International Journal of Current Biotechnology
ISSN: 2321 - 8371
Journal Homepage : http://ijcb.mainspringer.com

Primary and secondary stress responses in Indian major carps when exposed to heavy metals

Anbu K.

Principal (Rtd.), Rajah Serfoji Govt. College (Autonomous), Thanjavur- 613 005, Tamil Nadu.

ARTICLE INFO

Article History:
Received 10 August 2014
Received in revised form 18 August 2014
Accepted 22 August 2014
Available online 30 August 2014

Key words:
Stress response, cortisol, LDH, heavy metals, Indian major carps.

ABSTRACT

The discharges of toxic pollutants in the water ways, may result in acute or chronic toxicity and induce stress to fish. Environmental stress in aquatics mainly includes adverse physical and chemical condition of water. The generalized stress response exhibited in aquatic organism by impaired cortisol and metabolic enzymes like Lactate dehydrogenase activity (LDH). This work was to investigate the cortisol stress response and level of LDH under lab condition in Indian major carps, *Catla catla, Labeo rohita, Cirrhinus mirgala* treated with heavy metals like mercury (Hg) and lead (Pb) separately. The fishes were treated with different concentration of mercury and lead ranging from 0.5ppm to 2ppm to find out the LC50 value. The LC50 was recorded under different concentration of heavy metals treated fishes as 1ppm/24hrs for mercury, 1ppm/48hrs for lead. Elevated level of cortisol was found out in all treated groups of mercury and lead against control. The significant decrease of LDH was observed in all treated fishes. The influence of cortisol on LDH activity and impairment in the level of cortisol and LDH activity discussed. This study indicates that changes in cortisol and LDH can be used for monitoring toxicity due to mercury and lead exposure in aquatic organism like fish.

Introduction

Fishes are exposed to biotic and abiotic stresses with the wild as well as in captivity; environmental pollutants, disease and various types of intensive aquaculture practices are the potential stressors which affect the fishes under culture conditions. Environmental stress mainly include changes in water quality, metals and xenobiotics in the water can cause severe stress, health hazards and death of the fish (Iwama, *et al*., 1992). Fishes in captivity when exposed to certain chemicals also become physiologically stressed in turn the stressed fish exhibit a generalized stress response, i.e., characterized by impaired release of stress hormones as primary responses which in turn triggers a number of biochemical physiological changes and irregularity in metabolic enzymes termed as secondary stress response (Mazeaud, *et al*., 1977).

Under any physiological stress smaller fishes cortisol level is elevated and it is associated with higher resistance to environment change (Weil *et al*., 2001). Elevated blood cortisol is recognized as an indicator of general stress in fish (Adams, 1990). The sensitivity of the cortisol stress response to a variety of pollutants makes this hormone potentially useful as a bio indicator of general toxic stress. Hontela, (1992) reported laboratory studies of the acute cortisol response of fish to short term exposure (hours) to a single pollutant which have yielded interpretable results.

Enzyme activity can serve as valuable biomarker of pollutant exposure and effect (Mayer *et al*., 1992). Aquatic toxicologist paid greater attention towards muscle, liver and serum enzyme activities, which have been used extensively to provide simple accurate measures of oxygen dysfunction in animals. Enzyme assay is recently emerged as an important diagnostic tool in the field of environmental toxicology (Baskaran, 1991). LDH, the terminal enzyme in vertebrates anaerobic glycolysis, is one of the enzyme that have been employed for diagnosing liver, muscles damage caused by pollutants in fish (Naff, 1985). Anaerobic metabolism can be measured by LDH activity and this activity can be impaired after prolonged exposure to xenobiotics. On keeping these report this study was devised to investigate the cortisol and LDH activity in response to the actual exposure to heavy metals in Indian major carps under captive condition.

Heavy Metals

Mercury: Mercury is highly toxic element, its potential for toxicity is highly contaminated areas such as Minamata Bay and it is well documented in Japan. The

*Corresponding author.
Email address: anbudurai1956@gmail.com
Mobile: 9443866278

toxic effect of mercury depends on its chemical form and the root of exposure. Methy mercury (CH_3Hg) is the most toxic form. It affect the immune system, alters enzyme activity of fishes. Younger fish tend to have lower concentration of mercury than larger fish within the same water body. Mercury concentrates in the muscle tissue of fish (US Environmental Protection agency, 1997). Mercury emitted to the air and water by power plans, cement plants, chemical manufacturing industries. Where it binds in fish that we eat. Toxic metal, accumulate in the living organism through food chain, as large fish consume contaminated smaller fish (Natural Resource Defence Council, Illinois. USA. 2014) . Fish caught from aquatic contaminated with mercuric salts. The organic mercury compound- methyl dimethyl mercury is the neurotoxin compound to living organism. Methly mercury is healed tightly to fish protein, its level in carnivorus fish may bioaccumulate up to a million times greater than the surrounding water. Bioaccumulation in fish is influenced by the amount of methyl mercury present which in turn affected by local biogeochemical process and by mercury inputs from atmospheric pollution (Environment Canada, 2013).

Lead: one of the heavy metals widely used in industries has been subject of biological interest due to potential pollution properties (Jha and Pandey, 1989). Schenhammer and Norries (1996) reported that lead is a soft metallic element found naturally in all environmental media. The recent emergence of lead toxicology has resulted in the recognition of adverse effect of lead at lower level of exposure. Salomon *et al.,* (1984) recorded that among all heavy metals lead is one of the mostly used industrial metal, that anthropogenic release of lead to the environment is the highest of all heavy metals. All the existing data shows that its metabolic effect are adverse, most of the incorporated into suspended bottom sediments. The primary cause of leads toxicity is its interference with a variety of enzymes because its builds to sulfhydryl groups found in many enzymes (Natural Resource Defence Council, Illinois. USA. 2013).

Material and methods

Indian major carps: These are economically and commercially important fishes cultured in south India especially in Thanjavur district. These carps spawn spontaneously and it is available in good number throughout the year. It can be maintained and adaptable under laboratory condition. The toxicity study was carried out under laboratory condition.

Indian major carps *Catla catla, Labeo rohita* and *Cirrhinus mirgala* collected from culture farms near Thanjavur town. Forty five day old premature young fishes were selected (*Catla catla* Wt 41.93 ± 4.2gm, L 17.32 ± 1.1cm, *Labeo rohita* Wt 14.78 ± 2.1gm, L 10.78 ± 1.2cm, *Cirrhinus mirgala* Wt 37.4 ± 3.2gm, L 17.1 ± 0.4cm) and maintained under laboratory condition for about 48 hrs and fed with commercial feed in the ratio of 10% of body weight twice in a day at a fixed time. Physiochemical condition of the water during the experimental period were dissolved oxygen 5.5 - 6.0 ml/ lit, temperature 29 ± 1^0C , pH 7.0 ± 0.1 with 12 hours, 12 hours light and dark cycle. The experimental fishes were exposed to 0.5 ppm to 2 ppm concentration of mercury and lead separately. LC 50 was identified as 1 ppm/24 hrs for Hg and 1 ppm/48 hrs for lead. Control were maintained without any treatment under laboratory condition. Fishes were sampled at each concentration control, 0.5 ppm and 1 ppm. The muscles and liver tissue was removed for further analysis.

Cortisol assay

Cortisol was measured in muscles and liver by direst solid phase enzyme radio-immuno assay (RIA) as described by Levesque *et al*., (2003) using UBI magiwell cortisol kit (Uni-biotech, Canada). One gram of tissue was homogenized using one ml of Tris-HCL buffer at 7.2 pH, centrifuged at 5000rpm for five min, the superntant was used for cortisol assay in the tissue

LDH assay

LDH was measured using commercial kit (Agappae diagnostics Ltd, Kerala) with auto analyser. One gram of tissue was homogenized with sodium phosphate buffer at 7.4 pH,centrifuged at 5000rpm for 10 minutes and the supernatant was used for LDH assay.

Results

Toxicity experiments revealed that, the acute lethal concentration dose was 1ppm/24hrs for mercury and 1ppm/48hrs for lead as depicted in the fig 1-4.

Cortisol: At all doses of treatment cortisol level rose rapidly from low levels. Cortisol levels were significantly higher at 1ppm treatment of Hg (Fig -1). Peak cortisol level was observed in *Labeo rohita* muscles (400ng/ml) and liver (460ng/ml) at 1 ppm of mercury treatment. Differences of cortisol level in *C. mrigala* in the muscles and liver tissues were detected. Significant increase of the cortisol level was observed in all the treated doses. As same as above during - lead treatment (Fig -2) significant elevation of cortisol in the muscles and liver tissues of L. *rohita* and in the muscles of *C. catla* cortisol level significantly increased from 0.5ppm to 1ppm in all the tested and treated groups. Mercury treatment effect was more pronounced in cortisol elevation than lead. Significant increase in cortisol level was noticed in 1ppm lead treated fishes. 100% mortality was observed at 2 ppm/24hrs of mercury and 2 ppm/48hrs of lead treatment.

LDH: Based on the study results, the activity of LDH in crude muscles and liver tissue was recorded. Significant decrease of LDH activity was observed in all treated fishes. Dose dependent variation of LDH activity was also recorded. Maximum activity was recorded in the tissue of *Labeo rohita* (muscle 58 IU/L, liver 60 IU/L) followed by Catla *catla* (muscle 46 IU/L, liver 60 IU/L) than in the control (Fig -3). The maximum variation of LDH was observed in *Labeo rohita* between the controls and treated. Maximum decrease of LDH was observed at 1ppm treated groups of mercury and lead activity in the muscle and liver *of C. cattla* and *L. rohita* . The mercury effect was more pronounced in the liver tissue of *C. cattla* and *L. rohita*. On lead exposure pronounced effect was observed in muscles and liver tissue of *L. rohita* and *C. cattla* (Fig -4). Minimum variation of LDH was observed in *C. mirgala*, which was not much affected by heavy metal stress.

Discussion

Extensive industrialization and urbanization has increased pollutants into the aquatic environment. The discharge of toxic pollutant into the water ways may results in acute or chronic toxicity to fish. Thanjavur district, the granary of South India, more than fifty rice varieties is cultivated on season. Beyond that, cereals and pulses along with cotton and domestic vegetables are cultivated. A remarkable amount of pesticides and insecticides are used to control the insect and pest to protect the crops. The non-degraded pollutant drained into the water bodies used as a fish culture pond or the water used for fish

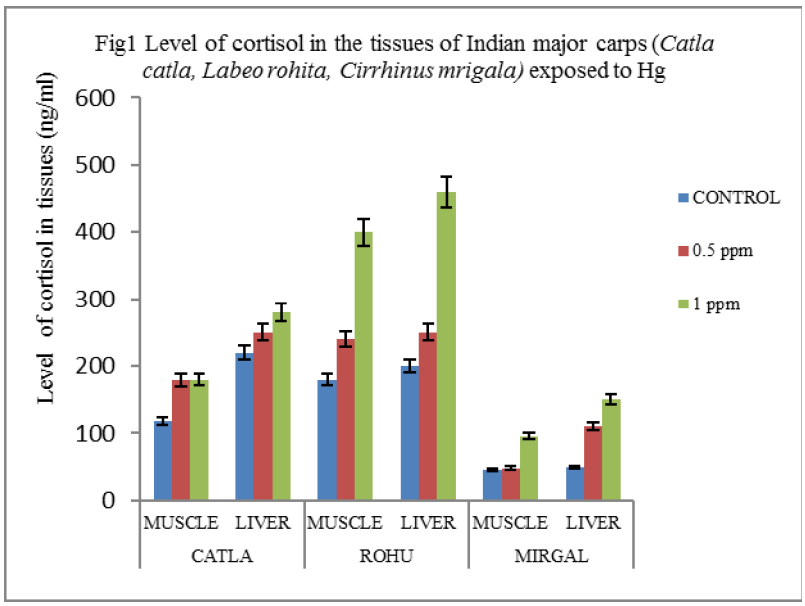


Fig1 Level of cortisol in the tissues of Indian major carps (Catla catla, Labeo rohita, Cirrhinus mrigala) exposed to Hg
Level of cortisol in tissues (ng/ml)
600
500
400
300
200
100
0
CONTROL
0.5 ppm
1 ppm
MUSCLE LIVER MUSCLE LIVER MUSCLE LIVER
CATLA ROHU MIRGAL

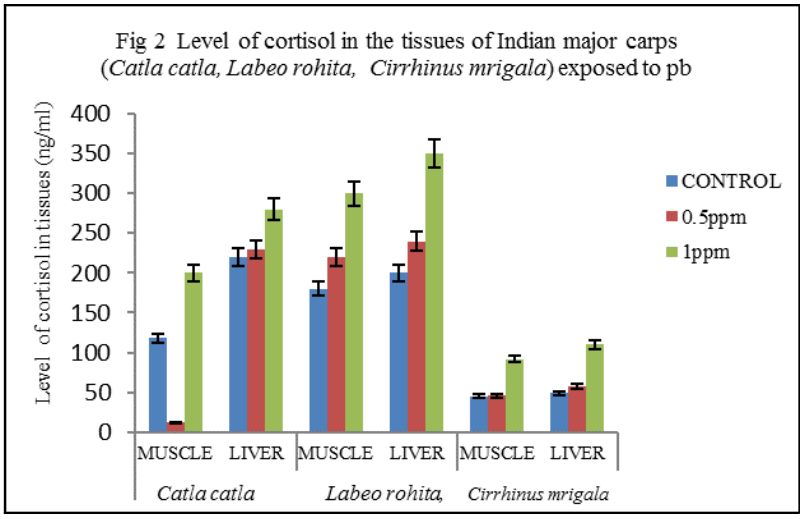


Fig 2 Level of cortisol in the tissues of Indian major carps (Catla catla, Labeo rohita, Cirrhinus mrigala) exposed to pb
Level of cortisol in tissues (ng/ml)
400
350
300
250
200
150
100
50
0
CONTROL
0.5ppm
1ppm
MUSCLE LIVER MUSCLE LIVER MUSCLE LIVER
Catla catla Labeo rohita, Cirrhinus mrigala

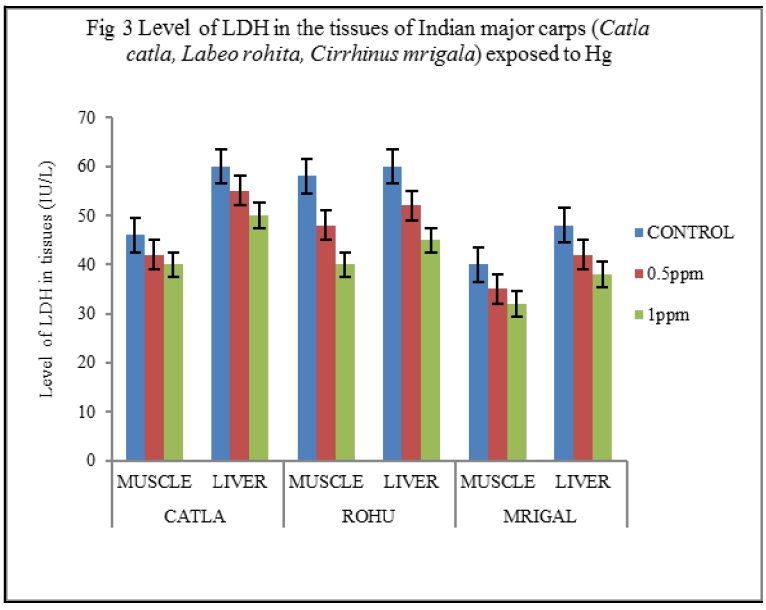
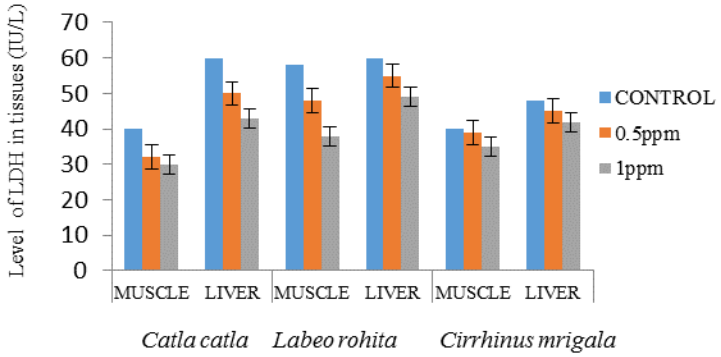


Fig 3 Level of LDH in the tissues of Indian major carps (Catla catla, Labeo rohita, Cirrhinus mrigala) exposed to Hg
Level of LDH in tissues (IU/L)
70
60
50
40
30
20
10
0
CONTROL
0.5ppm
1ppm
MUSCLE LIVER MUSCLE LIVER MUSCLE LIVER
CATLA ROHU MRIGAL

Fig 4 Level of LDH in the tissues of Indian major carps (*Catla catla, Labeo rohita, Cirrhinus mrigala*) exposed to pb



culture. Fish habitats influenced directly by rural agricultural and industrial activities. Indian major carps are fishes consumed by the people of Tamilnadu as a major protein source so these fishes are cultured throughout Thanjavur districts. To understand the toxicity of the heavy metal stress and impact of xenobiotics on Indian major carps *C. catla, L. rohita, C. mrigala* the present study was carried out.

Under this study some morphological and behavioural changes were observed in all treated groups at the beginning of mercury exposure such as uncontrolled swimming, feed avoidance and increased respiration movement towards the surface, mucous secretion and fade in colouration. Under this investigation the significant increase in cortisol level and corresponding decrease in LDH was observed on treatment of heavy metals. Maximum level increase in cortisol was observed, in L. *rohita* and minimum rise in *C. mrigala.* LDH activity was decreased in *C. catla* and *L. rohita*. The pronounced effect was observed in the muscle and liver tissue of mercury treated groups than lead treated.

The most domesticated fish cyprinids have a very high cortisol response to stress. (Pottinger *et al.*, 2000; Nematollahi, 2010). The present study revealed the elevated cortisol response to heavy metal stress. Exposure of heavy metals results in stress condition and the increased energy need under stress are compensated from stored glycogen in the tissues of muscle and liver (Wendelaar, 1997) and also from non carbohydrate sources through gluconeogenic pathway (Levesque *et al.*, 2002, Karaylug *et al.*, 2010). Abbas, 1998 and Zaki *et al.*, 2008 reported that acute exposure to contaminates such as heavy metals and herbicides (Abbas *et al.*, 2007), increase plasma cortisol in fish. Hontela (1997) and Barton (2002) mentioned that in chronic exposures to contaminants an increase plasma cortisol in general followed by decrease as the fish acclimates or as has been reported for cadmium. The most important implication of elevated environmental Hg and Pb is in property to accumulate in to aquatic food chain, potentially causing adverse effect: bio magnification of heavy metals with fresh water fishes and water bodies leads to fish muscle poison of edible fishes and totally affect the fish population. During the reproductive phase of fresh water fish *Notopterus notopterus* (Sundaraj and Gowswami, 1971, Sundaraj *et al.*, 1982 and Shanker and Kulkarni, 2006) it was e observed that a greater concentration of cortisol compared to estrogen may have role in oocyte maturation and ovulation it enchance vittellogenic activity of young oocytes. The ovarian somatic index and hepatosomatic index was reduced under cortisol stress. Sea water exposure caused an increase in plasma cortisol level and it is involved in the non regulatory mechanism (Young, 1986) cortisol acts directly on the gills of fishes. During the early development stage (Young fishes 30 to 45 days old) cortisol simply responding as a general stress factor induced by the living environment (Strange *et al.*, 1977; Schrek, 1981). The cortisol stress responses of surubim (*Pseudo platystoma*) acclimated to different rearing temperature, at 30^0 C the fish subjected to be under physiological stress evidenced by elevated cortisol level (Pankhurst and Van Derkraak, 1997). According to the above studies any stress induces cortisol elevation in fishes. The present study record supports the above observation and investigation.

Significant decrease of LDH was identified in Indian major carps on Nitrite toxicity (Das, *et al.*, 2004) on monocrotophos (Vimala and Anbu, 2011). The decrease in LDH activity was correlated with the present study. LDH activity decreased overtime both in the muscle and liver tissue possibility due to irregular metabolic activity after the exposure to heavy metal toxicity.Sastry and Shukla, (1994) studied the enzymological parameters of fresh water fish *Channa punctatus* exposed to acute and chronoic concentration of cadmium. They reported the decrease of LDH activity in muscles and liver. In the present study the significant decrease of LDH during heavy metals treatment may be due to impairment of glycolysis recalling the observation of above authors. LDH activity decreased over time both in the muscle and liver tissue possibility due to irregular metabolic activity after the exposure to heavy metals toxicity

Cortisol stress response has been associated with animals showing a high resistance to environmental change (Weil, *et al.*, 2001). When a fish chronically exposed or subjected to contaminant in their environment are available to respond normally to the acute stress of capture. Serum cortisol level of fishes was very low at the most contaminated environment. The role of cortisol

and LDH and their interaction is determining good adaptability to the living environment (Avella, *et al.*, 1990).

Stress studies in experimental animals described an initial alarm stage, the ensuring stages of resistance and final stage of exhaustion. The alarm reaction, characterized by sharp increase in blood cortisol level in fish (Schreck, 1981; Donaldsm, *et al.*, 1984). Lifelong exposure of heavy metal pollution like mercury results of prolonged cortisol elevation leads to muscle wasting and lower growth rate of fish as well as to disturb the reproductive activity (Billard *et al.*, 1981). In the absence of cortisol response implied that fish from the polluted sites lack the glyconeogenic and lipolytic energy mobilizing capacity. Fish in such a physiological state may fail to react and display the normal behavioural response, thus further jeopardizing their own survirorship (Hontela et *al.*, 1992). Endocrine parameters, through their integrative role in the maintenance of homeostastis are emerging as useful diagnostic in the detection of early or low level responses to pollutant (Hontela et *al.*, 1989) which may precede the outset of pathologies and mortality. The stress response develops as a function of fish size as well as age. Both size and age are among the most important factors that control the development of many physiological activities in fish (Hoar and Randall, 1988).

Conclusion

The present study concludes that the exposure of heavy metals mercury and lead at acute toxicity levels elevated the cortisol levels in the muscles and liver tissues of Indian major carps *C catla, Labeo rohita, Cirrhinus mrigala*. The elevated level of cortisol is previously established as a result of ionic disturbance as a mechanism of coping up with the stress. The decrease in LDH activity may be due to impaired carbohydrate a metabolism after the exposure of mercury and lead. Cortisol elevation as a primary stress response and change and decrease in LDH activity as a secondary response when Indian major carps treated with mercury and lead under sublethal and acute concentrations. Cortisol and LDH stress response has been associated with animals showing high resistance to environmental changes.

Acknowledgement

The author very much thankful to UGC, SERO Hyderabad for the financial assistance rendered towards Minor research project. The author express the gratitude to Prof.P.Subramanian for providing facilities to carry out the work partly in the Division of aquaculture, Department of Animal Biotechnology, Bharathidasan University, Tiruchirappalli, Tamilnadu.

References

Adams,S .M., 1990. Biological indicators of stress in fish. American fisheries Society, Symposium, 23-28.

Abbas, H.H., 1998. Toxicological effect of copper and lead on some physiological aspects in two fish species, blue tilapia, *Oreochromis aureus* and African catfish, *Clarias garispinus*. Ph.D. Thesis, Faculty of Science, Cairo University, Egypt pp: 214.

Abbas, H.H., M.M., Abumouras I.K., and El-Badwy., A, 2007. Studies on the effect thiobencarb herbicides on some biological, physiological biochemical, histological and genetics aspects of Nile tilapia, *Oreochromis niloticus*. Egyptian J. Aquatic Biol. Fish., (11)1: 123-150.

Billard B. Bry C. and Gillet C., 1981. Stress, environment and reproduction in teleost fish. In: Pickering A. D. (Ed). Stress in fish. Academic Press, London. Pp.185–208.

Das P.C. Ayyappan S. Das B.K. and Jena J.K. 2004. Nitrate toxicity in Indian major carps. Sub lethal effect on selected enzyme in fingerlings of *Catla catla, Labeo rohita* and *Cirrhinus mrigala*. Biochem. Physiol. Toxiocol. Pharmacol. 138(1): 3-10.

Hoar W.S. and Randall D.J., 1988. The physiology of developing fish. Part A. Eggs and larvae. Part B. Viviparity and Post hatching juveniles. In Fish physiology, Academic Press, London.

Hontela A. Rasmussen J.B. Audet C. and Chevalier G., 1992. Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs, and mercury. Arch. Environ. Contam. Toxicol. 22: 278-283.

Hontela A., 1997. Endocrine and physiological responses of fish to xenobiotics: role of glucocorticosteroid hormones. Environ. Toxicol. 1: 41-46.

Iwama G. K. Vijayan Mathilakath M. Forsyth B. and Ackerman Paige A., 1999. Heat shock proteins and physiological stress in fish. Soc. Integrative and comparative bio.Amer. Zool. 39: 901-909.

Jha B.S and Pandey S. 1989. Histopathological lesions induced by lead nitrate in the stomach of the air- breathinig teleost, *Channa paunctatus*. Environ. Ecol, 7(3): 721-723.

Karaytug S. Karayakar F. Ciftci N. Cicik B. Ozcan A. and Erdem C., 2010. Effects of Cadmium on Sera Glucose and Cortisol Levels in *Clarias gariepinus*. J. Anim. Veterin.Advanc. 9: 2159-2162.

Levesque H.M. Dorval J. Hontela A. Van Der Kraak G.J. and Campbell P.G., 2003. Hormonal, morphological, and physiological responses of yellow perch (*Perca flavescens*) to chronic environmental metal exposures. J Toxicol Environ Health. 66(7):657-76.

Mayer F. L. Versteeg D. J. McKee M. J. Folmar, L. C. Graney R. L. Mceume D. C. and Rattner, B. A. 1992. Physiological and nonspecific biomarkers. In: Biochemical, physiological and histological markers of anthropogenic stress. (Eds) Hugget, R. J., Kimerle, R. A., Mehrle , Jr.P.M and Bergman, H. L. Proceeding of eight pellston workshop, colorada Lewis publishers, Boca Raton, USA, pp 5-85.

Mazeaud M.M. Mazeaud F. and Donaldson E.M., 1977. Primary and secondary effects of stress in fish: some new data with a general review. Trans Am Fish Soc. 106:201–212.

Neff, J. M., 1985. Use of biochemical measurement to detect pollutant medicated damage to fish. ASTM spec Tech. publ. 854: 154–183.

Miller, L.L., F, Wang, V.P. Palace and Alice Hontela, 2007. Effect of acute and subchronic exposures stress response and oxidative stress indicators in juvenile rainbow and trout. Aquatic Toxicol., 57: 263 -271.

Nematollahi M. A., 2010. Net confinement stress response in common carp (*Cyprinus carpio*). J. Fisheries, 63(1): 39-47.

Nematollahi1 M. A. De Van Pelt H. and Komen H., 2013.Response to stress in 17" hydroxylase deficient common carp (*Cyprinus carpio L.)*. J. Agr. Sci. Tech, 15: 303-310

Pankhurst N.W. and Van Der Kraak G., 1997. Effects of stress on reproduction and growth of fish. In: (Iwama, G.K., Pickering, A.D., Sumpter J.P. and Schreck C.B.(Eds.) 'Fish stress and health in aquaculture Cambridge University Press: Cambridge, pp 73-99.

Pottinger T. G. Carrick T. R. Appleby A. and Yeomans W. E., 2000. High blood cortisol levels and low cortisol receptor affinity: is the chub, *leuciscus cephalus*, a cortisol-resistant teleosts. gen. comp. Endocrinol., 120(1): 108-117.

Salomons W., and Forstner, 1984. Metals in the hydrocycle Springer, Berlin. 394.

Sastry K.V. and Shukla V. 1994. Acute and chronoic toxic effect of cadamiun on some haematological, biochemical and enzymological parameters in the freshwater fish *Channa paunctatus.* Acta. Hydrobiol. 22:171-176.

Scheuhammer, A.M. and Norries, S.L. 1998. Canadian wild life service, Environ can Exotoxical 279-295.

Schreck C. B., 1981. Stress and compensation in teleostean fishes responses to social and physiological factors. In. Plckering, D. (ed.) Stress and fish. Academic Press, London, 295-332.

Shankar D.S., and Kulkarni R.S. 2006. Effect of cortisol on female freshwater fish *Notopterus notopterus*. J. Environ. Biol. 27(4): 727-731.

Strange R. J. Schreck C. B. and Golden, J. T. 1977. Corticoid stress responses to handling and temperature in salmonids. Trans Am. Fish. Soc. 106: 213-217.

Singh H. and Tripathi G., 2011. Effect of cortisol on lactic dehydrogenase, catalyse and protein of the snake headed fish, *Channa punctatus.* Nat. J. Life Sci. 8(2): 113-116.

Sundararaj B.I. and Goswami S.V., 1971. Effects of deoxycorticosterone and hydrocortisone singly and in various combinations in vitro maturation of oocytes of the catfish Heteropneustes *fossilis* (Bloch). Gen. Comp. Endocrinol. 17: 570-576.

Sundararaj B.I. Goswami S.V. and. Lamba V.J., 1982. Role of testosterone, estradiol and cortisol during vitellogenin synthesis in the catfish *Heterpneustes fossilis* (Bloch). Gen. Comp. Endocrinol, 48: 390 -397.

Vimala G. and Anbu K., 2011. Comparative study on the enzyme profile of monocrotophos treated Indian major carps. Proc. Nat. Acad. Sci. India Sec-B.18, Part IV, 389-395.

Weil L.S. Barry T.P. and Malison J.A., 2001. Fast growth in rainbow trout is correlated with a rapid decrease in post-stress cortisol concentrations. Aquaculture. 193: 373-380.

Zaki, M.S., S. Moustafa, H.Rashad and N.Sharaf, 2008. Assessment of the hazardous effect to lead pollution on *Oreochromis aureus* including hematological biochemical and immunological parameters. American – Eurasian J. Agric. Environ Sci., (3)1: 91-95.