamurthi, (1979) have reported that fall in muscle glycogen suggests an increased turnover of glycogen and that the muscle glycogen does not contribute towards hyperglycemia.

Different pollutants, including trace metals, colour pigments are known to enhance the cellular formation of reactive oxygen species which are responsible for oxidative stress leading to several toxic processes including protein degradation, enzyme activation, damage to DNA and chemical carcinogenesis (Halliwell and Gutteridge, 1989; Winston, 1991). Environmental stress like salinity, temperature or pollutant contamination can modify protein expression in different tissues of relevant aquatic organisms (Kultz and Somero, 1996; Shepard and Bradley, 2000; Shepard et al., 2000; Meiller and Bradley, 2000).

In the present study from table 6 and 9 it can be postulated that in the crabs treated with Bisphenol A, Protein content after administration of Bisphenol A for 15
days was observed to decrease significantly as the doses are increased up to 50 ppm and 300 ppm as compared to control. The exceptional observations were found in case of ovaries and testis. The observation table indicates that, a significant decrease in the protein content in all the tissues as well as blood serum was observed in the animals exposed to Bisphenol A. Significant increase in protein contents of ovary were observed, while a significant decrease was observed in the muscle tissue. This is in lieu with the fact that during vitellogenesis, yolk synthesis takes place in ovaries. It is presumed that the crabs were in vitellogenesis I as shown in light microscopy. The decrease in protein content of muscle tissue is correlated with its anaerobic metabolism where amino acids are generated by degradation of proteins to meet energy needs.

The same results were observed with the sets for 15 days, 30 days, 45 days and 60 days. Significant decrease was observed both in the hepatopancreas and muscle tissue while in the ovaries a significant increase was observed. The significant decrease in protein concentration in hepatopancreas may be due to its transport from hepatopancreas to ovary through hemolymph. This is supported by the increase in the concentration of proteins in ovaries. The observed changes in muscle tissue may be due to its mode of respiration during reproductive cycle. The damage caused to hepatopancreas may be due to the breakdown of proteins therein, resulting in decreased protein contents. It is also be correlated with the enhanced level of acid phosphatase activity is partially responsible for the destruction of protein content of tissues. Similar results were also observed by Reddy et al., 1983 in rice field crabs exposed to pesticide sumithion. As far as serum proteins were concerned, a consistent elevation in protein content was also observed by Reddy et al., 1983 and Bhagyalaxmi et al., 1983 in Oziotelphusa sensex sensex exposed to benzene hexachloride and sumithion. The muscles and gills with the breakdown of glycogen also use proteins to produce more energy to meet the stressful conditions because of Bisphenol A. This may also be due to increased proteolysis and enhanced efflux of proteins into the blood (Bhagyalaxmi et al., 1983).

Cholesterol is the predominant sterol in the cellular membranes of crustaceans and can account for between 5 and 12 % of the total membrane lipid (Krzynowek et al., 1982). Alterations in cholesterol level have already been reported earlier by Raizada et al., 1980, 1982; Bhattacharya et al., 1984, Ram et al., 1984.
In the present study from table 10 and 13 it can be concluded that in the crabs treated with concentration of Bisphenol A, Cholesterol for 15 days administration of Bisphenol A was observed to decrease significantly as the dose are increased upto 50 ppm and 300 ppm as compared to control in gills, shows significant increase in the muscle, testis and ovary. The significant decrease in cholesterol content of hepatopancreas may be due to its transport to the ovaries. The increase in cholesterol concentration is due to the up take of cholesterol by ovary. In crustacean ovaries are an established source of ecdysteroids in addition to Y-organ. Ecdysteroids are synthesized from cholesterol and are conjugated to the yolk, which are utilized during morphogenesis as a morphogen. In addition to this the ovaries also store cholesterol as crustaceans can not synthesize cholesterol and depend on the internal stored cholesterol. Cholesterol is also an integral part of membranes and an important constituent of lipid rafts which play a major role in signal transduction.

In 45 days exposure, an insignificant increase in cholesterol level was observed in the hepatopancreas and muscle and a significant increase was observed in the ovaries. The insignificant increase in cholesterol content in hepatopancreas and muscle can be attributed to the fact that the role of these organs in vitellogenesis may be complete with the transfer of cholesterol after which homeostasis is being achieved, while the cholesterol is still being taken up by the ovaries.

The results observed in the experiments for 15 days, 30 days, 45 days and 60 days shows significant and insignificant decrease in cholesterol concentration in both hepatopancreas and muscle tissue while a significant increase was observed in the ovaries. The significant decrease in cholesterol shows that it is still in the process of being transported and the increase in ovaries is indicative of the completion of conjugation of synthesized ecdysteroids after which cholesterol is being stored in the ovaries. In crabs treated for 45 days to 50 ppm and 300ppm, a significant increase in cholesterol concentration was observed in gills and hepatopancreas. This shows that homeostasis is reached in cholesterol metabolism in different organs.

Administration of Bisphenol A leads to expedited molting and vitellogenesis. This leads to gross changes in cholesterol metabolism. Cholesterol is transported from the organs of storage to the hemolymph. Y-organ from crustaceans plays a major role in cholesterol metabolism. The cholesterol is converted to ecdysteroids which is then
circulated through the hemolymph. In the ovaries this ecdysteroid is taken for conjugating the yolk, in addition to which it also help in oocyte maturation. In crustacean the ovary is also known to be the source of ecdysteroids. The cholesterol that is taken up is converted to ecdysteroid to conjugate the yolk as well as stored for use during development. Ecdysteroid hormone in crustaceans is synthesized from cholesterol in the Y-organs. Circulating cholesterol is bound to high-density lipoprotein (HDL). Kang and Spaziani, (1995) studied the mode of cholesterol uptake by Y-organ cells in the crab, *Cancer antennarius*. They propounded that cholesterol is taken up by endocytosis of the entire HDL-cholesterol complex.

Factors emanating from the eyestalks as well as other endocrine glands exert influence on the lipid and cholesterol metabolism in crustaceans. Rao and Surendranath, (1992) showed that in the crab, *Scylla serrata* total lipids, total fatty acids, unsaturated fatty-acids, phospholipids and cholesterol contents increased in the tissue of eyestalk ablated juvenile intermoult crabs, while the neutral lipase activity, free fatty acids, glycerol and acetoacetate contents including neutral lipase activity have been restored to control levels. This suggests the neurosecretory factors present in the eyestalks of *Scylla serrataII* which regulate lipid metabolism. Through radiolabeling of cholesterol, using inhibitors of lysosomes and protein synthesis and its uptake is depressed by the molt-inhibiting hormone secreted by the eyestalks. So in the present investigation the decrease in cholesterol content by its uptake by ovaries can be ruled out as Bisphenol A is responsible for transfer of cholesterol where it is used as stored form of energy. It can also be presumed that the Bisphenol A showing estrogenic effect which may be responsible for formation of mobilization of cholesterol for steroid synthesis.

The changes in pyruvic acid and lactic acid levels suggest metabolic disorders. A severe respiratory stress is indicated as there is an elevation in the activity of lactic acid levels (Dange and Masurekar, 1981). An upward trend in the lactic acid relative to pyruvic acid in the exposed animals results in an inadequate oxygen supply to the tissues thereby disturbing the normal metabolic functioning (Huckabee, 1958).

In the present study from table 14 and 17 it can be discerned that in the crabs exposed to Bisphenol A for 15 days, Lactic acid contents were observed increased significantly as the dose were increased from 50 ppm to 300 ppm as compared to
control in hepatopancreas and shows significantly increase in the gills, muscle, testis and ovary. The present investigation shows decrease in lactic acid contents in hepatopancreas whereas increase in the ovary and muscles. There is also significant increase seen in the enzymes like SDH and LDH. The increase in the levels of lactic and pyruvic acid together with corresponding changes in the activities of LDH and SDH enzymes clearly indicates that the crabs are resorting to both aerobic and anaerobic pathways of energy production to meet the increased demand for the process of synthesis of vitellogenin in hepatopancreas and conversion of vitellogenin to vitellin or yolk in the ovary. The muscular changes suggests of the increased activity of the animal due to hormonal changes taken place in the metabolism of the crab.

The same results were observed in the sets for 15 days, 30 days, 45 days and 60 days. The changes in pyruvic acid and lactic acid levels in the experimental animals as compared to the control animals suggest towards metabolic disorders. A severe respiratory stress is indicated as there is an elevation in the activity of lactic acid levels (Dange and Masurekar, 1981). An upward trend in the lactic acid relative to pyruvic acid in the exposed animals results in an inadequate oxygen supply to the tissues thereby disturbing the normal metabolic functioning (Huckabee, 1958). After the exposure of crab to 15 and 30 days, there was a significant increase in the lactic acid levels whereas the pyruvic acid content in all the tissues like hepatopancreas, gills, muscles, and blood showed an insignificant decrease. This decrease in the pyruvic acid is due to the conversion of pyruvic acid into lactic acid and hence the increase in lactic acid levels. This is because the hypoxic condition partially suppresses aerobic respiration. In experimental animals this condition may be attributed to the inability of the affected animals to derive enough oxygen from the surrounding water because of the damage to the gills, which may lead to hypoxia. Lactic acid levels rise under hypoxic condition in the crabs (Teal and Carey, 1967). Under stress of pollutants, lactic acid is known to rise in the blood (Helmy et al., 1979, Sastry and Subhadra, 1982) and tissues (Burton et al., 1972) of the exposed fish. Similar observations were found in the crab, Scylla serrata exposed to cadmium (Mayekar et al., 2003). On these observations it appears very evident that the crabs exposed to the Bisphenol A might enter the state of “Lactic acidosis” in which levels of lactic acid in blood of tissue get increased.

In the present study from table 18 and 21 it can be said that in the crabs exposed to Bisphenol A, Pyruvic acid contents for 15 days was observed increased
significantly as the doses were increased from 50 ppm to 300 ppm as compared to control in all tissues. The changes in pyruvic acid and lactic acid levels in experimental animals as compared to the control animals suggest metabolic disorders. A severe respiratory stress is indicated as there is an elevation in the activity of lactic acid levels (Dange and Masurekar, 1981). An upward trend in the lactic acid relative to pyruvic acid in the exposed animals results in an inadequate oxygen supply to the tissues thereby disturbing the normal metabolic functioning (Huckabee, 1958). In experimental animals this condition may be attributed to the inability of the affected animals to derive enough oxygen from the surrounding water because of the damage to the gills, which may lead to hypoxia. After the exposure of the crabs, there was a significant increase in the lactic acid levels whereas the pyruvic acid content in all the tissues like hepatopancreas, gills, muscles, and blood showed an insignificant decrease. This decrease in the pyruvic acid is due to the conversion of pyruvic acid into lactic acid and hence the increase in lactic acid levels. This is because the hypoxic condition partially suppresses aerobic respiration.

The increase in the levels of lactic and pyruvic acid together with corresponding changes in the activities of LDH and SDH enzymes clearly indicate that the crabs are resorting to both aerobic and anaerobic pathways of energy production to meet the increased demand for the process of synthesis of vitellogenin in hepatopancreas and conversion of vitellogenin to vitellin or yolk in the ovary. The muscular changes suggests of the increased activity of the animal due to hormonal changes taken place in the metabolism of the crab.

Acid phosphatase and alkaline phosphatase catalyze the hydrolysis of almost any phosphoester to release inorganic phosphate. Acid phosphatase is identified with other hydrolases in lysosome (Novikoff, 1961). It has limited activity but it is of great significance in some pathological conditions. Moreover, acid phosphatase activity at different pH optima is associated with growth, differentiation and the lyses of cells (de Duve and Wattauex, 1966; Varute 1970, Varute and Sawant, 1971). Alkaline phosphatase is also associated with the maintenance of the orthophosphate pool, the transfer of phosphoryl groups, the hydrolysis and the esterification of metabolites moving across membranes within the cell and between the cell and extra cellular space (Saev, 1963, Morton, 1965).
Present study shows, Acid Phosphatase (ACP) content 15 days exposure of crabs changes were observed to significantly increase and decrease as the dose are increased upto 50 ppm and 300 ppm as compared to control in all the tissues.

In the crabs administered with two different concentration of Bisphenol A for 15 days, a significant decrease in Acid Phosphatase activity was observed in the hepatopancreas of the crab treated with 50ppm and 300 ppm Bisphenol A, while an insignificant increase in activity was observed in the muscle tissue, testis and ovary also. The similar results were also observed in the sets exposed to 15 days, 30 days, 45 days and 60 days.

The changes in ACP activity may be correlated with the metabolic state of the cells. It might be that since these are degradative enzymes their expression is tightly regulated in the tissues. The enzyme is expressed during development for the degradation and subsequent assimilation of yolk. In addition to this it is quite likely that the increased activity of this enzyme in the muscle tissue may be correlated with the energy needs by way of protein degradation. There was an increase in the ACP and ALP level in the blood due to the necrosis of tissues as a result of which it is released into hemolymph. Also the increase in activity is due to catabolism in tissue to circumvent stress caused by metal ions so as to meet energy needs.

Acid phosphatase is synthesized at a high rate for the degradation of protein and other micromolecules that are irreversibly damaged during stress. The ACP activity in the crabs was increased comparatively which might be due to cellular damage in the tissues. It is reported to bring about an increase in ACP activity (Dhavale and Masurekar, 1984; Novikoff, 1961; Hapke, 1977; Sastry and Shah 1981; Shafi, 1982). ACP is a lysosomal enzyme that helps in the autolysis of the cell after its death. The increase in the lysosomal activity in the injured cell occurs as a part of the prenecrotic changes (Novikoff, 1961; de Duve, 1963; Dhavle and Masurekar, 1984).

Novikoff, (1961) identified ACP with other hydrolases in lysosomes. Moreover, acid phosphatase activity at different pH optima is associated with growth, differentiation and the lysis of cells (deDuve and Wattaux, 1966; Varute, 1970; Varute and Sawant, 1971). Hence changes in concentration of this enzyme during reproductive cycle are imperative. Cellular damage in tissues is reported to bring about an increase

Elevation in acid phosphatase activity in the hepatopancreas of exposed crabs followed by depletion in proteins observed in the present investigation is in conformity with the investigation carried out by Dubale and Shah, 1981; Dhavale, 1984. The muscles and gills with the breakdown of glycogen also use proteins to produce more energy to meet the stressful conditions created by Bisphenol A. This may also be due to increased proteolysis and enhanced efflux of proteins into the blood (Bhagyalaxmi et al., 1983).

Alkaline Phosphatase (ALP) content after administration of Bisphenol A for 15 days changes were observed to be significantly increased as the dose are increased upto 50 ppm and 300 ppm as compared to control in all tissues.

Alkaline phosphatase is a brush border enzyme involved in trans-phosphorylation reaction. It is an important enzyme participating in cell phospho-metabolism. The inhibition of alkaline phosphatase activity as a result of nickel stress both at acute and chronic levels is due to disruption in the process of oxidative phosphorylation during the formation of energy rich compounds as observed by Sastry and Gupta, 1978; Dalela et al., 1980. It is also known that the inhibitory action of the heavy metal on enzymes is due to the binding of the metal to the enzyme proteins (Passow et al., 1961).

The Alkaline Phosphatase activity in the animals exposed to two different concentrations of Bisphenol A for 15 days shows significant increase in activity compared to control. Similar results were observed in the sets exposed for 30 days, 45 days and 60 days. The significant increase may be attributed to the importance of controlling the phosphate pool as well as the energy needs during vitellogenesis.

Alkaline phosphatase is associated with the maintenance of the orthophosphate pool, the transfer of phosphoryl groups, the hydrolysis and the esterification of metabolites moving across membranes within the cell and between the cell and extra cellular space (Saev, 1963, Morton, 1965).

ALP is known to be associated with the maintenance of orthophosphate pool, the transfer of phosphoryl groups, the hydrolysis and the esterification of metabolites
moving across membranes with in the cell and between the cell and extracellular space which is in agreement with Saev, (1963) and Morton, (1965) determined that the activities of digestive enzymes and alkaline phosphatase from the hepatopancreas of *Macrobrachium nipponense* under different environmental factors. The ALP activity is sensitive to environmental stress. Further, it appears that the ALP activity is under neuroendocrine control since it was inhibited *in vitro* by $10^{-4}$ M dopamine. The response to both environmental salinity and dopamine suggests that ALP activity could be an important component of muscle regulatory mechanisms at the biochemical level secondary to hyper regulation of *C. angulatus*. This especially gains relevance as ALP is functionally coupled to Na$^+$/K$^+$ ATPase in muscles. ALP activity as a function of time will help to shed more light on their role in the biology of crustaceans (Pinoni and Lopez, 2004).

ACP and ALP activities were increased during the Bisphenol A exposure, suggesting enhanced breakdown of phosphates to release energy, presumably to compensate for the impaired ATPase system. In the present investigation inhibition of ATPase activity was found in all the tissues after exposure to Bisphenol A. This enzyme located in the cell membrane is responsible for the active transport of Na$^+$ and K$^+$ across the cell membrane (Skou, 1957). Adenosine triphosphatase (ATPase) is a mitochondrial enzyme and it carries out oxidative phosphorylations i.e. it catalyses the hydrolysis of Adenosine triphosphate (ATP) to Adenosine diphosphate (ADP) and Phosphoric acid. This brings about the release of enormous energy. It is also involved in osmoregulation. Schmidt-Nielsen, 1974 reported that mercuric compounds inhibit Na$^+$/K$^+$ ATPase activity and interfere with osmoregulatory mechanisms of aquatic organisms. The bulk of cellular energy in normal cell is derived from ATP. The observed inhibition might be related to the activity of the compound to alter cellular configuration by binding SH groups at active sites of the membrane (Verma *et al.*, 1983). Any impairment in ATPase activity therefore results in less availability of cellular energy in the form of ATP. Schmidt-Nielsen, 1974 also observed inhibition of Na$^+$/K$^+$ ATPase activity in gills and liver of rainbow trout after exposure to chromium and mercury and attributed similar reasons for the enzyme inhibition. Since ATPase is integral part of the membrane, the activity of the enzyme would be blocked, thereby disrupting the functions of organs where ATPase has been inhibited (Verma, 1983).
GOT and GPT are the key enzymes of nitrogen metabolism and are important in energy mobilization. Thus, amino transferases serve as the strategic link between carbohydrate and protein metabolism by inter-converting metabolites such as α-ketoglutaric acid, pyruvic acid and oxalic acid on one hand and alanine and aspartic acid on the other hand. Enzymes Alanine amino transferase (ALAT/GOT) and Aspartate amino transferase (AAT/GPT) is known to elevate activity of enzyme phosphorylase which plays an important role in glycogenolysis. They play a major role in the intermediary metabolism of the amino acids by participating in gluconeogenesis and by providing precursors via transaminases, glutamate synthetases and glutaminases (Sarojini et al., 1987).

GOT is very active and widely distributed of the transaminases. GPT is much more abundant in tissue like hepatopancreas than any other tissues and consequently an altered activity of this enzyme points to some disorder in those tissues. To study the alterations in protein and carbohydrate metabolism, assaying of the transaminases is an imperative necessity.

Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) content after administration of Bisphenol A for 15 days changes were observed to significantly increase as the dose are increased from 50 ppm to 300 ppm as compared to control in all tissues except hepatopancreas.

In animals exposed 50 ppm and 300 ppm Bisphenol A for 30 days, an insignificant decrease in GOT concentration was observed in the hepatopancreas while a significant decrease was observed in the muscles, while in the ovaries a significant increase in GOT activity was observed. In crabs treated for 45 days and 60 days, hepatopancreas and muscle tissues displayed a significant decrease while the ovary displayed an insignificant decrease.

The decreased activity in the hepatopancreas and ovaries may be due to the high availability of carbohydrates. These tissues rely on it as a source of energy, while the increased activity in the muscle is correlated with the metabolism in the muscles where amino acids are pooled to TCA cycle. The decrease in activity of GOT in muscle of 30 days exposed sets may be due to attaining homeostasis after vitellogenesis.
The significant decrease in GOT activity in muscles shows that the amino acids are pooled to TCA cycle or other pathways. Phosphorylase is known to play an important role in glycogenolysis. The activity of the enzyme GOT is shown to be elevated with the increase in activity of phosphorylase (Bagyalakshmi et al., 1983). Similar observations were made on fishes by Rao and Dayakar (1987) studied the significance of aminotransferase in brachyuran species of diverse habitats. They showed that in the tissues of the crab, *Scylla serrata* from brakish water, *Ocypode platytarsis* from seacost and *Oziotelpusa senex senex* from paddy fields the free amino acid (FAA) content is high. The FAA was highest in the muscles.

Aminotransferases are believed to have a role in protein metabolism. But the relationship between protein synthesis and the activities of these enzymes is not very clear. The aminotransferases activity in the exposed crabs showed increase in the tissues like hepatopancreas, muscles, gills and ovaries whereas there was an insignificant decrease observed in the serum. Increased GOT and GPT activity in the blood plasma of animals exposed to pollutants were observed and these changes may be due to damage of liver and other tissues in the exposed fishes. This is supported by light and electron microscopic structure of hepatopancreas, gills and muscles. Similar results were also observed by Mokim et al., 1970; Christensen et al., 1972; Sastry and Subhadra, 1982. These studies revealed that the increased activity of aminotransferases may be due to the lesions in the liver that must have released these enzymes in the plasma. The increase in GOT and GPT activity suggests that proteins are channeled into the metabolic pathway.

The tissue GOT and GPT are high in muscle indicating operation of gluconeogenesis of energy. The ratios of GOT / GPT in tissues largely demonstrate preponderance towards anaerobic metabolism. Phosphorylase is known to play an important role in glycogenolysis. The activity of this enzyme is shown to be elevated with the increase in activity of phosphorylase (Bagyalakshmi et al., 1983). Burton and Feldman, (1983) studied the regulation of cell volume during hyperosmotic stress, in the intertidal copepod, *Tigriopus californicus*. Like other aquatic crustaceans, it rapidly accumulates high levels of intracellular alanine, proline, and glycine. Glutamate-pyruvate transaminase (GPT) catalyzes the final step of alanine synthesis. Studies showed under conditions of hyperosmotic stress, individual adult copepods accumulate alanine.
The decreased activity in the hepatopancreas and ovaries may be because due to the high availability of carbohydrates these tissues rely on it as a source of energy, while the increased activity in the muscle is correlated with the metabolism in the muscles where amino acids are pooled to TCA cycle.

The conversion of pyruvate to lactate is in the control of lactate dehydrogenase (LDH), therefore it has been the subject of several studies involving environmental contaminants. The decrease in LDH activity and the increase in lactate content indicated reduced mobilization of pyruvate into the citric acid cycle.

Succinate dehydrogenase (SDH) and Lactate dehydrogenase (LDH) content after administration of Bisphenol A for 15 days showed significant increase as the doses are increased from 50 ppm to 300 ppm as compared to control in all tissues other than gills. There is also significant increase seen in the enzymes like SDH and LDH. The increase in the levels of lactic and pyruvic acid together with corresponding changes in the activities of LDH and SDH enzymes. Same results were obtained from the set of 30 days, 45 days and 60 days. It clearly indicates that the crabs are resorting to both aerobic and anaerobic pathways of energy production to meet the increased demand for the process of synthesis of vitellogenin in hepatopancreas and conversion of vitellogenin to vitellin or yolk in the ovary. The muscular changes suggests of the increased activity of the animal due to hormonal changes taken place in the metabolism of the crab.

No significant changes were observed in LDH and SDH level in blood and they were to be within the control range. Further it is observed that the exposure leads to inhibit SDH as well as LDH from the serum in the stressful condition.

Adenosine triphosphatase (ATPase) is a mitochondrial enzyme and it carries out oxidative phosphorylations i.e. it catalyses the hydrolysis of Adenosine triphosphate (ATP) to Adenosine diphosphate (ADP) and Phosphoric acid. This brings about the release of enormous energy. It is also involved in osmoregulation.

Present study Adenosin tri phosphatase content in the blood after administration of Bisphenol A for 15 days changes was observed significantly increased as the doses are increased from 50 ppm to 300 ppm as compared to control in all the tissues.
ACP and ALP activities were increased during the Bisphenol A exposure, suggesting enhanced breakdown of phosphates to release energy, presumably to compensate for the impaired ATPase system. In the present investigation inhibition of ATPase activity was found in all the tissues after exposure to Bisphenol A. This enzyme located in the cell membrane is responsible for the active transport of Na\(^+\) and K\(^+\) across the cell membrane (Skou, 1957). Any impairment in ATPase activity therefore results in less availability of cellular energy in the form of ATP. Schmidt-Nielsen, 1974 also observed inhibition of Na\(^+\)/K\(^+\) ATPase activity in the gills and liver of rainbow trout after exposure to chromium and mercury and attributed similar reasons for the enzyme inhibition. The bulk of cellular energy in normal cell is derived from ATP. The observed inhibition might be related to the activity of the compound to alter cellular configuration by binding SH groups at active sites of the membrane (Verma et al., 1983). Since ATPase is integral part of the membrane, the activity of the enzyme would be blocked, thereby disrupting the functions of organs where ATPase has been inhibited (Verma, 1983). Schmidt-Nielsen 1974 reported that mercuric compounds inhibit Na\(^+\)/K\(^+\) ATPase activity and interfere with osmoregulatory mechanisms of aquatic organisms.

4.2 Histological study:

The application of ecotoxicological studies on non-mammalian invertebrates is rapidly expanding. Crab have become a valuable indicator for the evaluation of various chemical compounds as well as mixture of pollutants. In a toxicity study histology and histopathology is used as a biomonitoring tool. Histopathological alterations are biomarkers of adverse effect of environmental contamination, revealing alteration in the physiological and biological functions. Histopathology provides a very useful data concerning changes in the cellular / sub-cellular structure of an organ / tissue much earlier than external manifestations. Thus, this investigation helps in determining the pollution stress well in advance to avoid future disasters. Histology has made progress along with the sophistication achieved in the field of microscopy. With the advent of electron microscopic changes at sub-cellular level are also easily noted and this helps in predicting the dangers and safeguarding the interests of the concerned individuals. In the light of these facts, histopathological changes have been observed by light and electron microscopy in the male/female crab, Paratelphusa jacquemontii exposed to Bisphenol A in the target organs such as gills, hepatopancreas, ovaries and testis.
In the present investigation gills of the crab, *Paratelphusa jacquemontii* exposed to 50 ppm and 300 ppm concentrations of Bisphenol A were studied by light and electron microscopy. In the light microscopic studies of gills, congestion of gill lamellae and gross enlargement of gill processes and gill rachis due to massive influx of hemocytes was seen thereby changing the profile of lamellae so as to distort the shape of the gill. The most obvious and notable change in the gills is enlargement of the tip of the secondary lamellae. Sloughing of gill lamellae and gill processes occurred in the gills heavily congested with large number of necrotic and blackened hemocytes, giving them empty appearance. Hypertrophy of epithelial cells and hyperplasia may lead to enlargement of the gill lamellae tip and reducing the haemal canal. Oikari *et al.*, (1983) suggested that among observable effects, the most noteworthy was the swelling of gill lamella.

Gills are not only for gaseous exchange they perform several other physiological functions including osmoregulation and excretion. Changes in environmental parameters often damage this delicate vital organ which has direct contact with aquatic environment. (Parashar and Banerjee, 2002) Dutta *et al.*, 1996; Wendelaar Bonga, 1997, have demonstrated increased concentrations of different pollutants including several heavy metals seriously damage the gills of teleostean fish.

The gills of the crab, *Paratelphusa jacquemontii* are composed of primary lamellae arranged serially in pairs, the gill is covered by thin layer of chitinous cuticle under which epithelial cell, after the exposure of 50 ppm Bispehol A exposure shows, alterations including, enormously thickened control rachies filled with large mass of granular haemocytes. The gill lamellae were also affected showing accumulation of hemocytes resulting in swelling and enlargement of lamellae, The gill lamellae also showed breakages of the epithelial cells. After exposure of 300 ppm it shows, swollen central axis with sloughed inner tissue. The central axis as well as lamellae are showing accumulation of haemocytes. The epithelium of lamellae is also disintegrated and broken at many places. The central axis as well as lamellae show disintegration of connective tissue with many vacuoles. The present results also shows the similar type of alteration in the histological structure of gills.

The gills are the primary initial target of toxicity, and the cytological changes in gill morphology in fish usually occur as a result of contaminant exposure. Gills have an
extensive surface area and minimal diffusion distance between dissolved O\textsubscript{2} and blood capillary for efficient gaseous exchange. The fusion occurred in gills of fishes exposed to 4-nonylphenol in this investigation may cause a drastic reduction in the respiratory surface area. However, very little is known about the toxic impact of 4-nonylphenol on the functional morphology of the gills. It has been reported that the immediate morpho-pathological response of the gills to xenobiotics is often manifested by a significant increase in the density of its mucous cells (Dutta, 1997, Hemalatha and Banerjee, 1997).

In the ultrastructural study of gills it was found that Bisphenol A exposure had an effect on the epithelial cells of the gill lamellae. Lifting of epithelial cells was another feature observed. Morgan and Tovell, (1973) suggested that the epithelial lifting might exert protective effect hindering the pollutant uptake by increasing the diffusive distance. The lifting up of epithelial cells in aquatic animals is also reported by Gilderhaus, (1966) and Eisler and Gardner (1973). Results were also supported by Parashar and Banerjee, (2002). Similar results were found in gills of *Lepomis macrochirus* exposed to malathion (Richmond and Dutta, 1989). Jamila and Fernandez, (1995) stated that the gills of nickel exposed *Etropus maculatus* showed lifting of epithelial layer. The mitochondria were affected tremendously showing decrease in its number. Disruption of the bounding membranes and loss of cristae of the mitochondria were seen. Histopathological changes in mitochondria and alteration in the cholesterol was is discussed by Denise Fernandes, (2007) who exposed the male sea bass, *Dicentrarchus labrax* to different concentrations of endocrine disrupting compounds. He suggested that the disruption of the mitochondrial membranes reduced the ability of the mitochondria to synthesize ATP that led to an increase in permeability, which might account for their swollen appearance. Kashiwada *et al.*, 2002 also observed pathological changes in the gills of the fish. Swollen mitochondria with flocculent-appearing matrices and fragmented cristae were seen and some other related changes were induced distension of the number of lysosomes.

The smooth endoplasmic reticulum as well as rough endoplasm reticulum were seen in more numbers and with dilated cisternane. There is also infiltration of haemocytes in the gill lamellae, suggesting necrotic changes in the cells. Few lysosomes are also seen in the cytoplasm engaged in the phagocytic activity. Similar results were reported by Camargo, (2007) in his research work he analyzed
histopathology of the gills, kidney and liver of a neotropical fish caged in an urban stream.

Hepatopancreas regulates metabolic processes in the body of the crab and thus any changes occurring in the hepatopancreatic tissue is a reflection of cellular damage to the organ. In the present study, the hepatopancreas exhibited necrotic changes. The high content of many enzymes in the hemolymph may be due to its release from the hepatopancreas. Our ultrastructural studies are supported by biochemical studies which are in agreement.

Light microscopic structure of hepatopancreas shows prominent histopathological changes. The acinia showed irregular shape and enlarged in size. The lumen size is reduced with scanty secretory material. The connective tissue is also disintegrated and showed secretory cells with lot of vacuols. Similar changes were reported by Radhaiah and Jayantha, (1992). The light photomicrographs of hepatopancreas shows that in the control crabs the vitellogenin synthesis is not started, while in the hepatopancreas of experimental crabs treated with 50 ppm and 300 ppm Bisphenol A the hepatopancreas showed enlarged in the size of acinar cells with prominent nucleus and secretory apparatus. The light microscopic data and estimated protein concentration in these tissues for different sets correlate with increased protein synthesis and secretion. The crustacean hepatopancreas and the subepidermal adipose tissue (SAT) are the major sites of vitellogenin synthesis during vitellogenesis. The synthesized vitellogenin is transported through hemolymph to the ovaries where it is taken up during vitellogenesis II by endocytosis. The changes occurred in hepatopancreas were correlated with changes in the ovary.

The ultra structure of hepatopancreas of the crab, Paratelphusa jacquemontii exposed to 300 ppm of BPA for 60 days showed many degenerative changes in the cellular structure. Changes like transformation of rER into whorls and vesicles, swollen mitochondria, presence of myelin bodies indicating intracellular lysis and pyknotic nuclei were observed in the crab, Scylla serrata studied by Mayekar et al., 2003. Our study reports, there are various cells seen in the acina epithelium such as secretory cells, fibrilical cells, embryonic cells. The secretory cells are in the phase of active secretion showing the prominent spherical nucleus and nucleus with euchromatin and heterochromatin. The observations are supported by changes observed in Gammarus
fossarum exposed to different endocrine disrupting chemicals by Mazurová et al., (2010).

Histopathological changes in the testis exposed with 50 ppm and 300 ppm Bisphenol A for 60 days shows the alteration. The seminiferous tubules showed irregular pattern of shapes. The connective tissue between the seminiferous tubules is loosely arranged. It shows presence of phase of spermatogenesis as primary spermatocytes, secondary spermatocytes and spermatids. The testis also showed accumulation of some debris in the connective tissue. The seminiferous tubules also showed marked vacuolation. All the stages of spermatogenesis as primary spermatocytes, secondary spermatocytes, spermatids and darkly stained full grown sperms, demonstrate that endocrine disruptors can abolish effect on the histology of the testis. The results were supported by Paulo silva et al., 2012.

The ultra structure of testis of the crab, Paratelphusa jacquemontii exposed to 300 ppm of BPA for 60 days showed alteration in the frequencies of spermatogenic stages in testis in male crab as compared to control male. Spermatogenesis appeared to be inhibited, as testis were characterized by enlarged area of mature sperm and reduced percentage of spermatocytes, Similar results were observed in fish exposed to 2-ethyl hexyl 4-trimethoxycinnamate an inhibition of testicular development was shown.(Verna christen et al., 2011).

Testicular factors, epididymal dysfunction may be involved in abnormal sperm mortality. Chemicals like chlorohydrins, methyl chloride may be classified as epididymal toxicants, either having direct or indirect effect on the epithelium (Hess et al., 2011). Vocualization is a common indication of apoptosis in the interstitial tissue cells and sertoli cells in the testicular injury (Han, et al., 2004). Bisphenol A becomes a hurdle on normal sperm maturation. It is possible that it will interfere with normal sperm maturation. Bisphenol A might be differentially act on epididymal sperm. This was supported by study carried out by Balak with endocrine disrupters causes tail abnormalities that would be interfearing with sperm motility and thus by it could possibly compromise male fertility (Balak et al., 2004).

ALP is used as marker associated with germinal epithelium (Quan Bian et al., 2006). In this study activity of ALP was decreased significantly in both the concentrations and there are marked changes in LDH which is associated with germinal
epithelium and SDH linked with germ cell maturation. The alteration in the biochemical enzymes associated with testis were well supported by histopathological observations.

An inhibition of testicular development was reported for fish exposed to weak estrogen 4-nonylphenol, which lead to significant reduction in fecundity (Harrier et al., 2000). These data leads to conclusion that the histological effects of Bisphenol A in the testis indicate an overall estrogenic effects.

The histological studies of the ovaries exposed with 50 ppm and 300 ppm Bisphenol A for 60 days showed the presence of two distinct cell types, the follicle cells and the oocytes. The oocytes in the mature crabs during vitellogenesis period weigh down the ventral side of the ovary consequently throwing the germinal zones into folds. Follicle cells occur as disorderly strands in the early and spent stage of ovaries, during rapid vitellogenesis. However, they are applied closely to the oocyte periphery. Changes also includes appearing a follicular alersia, fibrosis and infilteration. This could be explained by the fact that ovaries of female crab had more vitellogenic and mature follicles. Follicles in an advanced maturity stage had a high probability of becoming atretic. Despite not having been unbaiedly quantified, increased ovarian follicle was reported in the studies where fish has been exposed to estrogen (Gray et al., 1999, Lange et al., 2001, Papouliase et al., 1999, Zillouy et al., 2001, Paula Silva et al., 2012).

Varadrajan and Subramaniam, 1980, revealed that acid mucopolysaccharide nature facilitates the passage of macromolecules into oocytes. Intimate association of follicle cells and oocytes has also been noted by electron microscopic study in Orchestia gammarella (Zerbib, 1973). The present investigation also showed that follicle cells in cytoplasm is hypertrophied mitochondria with lack of cristae with endoplasmic reticulum and Golgi are also hypertosphid suggesting that in the exposed crabs the vitellogenic process is disturbed and might have led to depleted synthesis and storage of vitellogenin. Resorption of oocyte lacking follicle cells suggests that the latter may even regulate vitellogenesis by apportioning available hemolymph proteins to only those which they encircle (Varadrajan and Subramanian, 1980).

Fetal primary ovaries are sensitive to Bisphenol A and exhibits alteration in both meiotic phase and follicle formation (Patriag et al., 2012). Formation of multi oocytes follicles that contain more than a single oocytes has been reported in a wide variety of
species (Vandenberg et al., 2010). Early oocyte growth must therefore be dependent upon ecdysterone. Adiyodi and Adiyodi, (1970) have reported that the hepatopancreas of the crab, Paratelphusa shows cyclic fluctuations in the hepatopancreatic free sugars related to ovarian cycle. The sugars present in the hepatopancreas of Paratelphusa vanishes progressively as the proteins in the ovary become conjugated in the course of yolk formation, suggesting that the sugars may be utilized mainly in the buildup of yolk formation, by the ovary and perhaps also as fuel during that process. The ovarian maturation in these cirripedes advances through several molt cycles suggesting that the naturally fluctuating ecdysteroids, correlated with the molt cycle do not have any such inhibitory effect on ovarian maturation (Fyhn and Costlow, 1997).

In crabs, the ovarian inhibiting hormone does not act alone to control the full course of ovarian development. When the Y-organs are removed from the young female, Carcinus oogonial mitosis almost cease and no oocytes develop (Arvy et al., 1954; Charniaux-Cotton, 1973). Ovarian growth has been induced by thoracic ganglion implantation, while injection of aqueous extracts from brain and thoracic ganglion as well as in vitro incubation has proved that vitellogenesis is stimulated in many crustaceans (Eastman-reks and Fingerman, 1984; Takayanagi et al., 1986; Kulkarni et al., 1991; Yano, 1992). Follicle formation is a complex involved instruction between growth factors and other signaling path way regulated by hormone. Link between oocyte cells cycle and follicle formation has been responsible for delayed meiotic cell cycle progression in response to Bisphenol A. The oocyte is known to control the rate of follicle growth (Pepling, 2012).

In the present study changes have been observed in the hepatopancreas and ovary of the female crabs exposed to Bisphenol A. In many crustaceans the ecdysteroids are shown to play a vital role both in vitellogenesis and in the maturation of gonads. It is known that in brachyuran crab species where the entire vitellogenesis and hence the ovarian maturation is completed in the intermoult period, when ecdysteroid is kept at very low level. Results are supported by Miyashitu et al., 2011.

4.3 Genotoxicity study:

The Allium test has often been used for the determination of cytotoxic or genotoxic effect of various substances (Grant, 1982; Samaka et al., 1996). It is considered to be a standard procedure for quick testing and detection of toxicity and
pollution level in the environment. Result of the *Allium* test may indicate the presence of certain cytotoxic/genotoxic or mutagenic substance in the environment, which represents the direct or indirect risks for all living organisms (El-Shahaby *et al*., 2003). Findings of the present study reflecting the utility of root meristem cells of *Allium cepa* for monitoring of the cytotoxicity level of test compound can be determined based on the increase or decrease in the Mitotic index (Seth *et al*., 2008) which can be used as a parameter of cytotoxicity studies for biomonitoring. MI lower than the negative control may indicate that growth and development of exposed organisms have been affected by the compound. The frequencies of chromosomal aberration increase with increasing concentration (Kumar and Paneerselvam, 2007). The difference between concentrations have been significant, when compared with negative control. The most frequent aberrations are chromosomal break, bridge formation, stickiness formation and laggarding chromosome formation. Inhibition of mitotic activities is often used for tracing cytotoxic substances. MI decrease below the negative control causes lethal effects on test organisms, while a decrease below 50% (cytotoxic limit value) usually has sublethal effects. With regard to the MI values in Table ---, it is clear that there is reduction in mitotic activity in the root tips of *A. cepa*. The data presented in Table --- shows the cytogenic effect of BPA. The number of abnormal cells and aberrations are dependent. The frequency of CA increase with increase in concentration. Same thing happens with the mitotic aberrations( MA). Plant system has a verity of well defined genetic end points and includes decrease in MI, chromosomal aberration, etc. BPA has decreased MI in the treatment group compared with the negative control at all concentrations. The higher sensitivity of the chromosomal aberration test in *A. cepa* can be explained by the fact that chromosomal aberrations are more closely associated with DNA damage (bridges, laggards), whereas the induction of micronuclei in root meristems of *A. cepa* is the manifestation of chromosome damage and disturbance of the mitotic process. The micronucleus is formed by a new membrane developing around the chromatin matter that failed to move to either pole during the anaphase of mitosis. Such chromatin matter arises either from anomalous disjunction of chromosomes due to spindle abnormalities or from breakage of chromosomes resulting in the formation of acentric fragments, dicentric chromosomes and chromatin bridges. Therefore, the aberration in chromosome suggests that the BPA may have been either spindle inhibitor or a clastogens.
The marked reduction in mitotic activity was registered with increase in concentration from 0.005% to 0.020%. Among the tests carried out with *A. cepa* chromosomal aberration provides important information and may be considered an effective test to investigate the genotoxicity potential of Bisphenol A. Genotoxicity effect of BPA in both end point-like CA/MA are possible. Significant and dose-dependent inhibition of root growth and MI, as observed in the present study, suggests that the exposure of BPA prevent cells to enter into cell division, which indicates the cytotoxic potential of BPA. The interaction of BPA with the proteins essential for cell cycle progression may be the cause of inhibition of MI. Reduction in mitotic activity could be due to inhibition of DNA synthesis (Schneiderman *et al.*, 1971; Sudhakar *et al.*, 2001) or blocking in the G2 phase of cell cycle preventing the cell from entering mitosis (Van’t Hof, 1968). Beu *et al.* (1976) have also shown that exposure of root tips of *V. faba* to high concentrations of the herbicide paraquat has led to inhibition of DNA synthesis. This suggests that BPA may cause inhibition of DNA synthesis (Kumar and Paneerselvam, 2007). Sifa, (2005) reported that chromosome bridges may be due to the chromosomal stickiness and subsequent failure of free anaphase separation or may be attributed to unequal translocation or inversion of chromosomal segments. Chemicals that induce chromosome breakage are known as clastogens and their action on chromosomes is generally regarded to involve an action on DNA (Grant, 1978; Chauhan *et al.*, 1990). Thus, the induction of chromosome breaks by BPA may be independent of its effect on the amount of DNA. Our findings are in agreement with earlier studies which reported inhibition of MI (Seth *et al.*, 2008; El-Shahaby, 2003; Carta *et al.*, 2008; Fiskesjo, 1985; Grover and Kaur, 1999).

In conclusion, as has been stated Bisphenol A has harmful effect on the meristem cells of *A. cepa*. BPA is found at below detectable level in the environmental matrices can have its effect on plants as well as on animal system and, therefore, this type of work can give a first alert of an environmental hazard and a large scale monitoring work using the plant bioassay can give idea to protect ecosystem including human being.