



STRESS RELATED HISTOPATHOLOGICAL CHANGES IN THE HEPATOPANCREAS OF BOTH THE SEXES OF PALAEMONID PRAWN *MACROBRACHIUM DAYANUM* (HENDERSON) (CRUSTACEA : DECAPODA)

RASHMI TRIPATHI

Department of Biosciences and Biotechnology, Banasthali University, Banasthali, Rajasthan.

Email.Id: tripathi.rashmi@gmail.com

ABSTRACT

The palaemonid prawns *Macrobrachium dayanum* (Crustacea: Decapoda) are important species found in river Gomti and are very good bioindicators. These prawns are ideal animals to study the histopathological impairments caused by the effect of heavy metals prominent in river Gomti. The test animals were exposed to LC₅₀ value (0.15 mg/l and 0.16 mg/l respectively) at acute exposure for 24, 48, 72 and 96 h and at subacute exposure 25% of 96 hr LC₅₀ values of CdCl₂ for males and females (0.0375 mg/l & 0.04 mg/l) respectively for 10, 20 and 30 day exposure. The histopathological changes were studied in both the sexes in the animals. Marked histopathological changes were noticed in hepatopancreas of *M. dayanum* after cadmium chloride exposure. At 96 h acute exposure, hepatopancreas showed vacuolization in epithelial cells, necrosis in tunica propria, increased number of migratory cells, granuloma in intertubular connective tissue along with karyorrhexis and karyolysis in cells. Necrotic and degenerative changes in hepatopancreatic epithelium, which were mainly, noticed in R and B cells. Loss of histological architecture along with the heavy influx of haemocytes was recorded at this stage. After sub-acute exposure large vacuoles, particularly R and B cells were observed along with hypertrophy, necrosis and degenerative changes at most of the places in hepatopancreatic epithelium. Karyorrhexis and pyknosis were common. Increased number of migratory cells in intertubular spaces was a peculiar feature at this stage. Almost complete loss of the architecture of hepatopancreas was noticed and about 90 % of the hepatopancreatic tubules were found non-functional. The histopathological alteration in males was more pronounced than in females, both at acute and sub-acute exposure.

Key words: Hepatopancreas, Environmental stress, Cadmium chloride, Histopathological changes, *Macrobrachium dayanum*.

INTRODUCTION

The agricultural, industrial and many other man made activities are a major source of heavy metal pollution to the aquatic environment. Heavy metals required in trace amounts by the organisms but in excess or higher concentration are detrimental (Lorenzon *et al.*, 2000). The heavy metals important as a source of pollution are hazardous to target aquatic animals of economic importance (Chourpagar & Kulkarni, 2011). Crustaceans form an important link in the food chain and being sensitive to heavy metal toxicity play an important role in the study of heavy metal toxicity. Hepatopancreas being responsible for important metabolic processes plays a very important role in the detoxification activities and results in histological alterations on being exposed to xenobiotics (Manisseri and Menon, 1995 & 2006; Wu *et al.*, 2008). To assess the stress response to xenobiotics the histopathological techniques are very rapid, sensitive, reliable, and inexpensive. Hepatopancreas in prawns is recognized as an important target organ for studying the effects of heavy metal pollution, possessing the

capability of detoxifying heavy metals and displays considerable cytological cytochemical and ultra structural alterations at chronic exposure to low levels of heavy metals. Crustaceans form a group of animals, which are frequently sensitive to heavy metals and act as very good bioindicators (Manisseri & Menon, 1995; Sreeram & Menon, 2005).

MATERIALS AND METHODS

The fresh water prawn, *Macrobrachium dayanum* (Henderson) (Crustacea – Decapoda), were collected from river Gomti at different localities in and around Lucknow (U.P.) India and brought to the laboratory (N-26°5'59" E-80°56'17"). The animals were maintained in water having following physio-chemical characteristics (APHA, 1998; Sharma & Shukla, 1990). pH - 7.66 ± 0.27; Temperature - 27.66 ± 0.66°C; Partial Alkalinity - 18.75 ± 3.75 mg/l; Total Alkalinity - 425 ± 11.36 mg/l; Total Hardness - 268 ± 2.67 mg/l; Dissolved Oxygen - 6.6 ± 0.74 mg/l. Animals before being used for experimental purpose were acclimated to laboratory conditions for at least 5-7 days. The male animals having an average length - 4.86 ± 0.18 cm; average weight - 0.92 ± 0.06 gm



Dr. Rashmi Tripathi did her Ph.D. in the field of aquatic toxicology from Lucknow University and joined Banasthali University as Assistant Professor in 2010. She is engaged in taking classes at both PG and UG levels and plans to pursue research in Aquatic Toxicology. She has several research papers to her credit in journals of repute and presented papers at international and national conferences. She is the proud recipient of BPA (Best Poster Appreciation Award) at the 22nd Annual Session of the Academy of Environmental Biology, Award for the Best Presentation 2-3rd February 2012 Guru Ghasidas Central University Bilaspur, Award for the Best Oral Presentation 11th-13th March, Dayal Singh College, Karnal. She has also supervised seven research students for dissertation under her guidance.

and females having an average length- 3.12 ± 0.14 cm, average weight - 0.52 ± 0.03 gm) is used for the experimental purposes. For short-term toxicity, tests 96 hours LC_{50} values of $CdCl_2$ for male and female *M. dayanum* (0.15 mg/l and 0.16 mg/l respectively) is used in present study to evaluate histopathological studies. At the intervals of 24, 48, 72 and 96 hours the observations is studied. For sub-acute toxicity tests, 25% of 96hr LC_{50} values of $CdCl_2$ for males and females (0.0375 mg/l & 0.04 mg/l respectively) are used in present study. For histological observations, hepatopancreas is used as target organs in males and in female animal. Tissues were carefully dissected out under the stereo binocular on desired exposure time (24, 48, 72 & 96 h and 10, 20 & 30 day exposure) from both experimental and control animal. Tissues were washed in Crustacean Ringer's Solution and fixed in alcoholic Bouin's fluid for 24 h. Fixation was followed by routine dehydration with ethyl alcohol grades; clearing and embedding in paraffin wax ($58-60^\circ C$). The $5-6\mu$ thick sections were serially arranged and flattened on albumenized slides. The sections stained with Harris's Haematoxyline and Eosin stained were studied and photographed on Olympus microscope comparing with controls.

RESULTS

Marked alterations were observed in the hepatopancreas after exposure to acute concentration of cadmium chloride and the changes in males were more prominent than in females when compared with control (Figs- 3,4,5 & 6 and Fig-1&2) Vacuolization appears to be very prominent, hyperplasia and hypertrophy was seen. Necrotic and degenerative changes noticed were greater in males than in females. Nuclear pyknosis was also observed. Granular depositions was found common in intertubular connective tissue after 24 h exposure. After 48 h, of exposure Necrosis and degenerative changes in the cells became common in intertubular connective tissue and were more prominent than in females. Pyknotic nuclei were also common.

Haemocyte influx was found increased further. After 72 h, exposure granular deposition was prominent. Necrotic and degenerative changes were evident at this stage, showing cellular damage. Karyolysis and karyorrhexis were seen. The lumen of hepatopancreatic tubules were found filled with cell exudates and broken nuclei. Severe effects were noticed mainly in R & B cells of the hepatopancreatic tubules. Haemocyte influx in intertubular spaces was found increased and tunica propria of most of the tubules was found broken due to degenerative changes. After 96 h, complete loss of architecture of hepatopancreas was observed due to necrosis, lysis and degenerative changes in hepatopancreatic tubules. Haemocyte influx was reduced. Karyolysis and karyorrhexis were seen. About 90% tubules were broken due to severe degenerative changes leaving tissue debris and broken nuclei in intertubular connective tissue. Acute exposure of cadmium chloride showed marked alterations in the hepatopancreas of female prawn (Figs-7, 8, 9 & 10) Vacuolization begins to appear, hyperplasia and hypertrophy was observed. Necrotic and degenerative changes were seen. Nuclear pyknosis was observed in some of the cells. Granular depositions was found common in intertubular connective tissue after 24 h exposure. After 48 h of exposure, vacuolization became prominent and increased. Necrosis and degenerative changes in the cells became common in intertubular connective tissue, along with broken tissue and pyknotic nuclei were common. Haemocyte influx was increased further. After 72 h, exposure granular deposition was prominent. Necrotic and degenerative changes were evident at this stage, showing cellular damage. Karyolysis and karyorrhexis were seen at most of the places. The lumen of hepatopancreatic tubules were found filled with cell exudates and broken nuclei. Severe effects were noticed mainly in R & B cells of the hepatopancreatic tubules. Haemocyte influx in intertubular spaces was found increased and tunica propria of most of the tubules was found broken due to degenerative changes. After 96 h, complete loss of architecture of hepatopancreas was observed due to necrosis, lysis and degenerative changes in hepatopancreatic tubules. Separation of tubular epithelium was common. Haemocyte influx was reduced. Karyolysis and karyorrhexis were seen. About 90% tubules were broken due to severe degenerative changes leaving tissue debris and broken nuclei in intertubular connective tissue. Cadmium chloride severely damaged the hepatopancreas in *M. dayanum* on its sub-acute exposure (Figs-11, 12 & 13). After 10 day exposure vacuolization in B and R cells of the hepatopancreatic tubule was observed. Hypertrophy, degenerative and necrotic changes were observed in tubular epithelium. Haemocyte influx was also observed. After 20 day exposure vacuolization, degenerative changes and necrotic changes were

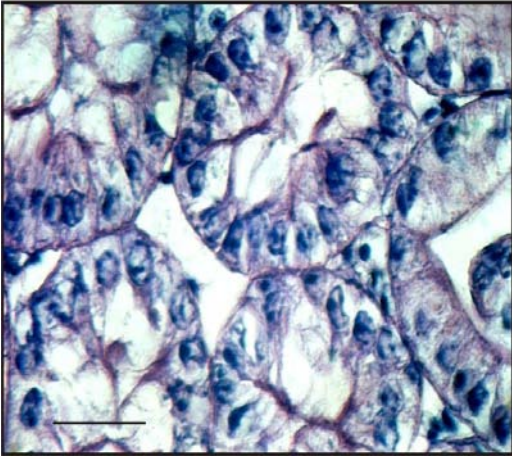


Figure-1: Photomicrographs of T.S. of Hepatopancreas of M.dayanum Male (Control : Scale Bar: 50 μ m)

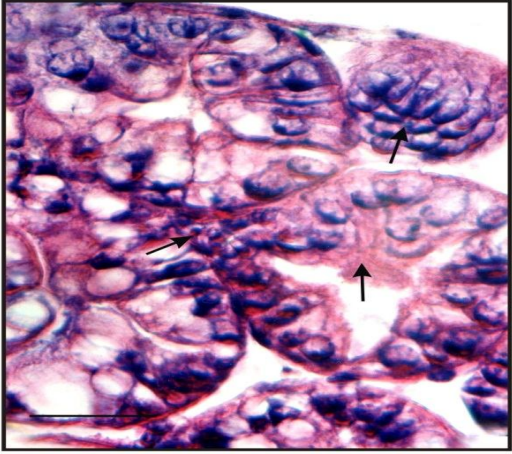


Figure- 3: Photomicrographs of T.S. of Hepatopancreas of male M.dayanum showing acute exposure (24h) of cadmium chloride exposure (Scale Bar: 50 μ m)

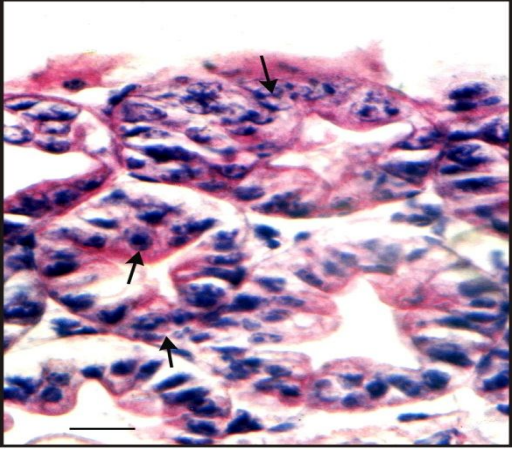


Figure-5: Photomicrographs of T.S. of Hepatopancreas of male M.dayanum showing acute exposure (72h) of cadmium chloride exposure (Scale Bar: 50 μ m)

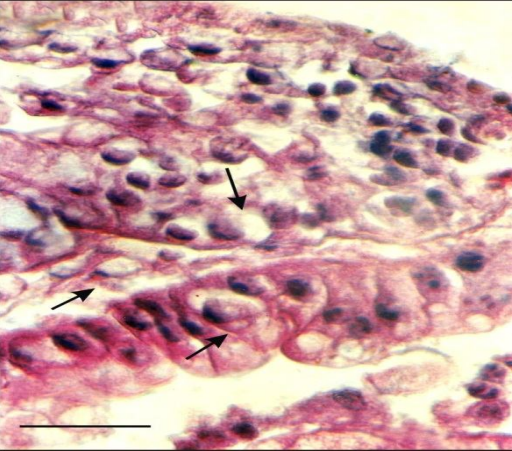


Figure-7: Photomicrographs of T.S. of Hepatopancreas of Female M.dayanum showing acute exposure (24h) of cadmium chloride exposure (Scale Bar: 50 μ m)

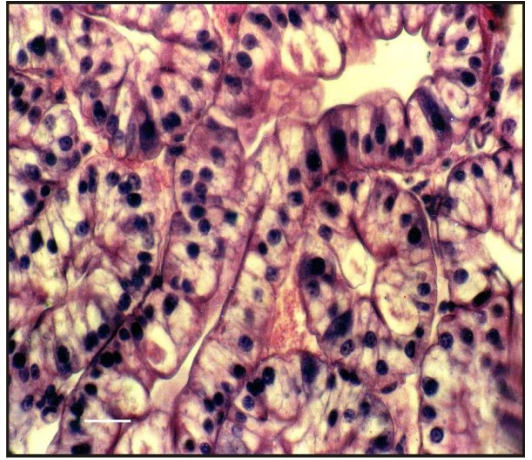


Figure-2: Photomicrographs of T.S. of Hepatopancreas of M.dayanum Female (Control : Scale Bar: 50 μ m)

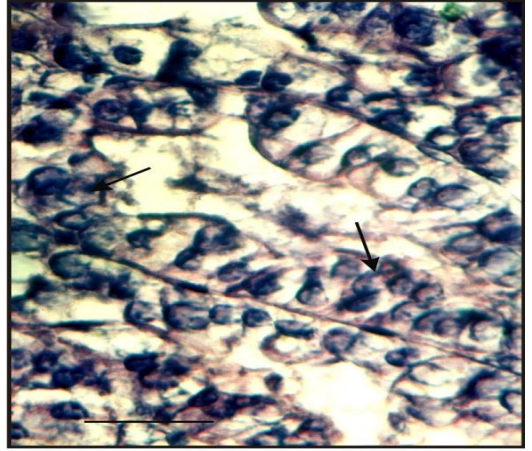


Figure-4 Photomicrographs of T.S. of Hepatopancreas of male M.dayanum showing acute exposure (48h) of cadmium chloride exposure (Scale Bar: 50 μ m)

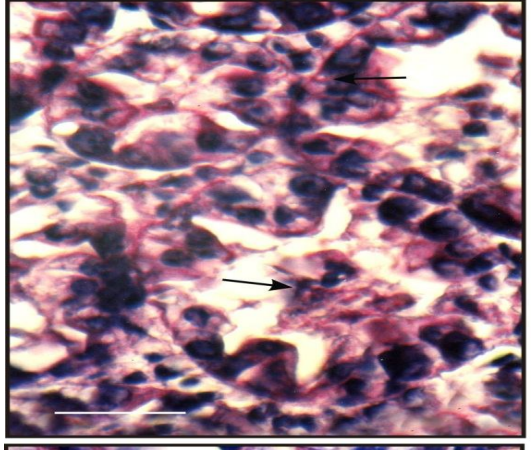


Figure-6: Photomicrographs of T.S. of Hepatopancreas of male M.dayanum showing acute exposure (96 h) of cadmium chloride exposure (Scale Bar: 50 μ m)

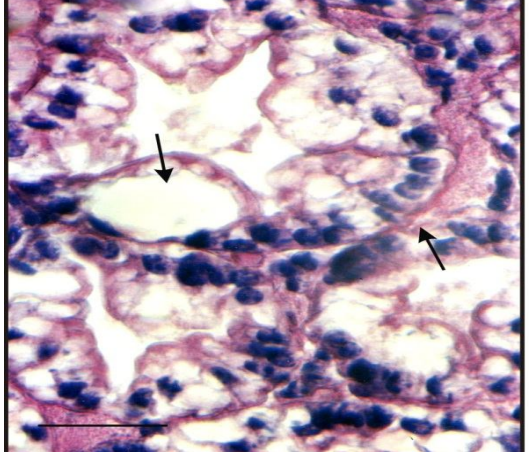


Figure-8: Photomicrographs of T.S. of Hepatopancreas of Female M.dayanum showing acute exposure (48h) of cadmium chloride exposure (Scale Bar: 50 μ m)

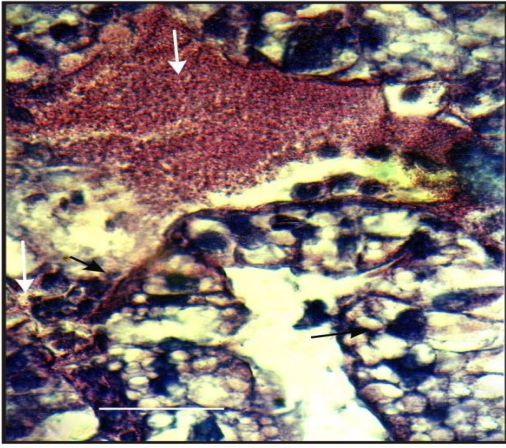


Figure-9: Photomicrographs of T.S. of Hepatopancreas of Female M.dayanum showing acute exposure (72h) of cadmium chloride exposure (Scale Bar: 50 μ m)

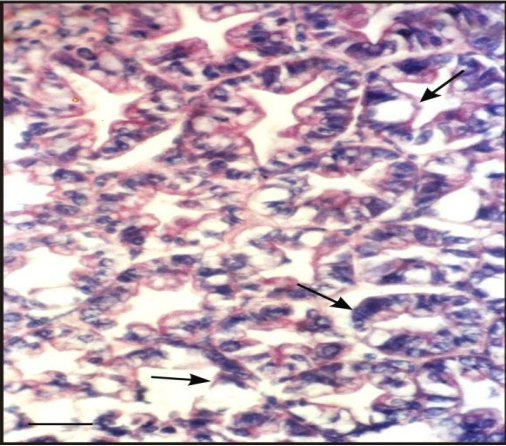


Figure-11: Photomicrographs of T.S. of Hepatopancreas of Male M. dayanum showing sub-acute exposure (10day) of cadmium chloride exposure (Scale Bar: 50 μ m)

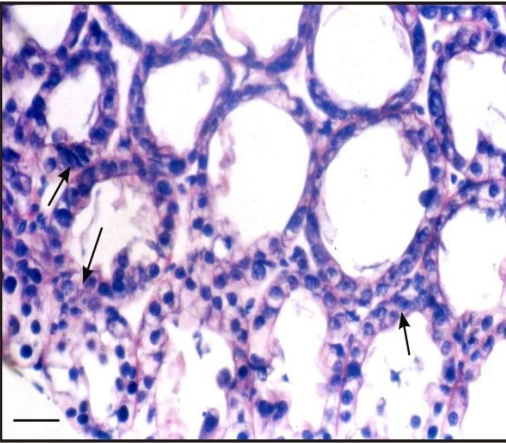


Figure-13: Photomicrographs of T.S. of Hepatopancreas of Male M. dayanum showing sub-acute exposure (30day) of cadmium chloride exposure (Scale Bar: 50 μ m)

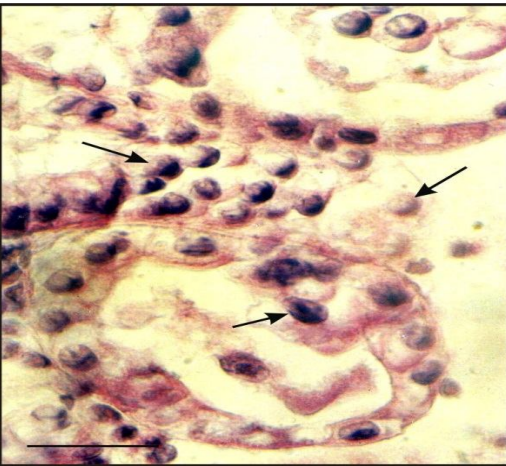


Figure-15: Photomicrographs of T.S. of Hepatopancreas of Female M. dayanum showing sub-acute exposure (20day) of cadmium chloride exposure (Scale Bar: 50 μ m)

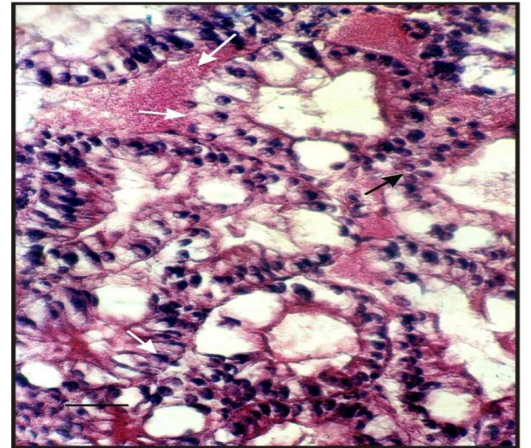


Figure-10: Photomicrographs of T.S. of Hepatopancreas of Female M. dayanum showing acute exposure (96h) of cadmium chloride exposure (Scale Bar: 50 μ m)

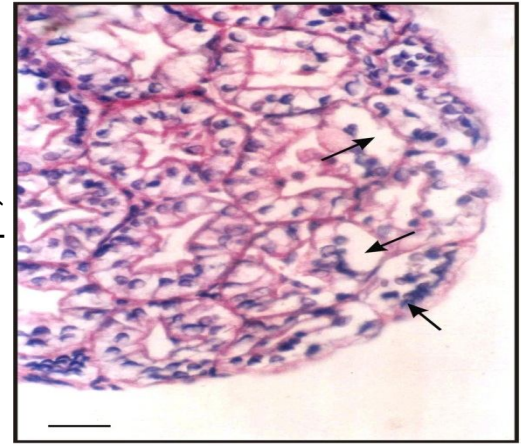


Figure-12: Photomicrographs of T.S. of Hepatopancreas of Male M. dayanum showing sub-acute exposure (20day) of cadmium chloride exposure (Scale Bar: 50 μ m)

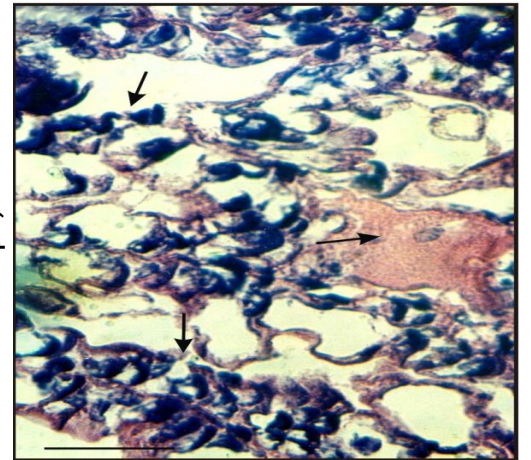


Figure-14: Photomicrographs of T.S. of Hepatopancreas of Female M. dayanum showing sub-acute exposure (10day) of cadmium chloride exposure (Scale Bar: 50 μ m)

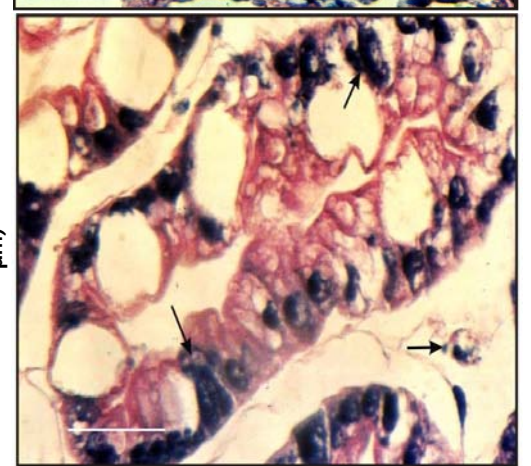


Figure-16: Photomicrographs of T.S. of Hepatopancreas of Female M. dayanum showing sub-acute exposure (30day) of cadmium chloride exposure (Scale Bar: 50 μ m)

observed. Pyknosis and karyolysis were common. Breakage of tunica propria was observed due to necrosis and lysis. Degenerative changes were observed in epithelial cells. Broken tissue debris was noticed in the lumen of hepatopancreatic tubules. Intertubular space was filled with granular depositions. Wandering cell number increased in the intertubular spaces. Whereas, after 30 day exposure almost 90% of the hepatopancreatic tubules lost their normal cellular organization due to necrosis and degenerative changes. Tubules were in the form of broken tissue mass containing haemocytes and broken nuclei showing karyorrhexis. Granular deposition in tubules was highly pronounced. Hepatopancreas of female *M. dayanum* (Figs -14, 15 & 16) was severely damaged on being exposed to sub acute exposure of cadmium chloride. After 10-day exposure, vacuolization in B and R cells of the hepatopancreatic tubule was observed. Tubular epithelium showed hypertrophy, degenerative and necrotic changes at some places. Haemocyte influx was also observed. After 20-day exposure vacuolization, degenerative changes and necrotic changes were observed. Large vacuoles were observed in epithelial cells. Pyknosis and karyolysis were common. Breakage of tunica propria was observed due to necrosis and lysis. Degenerative changes were observed in epithelial cells. Broken tissue debris was noticed in the lumen of hepatopancreatic tubules. Intertubular space was filled with granular depositions. Wandering cell number increased in the intertubular spaces. After 30 day exposure, almost 90% of the hepatopancreatic tubules lost their normal cellular organization due to necrosis and degenerative changes. Most of the tubules were in the form of broken tissue mass containing haemocytes and broken nuclei showing karyorrhexis. Granular deposition in tubules was highly pronounced and haemocyte influx was found increase in comparison to 20 day exposure. Most of the haemocytes were found deformed.

DISCUSSION

Hepatopancreas of crustaceans not only perform important role in secretion, absorption, and storage of food but also acts for metabolism of xenobiotics and their detoxification (Vonk, 1960; Gibson & Barker, 1979; Vogt & Quintio, 1994). Vacuolization, hypertrophy of tubular epithelial cells, granuloma in connective tissue, pronounced cell shedding in lumen, necrosis, karyolysis and increase in wandering cells population were the major histopathological alterations after acute exposure of cadmium. Vacuolization, hypertrophy, cell shedding in lumen, degeneration of tubules, increased interstitial cells, granuloma, thinning of tubular wall, Karyolysis and karyorrhexis were chief changes after acute and sub acute exposure of cadmium chloride. Haemocyte aggregation and brown to black colored granular deposition in tunica propria, hypertrophy, increased number of haemocytes and nuclear pyknosis

were the peculiar features after cadmium exposure in *M. dayanum*. The histopathological changes observed in present study are almost similar to the reports of other workers on fishes (*Anguilla anguilla*, *Heteropneustes fossilis*, *Garramullya*, *Tilapia mossambica*, *Channa punctatus*, *Bariliusvogra*, *Sarotherodon mossambicus*, *Anabas scandens*, *Clarias batrachus*, *Senegales sole*, *Soleasenegalensis*) after exposure to Cadmium, (Neol-lambat *et al.*, 1978; Gupta & Rajbanshi, 1982; Wani & Latey, 1983; Rani & Ramamurti, 1989; Ghosh & Chakrabarti, 1993; Cope *et al.*, 1994; Kumari & Kumar, 1995; Akram *et al.*, 1999; Bae, 1999; Naidu *et al.*, 1983; Ghosh & Chatterjee, 1985; Sastry & Tyagi, 1982; Venugopal & Reddy, 1992; Misra & Singh, 1997; Kumari & Kumar, 1997; Ray & Banerjee, 1998; Arellano *et al.*, 1999). Crustacean hepatopancreas has also shown similar histopathological effects as reported by Hopkin & Nott, (1979) in *Carcinus maenas*; Papathanassiou & King, (1986) in prawn *Palaemon serratus*; Narayanan *et al.*, (1994) in *Scylla serrata*; Guarino *et al.*, (1974) in *Idotea batlica*; Doughtie & Rao, (1984) in grass shrimp *Palaemonetes pugio*; Anderson & Batraup, (1988) in *Crangon crangon*; Krishnamoorthy & Subramaniam, (1996) in *Macrobrachium lamarrei*. The findings of present investigations are also similar to exposure of various toxicants on crustaceans and other invertebrates (Nagabhushnam *et al.*, 1987; Chandy & Kolwalker, 1984; Aiken & Beyard, 1972; Lajtner *et al.*, 1996). The cell shedding in lumen of hepatopancreatic tubules as observed in present study in case of both the metals is probably a indication of stress as indicated by Lozzi, (1971) and Gibson and Barker, (1979) where they emphasized that cell shedding in holocrine manner occurs during prolonged starvation and stress. The aggregation of haemocytes may be due to alterations in cell membrane leads to sequestration of hemocytes. As it is well known that due to similar ionic radii, Cadmium replaces Ca^{++} on the phospholipid headgroups of plasma membrane (Schaltz & Marinetti, 1972). Such similar and other alterations may be the reason for haemocyte aggregation but needs further confirmation. The hyperactivity of hepatopancreatic cells, nuclear pyknosis and chromatin condensation observed in present study might be due to hyper activity induced by metals as reported in from of inclusion formation and metal complexing with nuclear proteins (Lucas, 1942; Goyer *et al.*, 1970; Sorensen *et al.*, 1982).

Heavy metals accumulate in the biological system particularly in fishes and in soft tissues in decapod crustaceans (Gibson & Barker, 1979; Dall & Moriarty, 1983; Rainbow, 1988). Metal uptake, transport, distribution, sequestration within the body and metal excretion, these all process define metal accumulation strategy in invertebrates (Rainbow & White, 1989; Rainbow *et al.*, 1990; Rainbow & Dellinger, 1993). Metals not required by the animal even in traces include lead, cadmium, Mercury Rand, *et al.*, (1985). These metals are absorbed and accumulated in the soft tissues of organism

(Bryan, 1979; Rainbow *et al.*, 1990), and these required to be detoxified to avoid the toxicity. There are two possible mechanisms for the detoxification. Heavy metals can either bound to insoluble inert metaliferous granules (Mason & Nott, 1981; Brown, 1982; Taylor & Simkiss, 1982) or bind to soluble metal binding ligands such as metallothioneins (Olafson *et al.*, 1979; Engel & Brouwer, 1989; Roesijadi, 1992; Curtis, 2004). Vijayram & Geraldine, (1996) reported well pronounced metal regulation in marine crustaceans, lacking in fresh water; thus the freshwater crustaceans showed severe toxicological effects for metals, and this might be the reason for heavy tissue damage. Freshwater prawns due to the weaker metal regulation mechanism and higher metal accumulation tendency provide an indication of toxicity and therefore serve as a good biological indicator of metal pollution (Philips & Rainbow, 1993; Jenkins, 1980; Mushak, 1980; Anderson & Baatrup, 1988). Metallothioneins are as such a protective device, protecting cell from toxicity by binding with metals. When accumulates in excess binding with free metallic ions becomes highly toxic and lead to severe tissue damage (Webb & Etienne, 1977; Nordberg, 1978; Martinez *et al.*, 2002). Metallothioneins can bind about seven cadmium ions and this protects against toxicity and once metallothioneins saturate, cadmium is available to bind to other protein thiols leading to toxicity reported by Curtis, (2004). The tolerance to the metals accumulated in the animals is because of the active detoxification mechanism by the trapping of incoming metals by ligands such as metallothioneins in the cytosol (Viarengo, 1989). Metallothioneins are synthesized in the aquatic organism as a defense against the toxic metals (Couillard *et al.*, 1993). Metallothioneins considered central in the regulation of metals such as Cadmium, Copper and Zinc. Increased synthesis of metals is associated with increased capacity to bind with the metals leading to detoxification against metal toxicity (Roessijadi, 1992).

ACKNOWLEDGEMENT

Thanks are due to Prof K.C. Pandey, D.Sc. Head, Department of Zoology, Lucknow University, Lucknow, for providing the laboratory facilities.

REFERENCES

- Aiken, D.F. and Beyard, E.H. (1972): Histopathological changes in lobster *Homarus Americano* exposed to yellow phosphorous. *Sc. 76*: 1434-1435.
- Akram, M.; Hafeiz, M.A. and Nabi, G. (1999): Histopathological changes in the kidney of a fresh water cyprinid fish *Barilius vogra* following exposure to cadmium. *Pak. J. Zool.* 31 (1): 77- 80.
- Andersen, J.T. and Baatrup, E. (1988): Ultrastructural localization of mercury accumulation in the gills hepatopancreas, midgut and antennal glands of the brown shrimp, *Crangon crangon*. *Aquat. Toxicol.* 13: 309-324.
- APHA. (1998): Standard Methods for the examination of water and waste waters. 20th Edn. APHA, AWWA & WPCF Washington.
- Arellano, J.M.; Storch, V. and Sarasquete, C. (1999): Histological changes and copper accumulation in liver and gills of the *Senegales sole, Solea senegalensis*. *Eco. Toxicol. Environ. Safety.* 44: 62-72.
- Bae, I.H. (1999): Effects of zinc and cadmium on ultrstructure of hepatocyte of fish, *Acheilognathus yamatsutae*. *Korean, J. Limnol.* 32 (4): 295-302.
- Brown, B.E. (1982): The form and function of metal containing granules in Invertebrates' tissue. *Biol. Rev.* 57: 621-667.
- Bryan, G.W. (1979): Bioaccumulation of marine pollutants. *Phil. Tran. R. Soc. Lond.* B286: 483-485.
- Chandy, J.P. and Kolwalker, D.G. (1984): Histological changes in the gill and hepatopancreas of the marine crabs, *Charybdis lucifera* (Fabricin) and *Scylla serrata* (Forsk.) exposed to crude oil emulsion. *Indian J. Mar. Sci.* 13: 10-13.
- Chourpagar, R.A. and Kulkarni, K.G. (2011): Heavy metal toxicity to a fresh water crab, *Barytelphusa cunicularis* (Westwood) from Aurangabad region. *Rec Res in Sci and Tec* 3(3): 01-05
- Cope, W.G.; Weiner, J.G. and Atchison, G.J. (1994): Hepatic cadmium, metal-binding proteins and bioaccumulation in bluegills exposed to aqueous cadmium. *Environ. Toxicol. Chem.* 13 (4): 553-562.
- Couillard, Y.; Campbell, P.G.C. and Tessier, A. (1993): Response of metallothionein concentrations in a freshwater bivalve (*Anodonta grandis*) along an environmental cadmium gradient. *Limnol. Oceanogr.* 38(2): 299-313.
- Curtis. and Lawrence, R. (2004): Validating Modeling Parameters for risk assessment of metals in fertilizers. <http://www.atsdr.cdc.gov>
- Dall, W. and Moriarty, D.J.W. (1983): Functional aspects of nutrition and digestion. In: *The biology of crustacea*, Vol.5: Internal anatomy and physiological regulation (ed. L.H. Mantel), Academic press, New York. pp. 215-261
- Doughtie, D.G. and Rao, K.R. (1984): Histopathological and ultra structural changes in the antennal gland, midgut, hepatopancreas, and gill of grass shrimp following exposure to hexavalent chromium. *J. Invert. Pathol.* 43 (1): 89-108.
- Engel, D.W. and Brouwer, M. (1989): Metallothionein and metallothionein like proteins: Physiological important. *Adv. Comp. Environ. Physiol.* 5: 53.
- Ghosh T. K. and Chatterjee, S.K. (1985): Effect of Chromium on tissue energy reserve in a fresh water fish *Sarotherodon mossambicus*. *Environ. Ecol.* 3(2): 178-179.
- Ghosh, A.R. and Chakrabarti, P. (1993): Histopathological and histochemical changes in liver, Pancrease and kidney of the freshwater, *Heteropneustes fossilis* (Bloch) exposed to Cadmium. *Environ. Ecol.* 11(1): 185-188.
- Gibson, R. and Barker, P.I. (1979): The decapod hepatopancreas. *Oceanogr. Mar. Biol. Ann. Rev.* 17: 285-346.
- Goyer, R.A.; May, P.; Cates, M. and Krigmam, M.R. (1970): Lead and protein content of isolated intranuclear inclusion bodies from kidney of lead poisoned rats. *Lab. Invest.* 22: 245-251
- Guarino, S.M.; Guarino, F.M.; Tommonaro, G. and Nicolade, M. (1974): Cadmium induced lipofuscins and effect of zinc on hepatopancreas cells in *Idotea baltica*. *Experientia.* 51(9): 967-969.
- Gupta A.K. and Rajbanshi, V.K. (1982): Cytopathological studies resulting in Cadmium bioassay with *Heteropneustes fossilis* (Bloch) *Acta. Hydrochim. Hydrobiol.* 10(4): 345-351.
- Hopkins, P. and Nott, J.A. (1979): Studies on the digestive cycle of the shore crab, *Carcinus meanas* with special reference to the 'B' cell in the hepatopancreas. *J. Mar. Biol. Assoc. U.K.* 60: 891-907.
- Jenkins, D.W. (1980): Biological monitoring of toxic trace metals. Volume 2. Toxic trace metals in plants and animals of the world. Part II. Mercury. U.S. Environ. Protection Agency Rep. 600/3-80-09 1: 779-982.
- Lorenzon, S.; Ferrero, M. and Ferrero, E.A. (2000): Heavy metal toxicity and differential effects on the hyperglycemic stress response in the shrimp *Palaemon elegans*. *Arch. Environ. Contam. Toxicol.* 39: 167-176
- Mushak, P. (1980): Metabolism and systemic toxicity of nickel. pp 499-523 in J.O. Niragu, editor. *Nickel in the environment*. John Wiley and sons, New York.
- Krishnamoorthy, P. and Subramanian, P. (1996): Effect of sublethal

- dose of copper on the hepatopancreas of the fresh water prawn, *Macrobrachium lamarrei lamarrei*. *Geobios*.23: 16-18.
- Kumari, A.S. and Kumar, S.R. (1995): Effect of water pollution on the histology of fish *Channa punctatus* in Hussainasagar. Hyderabad. *Environ. Ecol.* 13 (4): 932-934.
- Kumari, S.A. and Kumar, N.S. R. (1997): Histopathological alterations induced by aquatic pollutant in *Channa punctatus* from Hussainasagar Lake (A-P) J. *Environ. Pathol.* 18(1): 11-16.
- Lajtner, J.; Erben, R. and Klobucar, G.V.I. (1996): Histopathological effect of phenol on the digestive gland of *Amphemelaniaholandrifer*. *Bull. Environ. Contam. Toxicol.*57: 458-464.
- Lozzi, R.F. (1971): Interpretation of crayfish hepatopancreatic function based on fine structure analysis of cell lines and muscle network. *Z. Zelforseh. Mikrosk. Anat. Bal.*1135: 420-440.
- Lucas, A.M. (1942): Effect of centrifugation on intranuclear inclusions produced by subcutaneous injection of Aluminum Oxide. *Amer.J.Pathol.* 18: 1051-1057.
- Manisseri, Mary.K. & Menon, N.R. (1995): Copper-induced damage to the hepatopancreas of the penaeid shrimp *Metapenaeus dobsoni*- an ultrastructural study. *Dis Aquat Org.* 22: 51-57
- Manisseri, Mary.K. & Menon, N.R. (2006): Ultrastructural aberrations in the hepatopancreas of *Metapenaeus dobsoni*(Miers) exposed to mercury. *J. Mar. Biol. Ass. India.* 48(1):89-94
- Martinez, T.L.; Gomez, O.; Galar, M. and Lopez, E. (2002): Stress produced by contaminated sediments with nickel in a pond with rainbow trout *Oncorhynchus mykiss* (Pisces: Salmonidae). *Rev. Biol.Trop.* 50(3-4): 1159-1168.
- Mason, A.Z. and Nott, J.A. (1981): The role of intra cellular biomineralised granules in the regulation and detoxification of metals in gastropods with special reference to the marine Prosobranch *Littorinalittorea*. *Aquat. Toxicol.*1: 239-256.
- Misra, R. and Singh, S.D. (1997): Histopathological studies on the stomach and liver of *Clarias batrachus* due to Lead Nitrate and dichromate. *Environ. Ecol.* 15(3): 641-616.
- Nagabhushanam, R.; Rao, K.S. and Sarojini, R. (1987): Histopathological changes in the gill and hepatopancreas of the marine crab, *Scylla serrata* induced by an organophosphate Dimecron. *J. Adv. Zool.* 8(1): 46-51.
- Naidu, A.K.; Naidu, A. and Ramamurthi, R. (1983): Histological alterations in liver and intestine of teleost *Sarotherodon mossambicus* in response to mercury toxicity. *Ecotoxicol. Environ. Saf.*7: 566-575.
- Narayanan, K. R.; Khan, S.A. and Pechimuthu, S. (1994): Histopathological changes due to effect of sublethal concentration of Copper sulphate on the hepatopancreas of edible crab, *Scylla serrata*. *J. Environ. Boil.* 15(4): 289-293
- Noel - Lambat, F.; Gerday, C.H. and Distech, A. (1978): Distribution of Cd, Zn, & Cu in liver and gills of the eel. *Anguilla anguilla* with special reference to metallothioneine. *Comp.Biochem.Physiol.*61c: 177-187.
- Nordberg, M. (1978): Studies on metallothionein and Cadmium. *Environ. Res.*15: 381-404.
- Olafson, R.W.; Kearns, A. and Sim, R.G. (1979): Heavy metal induction of metallothionein synthesis in the hepatopancreas of the crab *Scylla serrata*. *Comp.Biochem. Physiol.* 62(4): 417-424.
- Papathanassiou, E. and P.E. King (1986): Ultrastructural changes in the hepatopancreatic cells of the prawn *Palaemon serratus* induced by exposure to acute toxic cadmium concentrations. *Dis. Aquat. Org.*2: 39-47.
- Phillips, D.J.H. and Rainbow, P.S. (1993): Biomonitoring of trace aquatic contaminants. Elsevier Applied Science. 371pp.
- Rainbow, P.S. and Dallinger, R. (1993): Metal uptake, regulate & excretion in freshwater invertebrates. In: *Ecotoxicology of metals in invertebrates* (eds. R. Dallinger & P.S. Rainbow). Denis Publishers, Florida, pp. 119-131.
- Rainbow, P.S. and White, S.L. (1989): Comparative strategies of heavy metal accumulations by crustaceans: Zinc, copper and cadmium in a decapod, an amphipod and barnacle. *Hydrobiologia.*174: 245-246.
- Rainbow, P.S.; Phillips, D.J.H. and Depledge, M.H. (1990): The significance of trace metal concentration in marine invertebrates: a need for laboratory investigation of accumulation strategies. *Mar.Pollut. Bull.* 21: 321-322.
- Rainbow, P.S. (1988): The significance of trace metal concentrations in decapods. *Symp. Zool. Soc. Lond.* 59: 291-313.
- Rand, G.M. and Petrocelli, S.R. (1985): *Fundamentals of aquatic toxicology (methods and applications)*. Hemisphere publishing corporation, Washington.
- Rani, A.U. and RamMurthi, R. (1989): Histopathological alteration in the liver of fresh water teleost *Tilapia mossambica* in response to Cadmium toxicity. *Ecotoxicol. Environ. Saf.*17: 221-226.
- Ray, D. and Banerjee, S.K. (1998): Hepatic toxicity of Ni to *Clarias batrachus* exposed to nickel sulphate solution. *Environ. Ecol.* 16(1): 142-146.
- Roessijadi, G. (1992): Metallothioneins in metal regulation and toxicity in aquatic animals. *Aquat. Toxicol.*22: 81-114.
- Sastry, K.V. and Tyagi, S. (1982): Toxic effects of Chromium in a fresh water teleost fish, *Channa punctatus*. *Toxicol. Lett.*11: 17-21.
- Schultz, L. and Marinetti, G.V. (1972): Cadmium binding to the rat liver plasma membrane. *Biochem.Biophys.Acta.*290: 70-83.
- Sharma, U.D. and Shukla, S. (1990): Behaviour dysfunction of fresh water prawn, *Macrobrachium lamarrei* (Crustacea - Decapoda) following exposure to synthetic detergent, linear alkyl Benzene Sulphonate. *Biol. Mem.* 16(12): 58-61.
- Sorensen, E.M.B.; Smith, N.K.R. and Ramirez Michehel, R. (1982): Electron Prob. x-ray microanalysis of Arsenic inclusions in fish. *Arch. Environ. Contam. Toxicol.*11: 469-473.
- Sreeram, M.P. and Menon, N.R. (2005): Histopathological changes in the hepatopancreas of the penaeid shrimp *Metapenaeus dobsoni* exposed to petroleum hydrocarbons. *J. Mar. Biol. Ass. India* 47(2):160-168
- Taylor M.G. and Simkiss, K. (1982): Structural and analytical studies on metal ion containing granules, in the chemical perspective in Bio mineralization, Mann, s, webb, J, and williams. R.J.P, Eds. VCH.Publisher, Weinheim, Germany, 428.
- Venugopal, N.B.R.K. and Reddy, L.N. (1992): Neprototoxic and hepatotoxic effects of trivalent and hexavalent Chromium in teleost fish *Anabas scandens*: Enzymological and biochemical changes. *Ecotoxicol. Environ. Safe.*24: 287-293
- Viarengo, A. (1989): Heavy metals in marine invertebrate mechanism of regulation and toxicity at the cellular level. *Crit.Rev.Aquat.Sci.*1 (2): 295-371.
- Vijayaram, K. and Geraldine.P.(1996): Regulation of essential heavy metals (Cu, Cr & Zn) by the fresh water prawn *Macrobrachium malcolmsonii* (Milne Edwards). *Bull. Environ. Contam.Toxicol.*56: 335-342.
- Vogt, G. and Quintio T. E. (1994): Accumulation and excretion of metal granules in the prawn, *Peaneus mondon*, exposed to water borne Copper, Lead, Iron and Calcium. *Aquat.Toxicol.*28: 223-241.
- Vonk, H.J. (1960): Digestion and metabolism: In physiology of crustacea. (T.H. Waterman, Ce). PP. 291-316. Academic Press, New York.
- Wani, G. P. & Latey, A.N. (1983): Toxic effects of cadmium on the liver of a fresh water teleost Garramullya (Sykes). *Curr. Sci. India* 52 (21): 1034-1035.
- Webb, M. and Etienne.(1977): Studies on the toxicity and metabolism of chthonien. *Biochem.Pharmacol.*26:25-30.
- Wu, Jui-Pin.; Chen, Cheng-Hon and Huang, Da-Ji.(2008): Histopathological and biochemical evidence of hepatopancreatic toxicity caused by cadmium and zinc in the white shrimp, *Litopenaeus vannamei*. *Chemosphere.* 73: 1019-1026





STUDY OF NUTRIENT VALUE IN POST HARVESTED INFECTED ORANGE (*CITRUS SINENSIS*) FRUIT

ALKA SRIVASTAVA* AND SANJAY KUMAR**

Department of Botany, Govt.M.S.J.College Bharatpur-321001 Rajasthan, India

*E-mail : alka.041@ Gmail.com,9810101824

**E-mail : Kumardr. sanjay@ Ymail.com,9414315301

ABSTRACT

Orange (*Citrus sinensis*) is a very nutrient and beneficial fruit all over the world. Study were conducted on changes in the nutrient value like reducing sugar, non reducing sugar, starch, protein, phenol, vitamin C, alpha-amylase and total antioxidant activity of the post harvested infected orange fruit. In this analysis two fungi were isolated from infected orange fruits. The effect of these fungi indicate the reduction in non-reducing sugar, vitamin C, alpha-amylase and antioxidant activity but the amount of reducing sugar, protein and phenol increased in infected fruit. The nutrient value declined or increased due to the infection of *penicillium* and *phytophthora* fungus. From the present investigation it can be concluded that the fungus decrease or increase the nutrient value of fruit because they use them for their successful growth and establishment.

KEY WORDS: Orange, Protein, Phenol, Antioxidant activity, *Penicillium*, *Phytophthora*

INTRODUCTION

Oranges (*Citrus sinensis*) are produced all over the world. Orange ranked first among the citrus fruit. In India, it is mainly produced in Maharastra, Karnataka, West Bengal, Orissa, Assam, some parts of Rajasthan and other North-Eastern part. It is well established that orange or orange product are a rich source of vitamins, minerals, sugar etc. that are essential for normal growth, development and overall nutritional well being. Microorganisms influence the quality and quantity of fruit. According to Jay (2003) a single infected orange can be the source of infection to other oranges during storage and on transmit. Common air molds such as *Penicillium* species may gain entry into the susceptible tissue and cause loss during packaging (Ronald, 1988). The fungi influenced the stored substance or nutrient by absorbing them or by converting some of the substance complex form into simple ones (Sawant and Gawai, 2011). A number of post harvested disease attacks on these fruits which deteriorate its nutritional value. The objective of this study was to obtain information on the effect of fungus infection on the nutrient value of post harvested orange fruit.

MATERIALS AND METHODS

Fresh and infected samples of oranges were collected from the farmer's field of Kota district in Rajasthan for the biochemical and pathological analysis. Total 20 samples were collected for the study. Spoiled or diseased oranges were identified by physical examination. Healthy samples were used as control.

Isolation of fungi: In pathological study, 10 gm of samples was taken and blended with 100 ml of buffered peptone water. In initial suspension 1 ml was taken aseptically and transferred to the sterile petri dishes. 15 ml of Yeast extract-dextrose chloramphenical-agar medium was poured (previously melted and maintained at $45\pm 1^\circ\text{C}$ in water bath from culture bottle) into each petri dish. Inoculums was carefully mixed with medium and allowed to solidify by leaving petri dishes to stand on cool horizontal surface of bio safety cabinet (Indian standard method for yeast and mould count of food stuff IS 5403:1999). A separate controlled plate was made with 15 ml of medium to check its sterility. Petri dishes were placed inverted in the BOD incubator at $25\pm 1^\circ\text{C}$ for five days. After 5 days a loop full of fungus was taken from petri dishes on slide. Staining was done by cotton blue stain and morphological characters were observed under microscope.

Biochemical study: Estimation of vitamin C or ascorbic acid was done by Aberg method of Johnson (1948). The changes in non reducing sugar and reducing sugar was estimated by following the phenol sulphuric method of Dubois et al., (1956) and Miller (1972) respectively. Alpha amylase was detected by the method of Bernfield (1955) and starch was detected by the method of Mc Cready et al., (1950). Total phenols were estimated using standard method described by Bray and Thorpe (1954). Protein was separated by the method described by the Lowry et al., (1951). The estimation of total antioxidant activity has done by following the method described by Choong et al., (2007).