CIBTech Journal of Zoology ISSN: 2319–3883
Online, International Journal, Available at http://www.cibtech.org/cjz.htm
2024 Vol.13, pp.404-411/Remya et al.
***Research Article** (Open Access)*

CADMIUM-INDUCED PHYSIOLOGICAL AND CHROMATOPHORE ALTERATIONS IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

***Remya V. K, Smina M.S. and Sony Albert**
Sree Narayana College Nattika, P.O Nattika, India- 680566
**Author for Correspondence: remyavariath@rediffmail.com*

ABSTRACT
The global issue of heavy metal contamination in water bodies has raised serious environmental concerns. The objective of this study was to determine the effects of cadmium on various physiological parameters of *Oreochromis niloticus*, including muscle total protein, hemoglobin, thyroid hormones, and chromatophores. The study employed two groups: an experimental group exposed to a sub lethal cadmium concentration (0.01 mg/l) for a 30-day period, and a control group. Notably, the presence of cadmium led to a significant reduction in muscle total protein levels. Additionally, exposed fish displayed a marked decrease in plasma concentrations of thyroid hormones (T3, T4), thyroid-stimulating hormone (TSH), and hemoglobin (Hb). These findings suggest that short-term exposure to cadmium disrupts the endocrine balance and protein metabolism in *O. niloticus*. In addition, the study identified remarkable toxicopathological changes in chromatophores, including statistically significant changes in the size, shape, and morphology of melanophores. Specifically, melanophores cells exhibited smaller sizes and greater numbers than their normal counterparts.

Keywords: *Cadmium, Oreochromis niloticus, water bodies, thyroid hormones, protein metabolism, chromatophores*

INTRODUCTION

The issue of heavy metal contamination in aquatic systems stands as a pressing environmental concern today (Nriagu *et al.,* 1998; Silva *et al.,* 1999). Heavy metals wield varying effects on fish, contingent upon the specific metal, its concentration in water, and the duration of exposure (Boscher *et al.,* 2010). While metals like copper, chromium, and iron serve essential roles in the environment, others such as cadmium and lead, even in minimal concentrations, pose toxicity (Rubio *et al.,* 2016). Cadmium, widely distributed and highly toxic, is frequently used in environmental studies (Faroon *et al.,* 1994; Kuehl and Haebler, 1995; Camusso *et al.,* 1995; Cinier *et al.,* 1999) and typically enters water bodies via industrial discharges or corroded galvanized pipes (De, 1996).

Fish skin, directly exposed to environmental toxins, serves as a valuable indicator of aquatic contamination (Rajan and Banerjee, 1991; Paul and Banerjee, 1996; Hemalatha and Banerjee, 1997). Studies indicate varying rates of cadmium accumulation in fish organs like the liver, kidneys, and gills based on exposure concentrations (Klinck *et al.,* 2007). This metal induces metabolic alterations and a range of biochemical and physiological effects (Szebedinszky *et al.,* 2001). The fish utilize enzymatic processes to transform these foreign compounds into less harmful forms, emphasizing the importance of xenobiotic biotransformation in mitigating their impact (Persch *et al.,* 2017).

Due to their ease of culture, maintenance, and handling in laboratory conditions, Nile tilapia (Oreochromis niloticus) are useful experimental fish for studying environmental pollution adaptations (Garcia-Santos *et al.,* 2013). Within this framework, this experimental study aimed to explore the impacts of acute cadmium exposure on thyroid function, total tissue protein, and haemoglobin content in *O.*

niloticus. However, there is limited research regarding the influence of cadmium on melanophores (Banerjee and Mukherjee, 1994). Hence, this study endeavors to investigate the detrimental effects of cadmium chloride on the melanophores of *Oreochromis niloticus.*

MATERIALS AND METHODS

Regardless of sex, live specimens of *O. niloticus* were collected locally and acclimated to laboratory conditions for 15 days. During the acclimation period, the fish were regularly fed pellet feed. The water was renewed every 24 hours, leaving no fecal matter, unconsumed food, or dead fish, if any. For the present study, five groups of 10 fish each were separately exposed to 20 litres of a 0.01 mg/l cadmium chloride solution prepared in tap water. Parallel control groups were also kept alongside the experimental groups. Feeding was allowed in both the experimental and control groups for 3 hours before the renewal of water throughout the experiment. The duration of the experiment was 30 days. Blood was collected via the caudal vessel into heparinized tubes. Immediately after blood collection, the hemoglobin concentration of the blood was determined with a Sigma Diagnostics total hemoglobin kit. The hemoglobin content was measured in g/Dl. Plasma was obtained by centrifugation (5 min at 10,000g, 4 °C) and kept at -80°C for later analysis. The plasma T4 and T3 levels were determined using commercial immunoassay kits.

The muscle was excised and processed to analyze the levels of total protein. The total protein was estimated using the Lowery *et al.,* (1995) method. The color developed was measured using a spectrophotometer at 650 nm. The result obtained was expressed as the mean ± SD (standard deviation) of three individual observations. For the melanophore study, small fragments of skin were excised from above the lateral line canal. This tissue is then dipped in physiological saline for about 2 minutes. After 2 minutes, place it on a clean glass slide, mount it with glycerin or DPX, and place a cover slip over it. Then we can observe the chromatophore using a compound microscope.

The results from all treatments were compared using one-way ANOVA (significance was determined at $P<0.05$). Differences among means were determined with Tukey's multiple comparison tests. Statistical analyses were performed using SPSS version 16.

RESULTS

During the experimental period, neither mortality nor abnormal behavior was observed in either the control or Cd-exposed fish. However, significant physiological alterations were noted in the experimental group compared to the control group, specifically in hemoglobin, protein, and thyroid hormone levels (T3, T4, and TSH).

The hemoglobin levels in the experimental group (6.50 g/dL) were notably lower than those in the control group (10.17 g/dL). Additionally, the experimental group (0.71µg/ml) exhibited significantly reduced protein levels ($P < 0.05$) compared to the control group (1.23µg/ml).

Moreover, T3 levels were significantly decreased in the experimental group (0.56ng/m) compared to the control group (1.53ng/m), along with notably lower T4 levels (0.21) compared to the control group (1.42ng/m). The experimental group (0.32ng/m) also demonstrated significantly ($P < 0.05$) reduced TSH levels compared to the control group (0.62ng/m). These findings collectively indicate substantial physiological impacts resulting from experimental conditions.

Melanophores resemble asteroid cells, with tentacle-like processes of significantly variable length extending from a central body. They seem to exhibit a uniform distribution. These cells are filled with pigment (melanin) granules, and the spacing between adjacent melanophores appears relatively consistent.

Table 1: Changes in the muscle total protein and hematological parameters of *O. niloticus* after the exposure of cadmium.

Parameters	Control	Experiment	P value
Hb (g/dL)	10.17 ± 0.06^{b}	6.50 ± 0.10^{a}	$P < 0.05$
Protein(µg/ml)	1.23 ± 0.02^{b}	0.71 ± 0.01^{a}	$P < 0.05$
T3(ng/ml)	1.53 ± 0.04^{b}	0.56 ± 0.05^{a}	$P < 0.05$
T4 (ng/ml)	1.42 ± 0.04^{b}	0.21 ± 0.02^{a}	$P < 0.05$
TSH (ng/ml)	0.62 ± 0.03^{b}	0.32 ± 0.04^{a}	$P < 0.05$

The data represent the means ±standard deviation (SD) of three replicate cages.

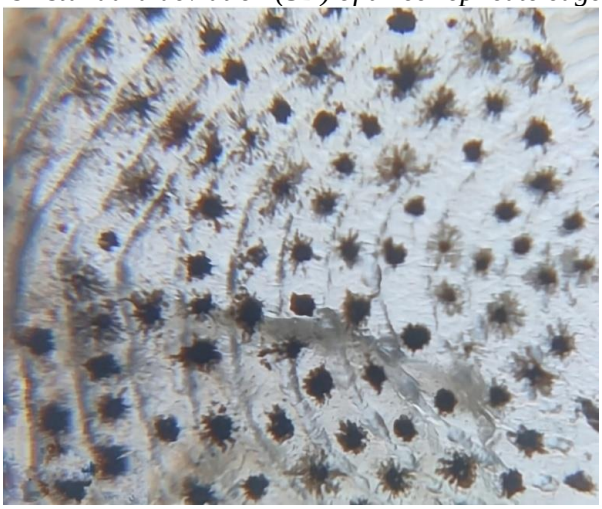

Figure 1: Whole mount preparation of dorsal skin epidermis of *Oreochromis niloticus* showing the normal distribution pattern of chromatophores.

In experimental fish, there is a reduction in the distinctiveness of the central body and tentacle-like processes of melanophores compared to the control group. Additionally, both the size and number of melanophores decreased in the experimental group.

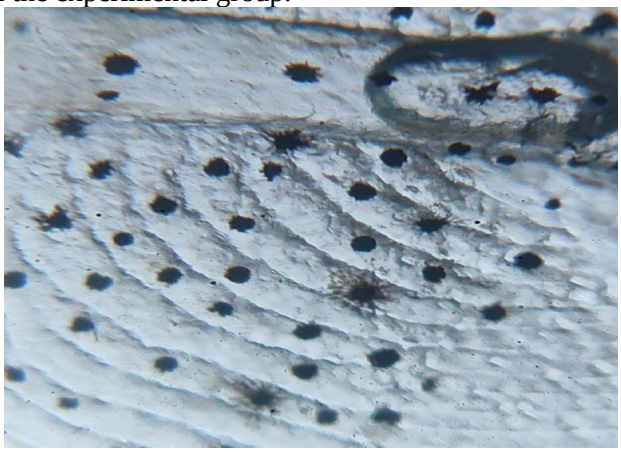

Figure 2: Whole mount preparation of cadmium exposed dorsal skin epidermis of *Oreochromis niloticus*

DISCUSSION

The presence of heavy metal contamination exerts additional stress on metabolically active tissues and organs within fish. Depending on factors such as fish species, exposure concentration, and duration, these metals can either elevate or reduce total protein levels. Many fish species undergo a natural depletion phase during parts of their life cycle, and proteins within their systems are notably sensitive to the effects of heavy metal poisoning (Jacobs *et al.*, 1977). Analyzing the biochemical alterations in fish provides insights into pollution effects, aiding our understanding of the mode of action and the specific pollutants involved.

In India, studies analyzing the biochemical components of freshwater fish have emphasized their nutritional value as protein-rich food sources for humans (Anon, 1962). Harper *et al.*, (1978) highlighted the versatility and abundance of proteins as biological macromolecules, underscoring their pivotal roles in protein metabolism, involving amino acids, enzymes, and coenzymes. These proteins are integral to various physiological processes and can serve as diagnostic indicators, reflecting different phases within an organism's physiology. Notably, proteins play a crucial role in cell architecture and serve as an energy source during chronic stress exposure (Umminger, 1977). Fish, particularly those lacking in carbohydrates, turn to proteins as an alternative energy source to cope with increased energy demands during stress. This heightened energy demand is often necessary for detoxifying toxicants and overcoming stressors in their environment. The total protein level in the current study decreased in the experimental group compared to the control group. A significant decrease in total protein was also observed in *Clarias graiepinus* exposed to 0.5 mg/l or 1.0 mg/l cadmium (Kori-Siakpere *et al.*, 2006). Moreover, *Clarias batrachus* exposed to 1 mg of CdSO4 for 32 days showed a significant reduction in protein levels (Kumar, 2016). On the other hand, in the presence of high cadmium concentrations, total protein levels were elevated in *Clarias graiepinus* (Gaber *et al.*, 2013). Alterations in protein levels observed in the study groups may be due to liver or kidney damage (El-Boshy *et al.*, 2014b).

Hemoglobin serves as the primary carrier of oxygen in fish blood. Studies by Dhanapakium *et al.*, (2001), Sjobeck *et al.*, (1984), Nanda *et al.*, (1996), and Bersenyi *et al.*, (2003) have indicated a substantial decrease in hemoglobin levels at sub lethal concentrations, suggesting a potential indicator of anemia in fish. Beena and Viswaranjan (1987) and Johansson-Sjobeck and Larsson (1978) reported a slight decrease in red blood cell (RBC) count after 30-day treatment, alongside significant reductions in hemoglobin concentration, packed cell volume (PCV), and mean corpuscular volume (MCV). These changes could be attributed to factors such as hemolytic or hemodilution induced by metal exposure. The influence of metal exposure on hematological values has been highlighted in studies by Tort and Torres (1988) and Dick and Dix (1985). These studies suggest that alterations in blood water content due to metal exposure lead to changes in ion exchange, causing hemodilution in freshwater environments and haemo concentration in saltwater environments. In the current study, Hb was significantly lower in the experimental group compared to the control group. Possibly, these changes resulted from defects in both the hemopoietic and metabolic states of the fish exposed to sublethal cadmium concentrations, which caused hemopoietic organ dysfunction, which in turn resulted in low Hb concentrations (Sharma and Langer, 2014).

The effect of heavy metals on the HPT (Hypothalamus-Pituitary-Thyroid) axis in teleosts has been demonstrated in several studies, both in controlled laboratory environments and in field settings (Hontela *et al.*, 1996; Ricard *et al.*, 1998; Zhou *et al.*, 2000; Levesque *et al.*, 2003; Oliveira *et al.*, 2008; Oliveira *et al.*, 2011), which results in altered levels of thyroid hormone in the bloodstream. However, the outcomes of these studies sometimes present inconsistencies, and for cadmium (Cd) specifically, the available information remains limited (Hontela *et al.*, 1996; Ricard *et al.*, 1998). For instance, Hontela *et al.*, (1996) observed elevated plasma T4 levels shortly after acute exposure of rainbow trout to cadmium, which returned to control levels after 96 hours and decreased after a week, while plasma T3 levels remained stable throughout the experimental period. Conversely, Garcia-Santos *et al.*, (2006) found that plasma T4

levels remained unaffected by cadmium exposure, but T3 levels decreased. Oliveira *et al.,* (2008) found decreased T3 levels in eels exposed to copper (Cu) for seven days, despite unchanged T4 levels.
In contrast to these findings, the present data demonstrate a decrease in both plasma T4 and T3 levels following cadmium exposure. There has been evidence of decreased plasma T3 and T4 levels in rainbow trout (Ricard *et al.,* 1998) and yellow perch (Levesque *et al.,* 2003) after sub chronic exposure to cadmium, which is in agreement with the observations made in perch collected from heavy metal-contaminated lakes. However, Zhou *et al.,* (2000) noted higher T4 levels in mummichogs from a polluted site without significant differences in T3 levels compared to a reference group. The discrepancies in these findings might stem from variations in the type of toxicant, dosage, exposure duration, fish species, protocols, timing of exposure, and metal concentrations utilized. Interpreting plasma thyroid hormone levels alone proves challenging, as they may reflect changes in thyroid status or alterations upstream or downstream of released thyroid hormones. These alterations could include changes in the hypothalamus, pituitary gland, hormone secretion, deiodination, or clearance rates (Oliveira *et al.,* 2008; Carr and Patino, 2011).
Skin pigmentation patterns in vertebrate animals are known to be complex and often influenced by various factors. Bagnara and Hardley (1973) highlighted that the distribution pattern of chromatophores is a crucial determinant in shaping the final pigmentation pattern of a species. Interestingly, our study observed a gradual increase in chromatophore density over successive stages, peaking at 45 days of exposure, accompanied by noticeable darkening of the skin. This finding aligns with previous research suggesting a correlation between chromatophore density and pigmentation intensity. Moreover, studies by Verbost *et al.,* (1989), Wicklund and Olsson (1991), and Hogstrand *et al.,* (1994) have indicated that cadmium disrupts ion regulation and osmotic balance, potentially affecting pigmentation processes. Conversely, investigations of the fiddler crab Uca pugnax (Fingerman *et al.,* 1963) emphasize the role of osmotic and ionic factors in melanization activity. In our study, the observed increase in melanization could be interpreted as a defense mechanism against cadmium toxicity, supported by melanin's known ability to bind aromatic compounds and cations (Banerjee and Mukherjee, 1994; Rajan, 1992). This suggests a potential adaptive response of the fish to environmental stressors, highlighting the intricate interplay between pigmentation dynamics and physiological responses to environmental challenges.

REFERENCES

Anonymous (1962). The wealth of India Raw Material of vol. IV supplement fish and fisheries *Council of Scientific and Industrial Research New Delhi*. 132 pp.
Bagnara JT & ME Hardley (1973). *Chromatophores and Color Changes.* Englewood Cliffs, New Jersey, Prentice Hall.
Banerjee TK & D Mukherjee (1994). Melanophore indexing: a quick bio-assay technique for detection of heavy metal toxicity. *Current Science,* **6** 177-182.
Beena S & Viswaranjan S (1987). Effect of cadmium and mercury on the hematological parameters of the "sh *Cyprinus carpio*. *Environmental Ecology,* **5** 726-730.
Bersenyi A, Fekete Z & Berta E (2003). Effect of ingested heavy metals (Cd, Pb and Hg) on haematology and serum biochemistry in rabbits. *Acta Veterinaria Hungarica*, **51** 297-394.
Boscher A, Gobert S, Guignard C, Ziebel J, L'Hoste L, Gutleb AC, Cauchie HM, Hoffmann, L. & Schmidt, G. (2010). Chemical contaminants in fish species from rivers in the North of Luxembourg: Potential impact on the Eurasian otter (*Lutra lutra*). *Chemosphere*, **78** 785-792.
Canli M (1996). E!ects of mercury, chromium, and nickel on glycogen reserves and protein levels in tissues of *Cyprinus carpio*. *Turkish Journal of Zoology* **20**(2) 161-168.

Carr JA & Patino R (2011). The hypothalamus–pituitary–thyroid axis in teleosts and amphibians: endocrine disruption and its consequences to natural populations. *General and Comparative Endocrinology*, **170**(2) 299–312.

De, A.K. (1996). Environmental Chemistry. New Age International Publishers (P) Ltd. New Delhi, 364 pp.

Dhanapakiam, P. & Ramasamy, V.K. (2001). Toxic effects of copper and zinc mixtures on some haematological and biochemical parameters in common carp, *Cyprinus carpio* (Linn). *Journal of Environmental Biology*, **22**(2) 105 - 111.

El-Boshy, Gadalla, H.A. & El- Hamied, F.M.A. (2014b). Immunological, hematological and biochemical changes induced by short term exposure to cadmium in catfish (*Clarias gariepinus*). *Journal of Coastal Life Medicine*, **2** 175-180

Fingerman, M., Miyawaki & C. Oguro (1963). Effect of osmotic pressure and cations on the response of the melanophores in the Fiddler crab *Uca pugnax* to the melanin dispersing principle from the sinus gland. *General and Comparative Endocrinology*, **3** 495-504.

Gaber, H.S.,El-Kasheif, M.A., Ibrahim, S.A. & Authman, M (2013). Effect of water pollution in El-Rahawy drainage canal on hematology and organs of freshwater fish. *World Applied Sciences Journal*, **21**(3) 329-341.

Garcia-Santos, S., Fontaı´nhas-Fernandes, A. & Wilson, J.M (2006). Cadmium tolerance in the Nile tilapia (*O. niloticus*) following acute exposure: assessment of some iono regulatory parameters. *Environmental Toxicology: An International Journal*, **21** 33–46

Garcia-Santos, S., Fontaínhas-Fernandes, A., Monteiro, S. M. & Wilson, J. M. (2013). Effects of exposure to cadmium on some endocrine parameters in tilapia, *Oreochromis niloticus*. *Bulletin of Environmental Contamination and Toxicology*, **90** 55-59.

Hemalatha, S. & T.K. Banerjee. (1997). Estimation of sublethal toxicity of zinc chloride by histopathological analysis of fish *(Heteropneustes fossilis*, Bloch.) epidemis. Current science, **73** 614-621.

Hogstrand, C, R.W. Wilson., D. Polgar and C.M. Wood . (1994). Effects of zinc on the kinetics of branchial calcium uptake in freshwater rainbow trout during adaptation to waterborne zinc. *Journal of Experimental Biology*, **186**(1) 55-73.

Hontella, A., Daniel, C. & Ricard, A.C. 1996(). Effects of acute and subacute exposures to cadmium on interrenal and thyroid function in rainbow trout, *Oncorhynchus mykiss. Aquatic Toxicology*, **35**(3-4) 171–182.

Jacobs, J.M., Carmicheal, N. & Cavanagh, J.B. (1977). Ultra structural changes in the nervous system of rabbits poisoned with methyl mercury. *Toxicology and Applied Pharmacology*, **39**(2), 249-261.

Jahansson-Sjobeck. & Larsson. (1978). The effect of cadmium on the hematology and on activity of d-molevulinic and dyhydratase (ALA-D) in blood and hemopoietic tissues of the flounder, *pleuronectes aerus* . Environmental research, **17**(2) 191-204.

Klinck, J.,Green, W., Mirza, R., Nadella, S., Chowdhury, M., Wood, C. & Pyle, G. (2007). Branchial cadmium and copper binding and intestinal cadmium uptake in wild yellow perch (*Perca flavescens*) from clean and metal-contaminated lakes. *Aquatic toxicology*, **84**(2) 198-207.

Kori-Siakpere, O.. Ake, J. & Avworo, U. (2006). Sublethal effects of cadmium on some selected haematological parameters of Heteroclarias (a hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*). *International Journal Zoological Research*, **2** 77-83.

Kumar, K. 2016. A review of heavy metals toxicological studies on mangur (*Clarias batrachus*) Fish. *International Journal of Engineering and Management Research (IJEMR)*, **6**(5) 577-583.

Levesque, H., Dorval, J., Hontela, A., Kraak, G. & Campbell, P. (2003). Hormonal, morphological, and physiological responses of yellow perch (*Perca flavescens*) to chronic environmental metal exposures. *Journal of Toxicology and Environmental Health Part A*, **66**(7) 657–676.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R. J. 1951(). Protein measurement with the Folin phenol reagent, *Journal of biological Chemistry*, **193**(1) 265-275.
Nanda, P.& Behera, M.K. 1996. Nickel induced changes in some haematological parameters of a catfish Heteropneustes fossilis (Bloch). *Environmental Ecology* **14** 82-5.
Oliveira, M., Pacheco, M. & Santos, M. (2011). Fish thyroidal and stress responses in contamination monitoring—an integrated biomarker approach. *Ecotoxicology and Environmental Safety*, **74**(5) 1265–1270.
Oliveira, M., Serafim, A., Bebianno, M.J., Pacheco, M.& Santos, M.A. (2008). European eel (*Anguilla anguilla* L.) metallothionein, endocrine, metabolic and genotoxic responses to copper exposure. *Ecotoxicology and environmental safety*, **70**(1) 20–26
Paul, V.I. & T.K. Banerjee. (1996). Analysis of ammonium sulphate toxicity in the catfish *Heteropneustes fossilis* using mucocyte indexing. *Polskie Archiwum Hydrobiologii*, **43** 111-125.
Persch, T.S.P., Weimer, R.N., Freitas, B.S. & Oliveira, G.T. (2017). Metabolic parameters and oxidative balance in juvenile *Rhamdia quelen* exposed to rice paddy herbicides: Roundup®, Primoleo®, and Facet®. *Chemosphere* **174**, 98-109.
Rajan, M.T. (1992). Histopathological Analysis of the toxicity induced by the heavy metal salt mercuric chloride on the skin and accessory respiratory organ of the freshwater catfish *Heteropneustes fossilis* (Bloch.). *PhD Thesis, Banaras Hindu University*, Varanasi, India.
Rajan, M.T. & T.K. Banerjee. (1991). Histopathological changes induced by acute toxicity of mercuric chloride on the epidermis of freshwater catfish *Heteropneustes fossilis* (Bloch.). *Ecotoxicology and Environmental safety* **22**(2) 139-152.
Ramalingam, K. & Ramalingam, K. (1982). Effects of sublethal levels of DDT, malathion and mercury on tissue proteins of *Sarotherodon mossambicus* (Peters). Proceedings: *Animal Sciences*, **91** 501-505.
Reddy, P. S. & Bhagyalakshmi, A. (1994). Changes in oxidative metabolism in selected tissues of the crab (*Scylla serrata*) in response to cadmium toxicity. *Ecotoxicology and Environmental safety*, **29**(3) 255-264.
Ricard, A.C., Daniel, C., Anderson, P. & Hontela, A., (1998). Effect of sub chronic exposure to cadmium chloride on endocrine and metabolic functions in rainbow trout *Oncorhynchus mykiss*. *Archives Environmental Contamination and Toxicology* **34**,377-381.
Rubio, I., Martinez-Madrid, M., Méndez- Fernández, L., Galarza, A. & Rodriguez, P. (2016). Heavy metal concentration in feathers of Little Egret (*Egretta garzetta*) nestlings in three coastal breeding colonies in Spain. *Ecotoxicology* **25** 30-40.
Sjobeck, M. L., Haux, H., Larsson, A. & Lithner, C. (1984). Biochemical and haematological studies on perch, Perca fluviatilis from cadmium contaminated river. *Eman. Ecotoxicology and Environmental Safety*, **8**(3) 303-312.
Suresh, A., Sivaramakrishna, B., Victoriamma, P. C., & Radhakrishnaiah, K. (1991). Shifts in protein metabolism in some organs of freshwater fish *Cyprinus carpio* under mercury stress. *Biochemistry international*, **24**(2) 379-389.
Szebedinszky, C., McGeer, J.C., McDonald, D.G. & Wood, C.M. (2001). Effects of chronic Cd exposure via the diet or water on internal organ-specific distribution and subsequent gill Cd uptake kinetics in juvenile rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry: An International Journal*, **20**(3) 597-607.
Tort, L. & Torres, P. (1988). Three coastal breeding colonies in Spain. *Ecotoxicology*, **25**, 30-40.
The effects of sub lethal concentrations of cadmium on haematological parameters in the dogfish, Scyliorhinus canacula. *Journal of Fish Biology*, **32**(2) 277-282.
Umminger, BL (1977). Relation of whole blood sugar concentration in vertebrate to standard metabolic rates. *Comparative Biochemistry and Physiology*. **55**, 457- 460.

Verbost PM, G Flik, R.A.C. Lock & Wendelaar (1989). Cadmium inhibits plasma membrane calcium transport. *The Journal of Membrane Biology*, **145** 185-197.
Wicklund Glynn, A. & P.E. Olsson. 1991. Cadmium turnover in minnows, *Phoxinus phoxinus* pre-exposed to waterborne cadmium. *Environmental Toxicology and Chemistry: An International Journal*, **10**(3) 383-394.
Zhou, T., John-Alder, H.B., Weis, J.S. & Weis, P. (2000). Endocrine disruption: thyroid dysfunction in mummichogs (*Fundulus heteroclitus*) from a polluted site. *Marine Environmental Research*, **50**(1-5) 393–397.