ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2019; 5(3): 314-319
www.allresearchjournal.com
Received: 16-01-2019
Accepted: 21-02-2019

Dr. Chanda Kumari
PG Department of Zoology, TMBU, Bhagalpur, Bihar, India

Corresponding Author:
Dr. Chanda Kumari
PG Department of Zoology, TMBU, Bhagalpur, Bihar, India

Immune response against pollutants (SSP) in certain air breathing fishes

Dr. Chanda Kumari

Abstract
The quality of human life is directly/indirectly related with the environment around us. Deterioration in the health of any of these components can have serious effects on the continuity of life. Fishes as an important aquatic fauna, serves as an important source of food item. Paddy-cum-fish culture is in great practice in India, where various air-breathing fishes are cultured besides paddy. Spread of agrochemicals in these paddy fields expose such fishes and increase the mortality rate of fishes affecting the economy of our country. Long term exposure of contaminants might increase tolerance in such species. In our experiment, the role of phosphate has more tolerance level in *Channa punctatus* than *Anabas testudineus.* So, it is preferable to use such tolerant varieties in these paddy-cum-fish culture and strengthen our economy.

Keywords: *Channa punctatus, Anabas tesudineus,* Paddy-cum-fish culture, SSP.

1. Introduction
The quality of human life is directly or indirectly related with the environment around us. Abiotic and biotic components including air, water, soil, food, plants and animals constitute our environment, and through complex interactions, life is sustained. Deterioration in the health of any of these components can have serious effects on the continuity of life.

Two third of the earth's surface constitute of the water bodies including rivers, lakes, ponds, streams etc., both marine and the freshwater body. These water bodies are inhabited by the majority of the extant species (both flora & fauna) in different ecological niches; moreover many of them are important source of human food. They play an important role in the ecological balance and the eutrophication of the water body. The aquatic environment therefore plays vital roles for ecosystem functioning, human health and civilization.

Aquatic environment are subjected to low-level and long term exposure of increasing number of new chemicals released continuously (Maccubin & Earsing, 1990; Folmer *et al.*, 1993) [17]. As a consequences of human population growth and industrial development, the production, consumption and disposal of anthropogenic chemicals (Chanda Kumari, 2008; Bruna *et al*., 2008; Bushra *et al.*, 2002; M. Abul Farah, 2003; Bushra Ateeq, 2005; Sanjay *et al.*, 2006; Sanjay *et al.*, 2005; Daoud Ali *et al.*, 2008; Daniela *et al.*, 2008; Chaurasia *et al.*, 2005) [1-9, 18, 22, 26, 27] continue to increase. The aquatic environment remains the ultimate recipient of increasing number of the anthropogenic chemicals, the large proportion of which are potentially genotoxic and carcinogenic substances, where the aquatic organisms are the ultimate sufferers (Lakra, 2009) [16].

28,000 of fish species inhabit these aquatic bodies (Nelson, 2006) [20]. Among them, there are varieties of fish species that are both marine and fresh water, and are being faced by the regular increase in the level of such pollutants. (Jha A.N., 2004; Wirgin and Waldman, 2004; Chanda Kumari, 2008; Bruna *et al.*, 2008; Bushra *et al.*, 2002; M. Abul Farah, 2003; Bushra Afeeq, 2005; Sanjay *et al.*, 2006; Sanjay *et al.*, 2005; Daoud Ali *et al.*, 2008; Daniela *et al.*, 2008) [2-5, 8, 9, 13, 18, 22, 26, 27, 35].

Fishes as an important aquatic fauna, serves as an important source of food item. On, the other hand feeding a large number of increasing population, man depends largely on the agricultural system. To increase the yield of the crop, large number of agrochemicals (pesticides as well as fertilized) are being used. Paddy-cum-fish culture is in great practice in some parts of India as well as Far-East Asia. These paddy-fields are inhabited by many of the air-breathing fish species such as *Anabas* sp., *Channa* sp., *Clarias* sp., Heteropneustes, *Trichogaster* sp. and shrimps. These fishes can live in the shallow water and since they have well developed breathing capabilities, can survive the conditions of the paddy fields. Hence, the spread of such agrochemicals in these paddy fields expose these fishes directly/indirectly to the high concentrations of these agrochemicals. These synthetic fertilizers has led to the large scale contamination of our living environment (http://www.fao.org/docrep/ W2598E/w2598e0.4htm,6.22.2005), through agricultural runoff, leaching grazing etc. The fishes thus are being affected by the residual effects of the fertilizers resulting into regular declination of fish population. Thus, by and large such contaminants impacts not only our economy by increase in mortality rate of the fishes but also our health by producing different types of anatomical, physiological, reproductive and cytogenetical changes (Chanda Kumaria, 2008; Sah *et al.*, 2006; Thakur & Sah 1989a,b, Ram & Sathyanesan, 1987b, Sarkar, 1991; Varadachari, 1992; Wood C.M., 1993; Sah & Ratha, 1998; Srivastava & Srivastava, 1979; Gupta V.K., 2000; Sah *et al.*, 1995) [5, 11, 21-25, 28, 30, 31-33, 36].

Other than physiological effect, very few genotoxic effects are available in air breathing fishes (Chanda Kumari, 2008) [5, 22], or the fresh water fishes even from the most polluted locales. Some substances, when present in low concentration may not cause acute detectable effects in organisms, but may in the long turn, reduce their life span (Nehl S, 2001) [19]. Facing such conditions for a long term, in turn may have developed resistant to such pollutants that bio-accumulate in their tissues. *Atlantic tomcod, Microgadus tomcod* from the lower Hudson River Estuary displayed resistant to AH contaminants at the molecular and organismic levels. Simlarly, Killifish *F. heteroclitus* exhibited resistant at the biochemical and oragnismic levels (Wirgin I & Waldman J.R., 2004) [35]. With, the physiological damage, the damage is lost by the death of the organisms, but once the damage occurs at the molecular level, the damage is subsequently passed from one generation to other and in turn affect those organisms that feed upon such food items. Since so many air-breathing fishes are being cultured in the paddy-field, and living in the similar habitat and are regularly facing the same conditions in the paddy fields, which of them are sensitive or resistant against the effects of the fertilizers, so fish cultivators would like to choose a tolerant variety of fishes for culturing and harvesting to strengthen their economic status. On that basis, we are performing an experiment to find out the genotoxic effect of SSP (Single Super Phosphate as Fertilizer) on chromosomal abnormalities in the two air-breathing fishes i.e. *Channa punctatus* and *Anabas testudineus*, compare the sensitiveness of the two fishes and find the tolerant variety to culture in the paddy fields & thus strengthen their economic status. So, the present study is carried on the conservation and management of the paddy-cum-fish culture, so that we could save the fishes by the unusual effect of fertilizers and also save the human life that feed upon such affected food varieties.

2. Materials and Methods

Specimen

Fresh water fish *Channa punctatus* and *Anabas testudineus* was procured from local market. They are common air-breathing fishes available round the year in pools and water logged marshy areas. The specimen were kept in the aquarium for 10-15 days for acclimatization and fed on minced liver. Healthy individuals having body weight of 25±5kg were randomly allocated to various treatment groups.

Chemicals

SSP is very important and conventional fertilizer containing 16% P_2O_5 (Soluble phosphate), while the triple phosphate yields 46% of soluble phosphate. Upon addition to soil this soluble phosphate are rapidly transformed into an insoluble form either as Ca, Fe or Al phosphate depending on the nature of the soil.

Doses and Treatment

Lethal dose was determined and three relevant sub-lethal doses (SL, HSL and QSL) of phosphate (i.e. 1%, 0.5% and 0.25%) were taken for both the fishes. In each group 12 fishes were taken. To maintain the exposure for the same concentration, the fishes were provided with freshly prepared doses of SSP. The animals were sacrificed immediately after 7 days of termination of treatment. A separate concurrent control were carried out.

Chromosomal abnormalities

0.3% colchicine was injected intra-muscularly at the rate of 1ml/100gm body weight to arrest the cells at the metaphase stage. The head kidney were taken out and macerated finally. Cell suspension was formed by the addition of phosphate buffer, incubate if for 40 minutes at room temperature, few drops of fixative (3 parts methanol + 1 part glacial acetic acid) were added followed by centrifugation at 1000 rpm for 10 minutes thrice. Pellets were obtained, mixed with 1 ml fixative and the slides were prepared by dropping the cell suspension from the height of about 40-50 cm on the grease free clean slides previously chilled in 70% ethanol Giemsa stain for 30-40 minutes; followed by washing in running tap water and air-dried. 300 metaphase plates were screened at the rate of 20 metaphase plates per slides both for treated and control variants.

3. Results and Discussion

For chromosomal abnormalities, 300 well spread metaphase plates were studies. After phosphate treatment, 16.3%, 18.0% and 20.6% abnormalities in *Channa* with respect to (5.33%) of control variant. Here, the abnormalities were increased by four fold. Whereas, 18.3%, 21.0% and 24.6% abnormalities were increased in contrast to (6.6%) of control. Here also four fold abnormalities were increased (Table-1-2) (Graph-1-2).

A quantitative estimation reveals that the abnormalities increased with the increase of the doses. Thus, the effect was dose dependent. The individual type of damages was more prominent than the gross type (Plate-I, 1-4) (Plate-II, 1-4) because SSP is synthetic in nature. While Chaurasia and Sinha, 1990 [1, 5-7, 22]; Chaurasia *et al.*, 2005 [1, 5-7]; Chanda Kumari, 2008 [5, 22] were studying on genotoxicity induced by fertilizers & silk dying wastes; they observed that the individual type of damages were more frequent than the gross type. The differential sensitivity might be occurred at two different levels. First, the damages at the protein level, either on spindle protein or on protein packing. Second, by the production of electrophilic ions & reactive radicals during the metabolization of mutagens (Chanda Kumari, 2008) [5, 22]. Such electrophilic reactive ions/radicals might attack to the nucleophilic site of the DNA leading to structural changes in chromosomes (Awasthy *et al.,* 1999)

[1]. Phosphate is present in the nucleic acid (nucleotide bases), so it can bring about conformational changes in the components of DNA.

When the overall effect of SSP was evaluated in both the air-breathing fishes, it was found that *Anabas testudineus* was more sensitive than the *Channa punctatus*. Since, *Channa punctatus* have an efficient respiratory system and can tolerate higher concentrations of pollutants than *Anabas testudineus* (Saha *et al*., 1994) [23, 24].

The degree of tolerance exhibited by any fish is dependent upon inherent ability (Wirgin and Waldman, 2004) [35]. The acclimation occurs at the level of the individual, where organisms become more resistant as a consequence of earlier exposure (Weis *et al*., 1999, Klerks *et al*., 1997) [14, 15, 34], and the genetic adaptation may result in altered allele frequencies at selected loci (Shaw 1999, Klerks and Bathalomew, 1991) [14, 15, 29]. As above mentioned, it might be possible that *Channa's* inherent ability can create a tolerance in the genomes resulting into less damage in the genetic material than the *Anabas testudineus.*

From the conservation point of view, since, both the fishes live almost in the similar habitat, cultured in the same paddy crops, being faced by same amount of fertilizers, and from my experiment it has been viewed that *Channa punctatus* is more resistant/tolerant against such pollutant than *Anabas testudineus*, so *Channa* is suitable and safer for the paddy-cum-fish culture rather using *Anabas*, and commercially beneficial for the farmers, fish cultivators and ultimately saving the health of the human being.

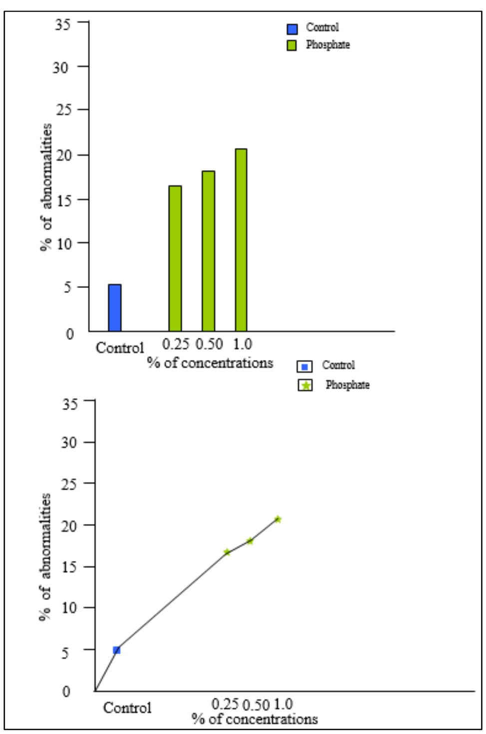
Table 1: Frequency of Chromosomal abnormalities (% S.E., N = 300) induced by SSP in *Channa punctatus*

Treatment	Abnormal Metaphase				Chromosomal Abnormalities																Grand Total			
					Gross								Individual											
	No.	%	±	S.E.	Poly ploidy	Hypo ploidy	C-mitosis	Sticki-ness	No.	%	±	S.E.	M.F.	A.F.	CTB	CTG	No.	%	±	S.E.	No.	%	±	S.E.
Control	15	5.00	±	1.25	1	-	-	-	1	0.33	±	0.33	9	4	2	-	15	5.00	±	1.25	16	5.33	±	1.29
SSP																								
0.25%	49	16.3	±	2.13*	1	2	-	-	3	1.00	±	0.57	18	21	6	1	46	15.3	±	2.07*	49	16.3	±	2.13*
0.50%	54	18.0	±	2.21*	2	1	-	1	4	1.33	±	0.66	20	23	4	3	50	16.6	±	2.14*	54	18.0	±	2.21*
1.00%	62	20.6	±	2.33*	4	2	-	-	6	2.00	±	0.76	21	25	8	2	56	18.6	±	2.24*	62	20.6	±	2.33*

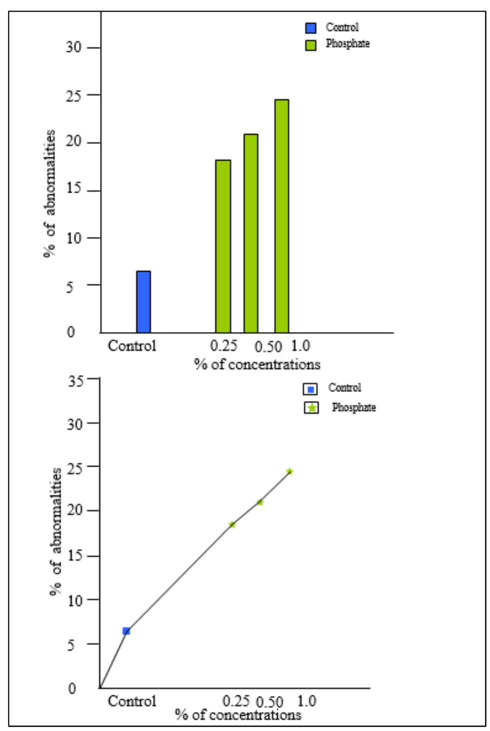
* Indicates significant difference at 5% level with corresponding value in the control.

Table 2: Frequency of Chromosomal abnormalities (% S.E., N = 300) induced by SSP in *Anabas testudineus*

Treatment	Abnormal Metaphase				Chromosomal Abnormalities																Grand Total			
					Gross								Individual											
	No.	%	±	S.E.	Poly ploidy	Hypo ploidy	C-mitosis	Sticki-ness	No.	%	±	S.E.	M.F.	A.F.	CTB	CTG	No.	%	±	S.E.	No.	%	±	S.E.
Control	20	6.6	±	1.43	-	2	1	-	3	1.00	±	0.57	10	3	4	-	17	5.6	±	1.32	20	6.6	±	1.43
SSP																								
0.25%	55	18.3	±	2.23*	1	2	-	-	3	1.00	±	0.57	20	29	10	3	52	17.3	±	2.18*	55	18.3	±	2.23
0.50%	63	21.0	±	2.35*	2	1	1	-	4	1.33	±	0.66	20	26	13	-	59	19.6	±	2.29*	63	21.0	±	2.35*
1.00%	74	24.6	±	2.48*	7	-	-	-	7	2.33	±	0.87	24	27	14	2	67	22.3	±	2.40	74	24.6	±	2.48*



Graph 1: Graph showing Chromosomal abnormalities induced by SSP in *Channa punctatus*



Graph 2: Graph showing Chromosomal abnormalities induced by SSP in *Anabas testudineus*

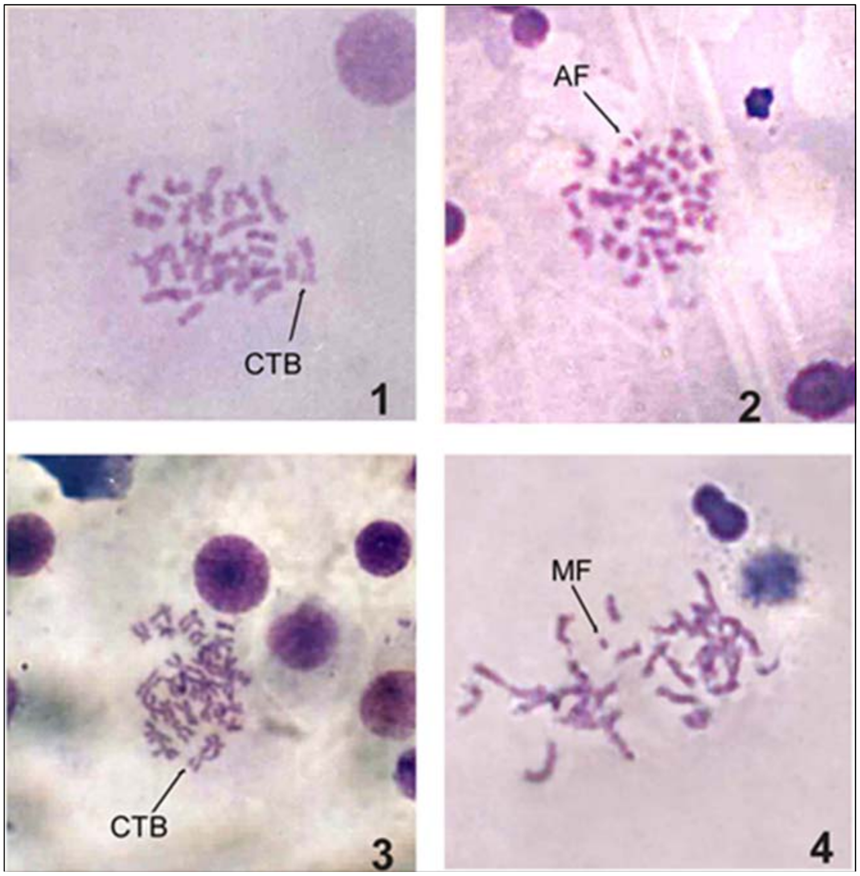


Plate 1: Abnormalities in *Channa punctatus*

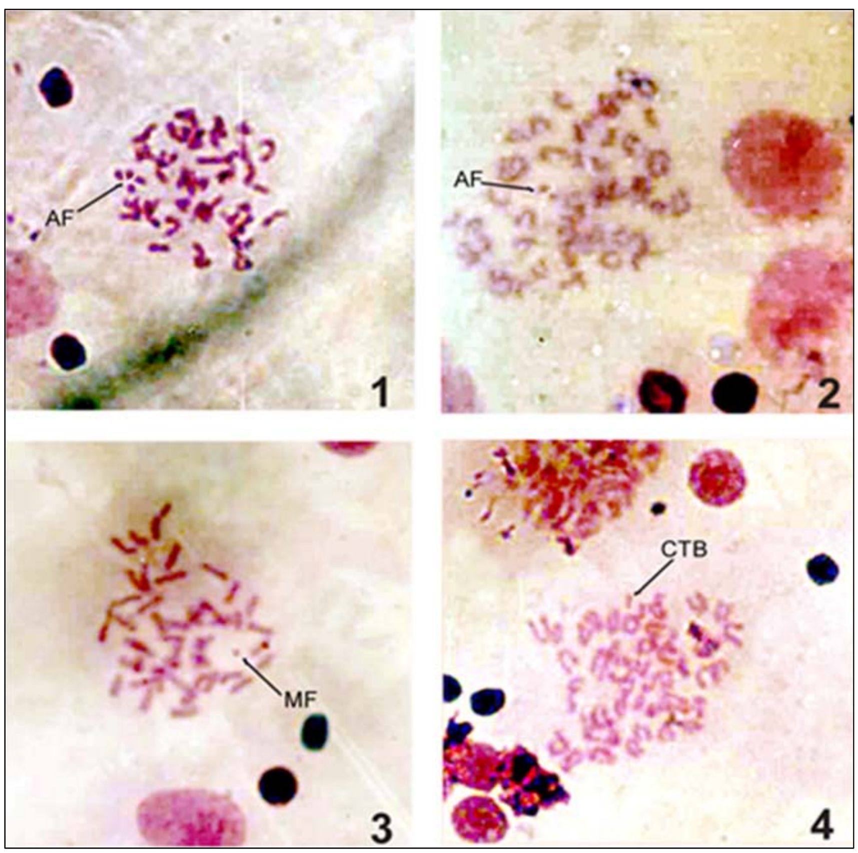


Plate 2: Abnormalities in Anabas testudineus

4. References

1. Awasthy KS, Chaurasia OP, Sinha SP. Prolonged Murine Genetoxic effects of Crude Extracted from Neem. Pytotherapy Research 1999;13:81-83.
2. Bruna de Campos Ventura, Dejanira de Fransceschi de Angelis, Maria Aparecida Marin – Morales Mutagenic and genotoxic effects of the Atrazine herbicide in *Oreochromis niloticus* (*Perciformes*, *Cichlidae*) detected by the micronucleus test and the comet assay, Pesticide Biochemistry and Physiology 2008;90:42-51.
3. Bushra Ateeq, M. Abul Farah, M. Niamat Ali, Waseem Ahmad. Induction of micronuclei and erythrocyte alterations in the catfish *Clarias batrachus* by 2, 4-dichlorophenoxyacetic acid and butachlor, Mut. Res 2002;518:135-144.
4. Bushra Ateeq, M. Abul Farah, Waseem Ahmad. Detection of DNA damage by alkaline single cell gel electrophoresis in 2, 4-dichlorophenoxyacetic acid and butachlor exposed erythrocytes of *Clarias batrachus*, Ecotoxicology and Environmental Safety 2005;62:348-354.
5. Chanda K, Chaurasia OP. Induction of Mitotic-Chromosome Anomalies and Micronucleus Test in *Channa punctatus* by Single Superphosphate 2008;9:70-73.
6. Chaurasia OP, Sinha SP. Induction of Mitotic Chromosome anomalies in mice by muriate of potash, Cytologia 1990;55:87-90.
7. Chaurasia OP, Alok Kumar, Moushmi Kumari. Genotoxic Effect of Silk Dyeing Wastes in Bone Marrow Cells of Mice, *Mus musculus*, Cytologia 2005;70(4):381-384.
8. Daniela Morais Leme, Maria Aparecida Marin – Morales. Chromosome aberration and micronucleus frequencies in *Allium cepa* cells exposed to petroleum polluted water, Mut. Res 2008;650:80-86.
9. Daoud Ai, Nagpure NS, Sudhir Kumar, Ravindra Kumar, Kushwaha B. Genotoxicity assessment of acute exposure of chlorpyrifos to fresh water fish *Channa punctatus* using micronucleus assay and alkaline single-cell gel electrophoresis, Chemosphere 2008;71:1823-1831.
10. Folmar LC, Gardener GR, Hickey J, Bonomelli S, Moody TY. Serum chemistry and histopath logicalo evaluation of brown bull heads (*Ameriurus nobulosus*) from Buffalo and Niagra rivers, New York, Arch. Environ. Contam. Toxicol 1993;25**:**298-303.
11. Gupta VK, Sharma JP, Verma AK. Rural Dev Unit, Regl Res Lab (CSIR), Canal Rd, Jammu (180001). Effect of some fertilizers on the early developmental stages of *Cyprinous carpio.* Linn. Poll. Res 2000;19(3):369-375.
12. (http://www.fao.org/docrep/W2598E/w2598e,0.4htm,6-22.2005). Introduction to agriculture water pollution page 1 of 19.
13. Jha AN. Genotoxicological studies in aquatic organisms, Mut. Res 2004;552:1-17.
14. Klerks PL, Batholomew PR. Cadmium accumulation and detoxification in a Cd-resistant population of the oligochaete Limnodrilus hoffmeisteri, Aquat. Toxicol 1991;19:97-112.
15. Klerks PL, Leberg PL, Lance RF, Mc Millin DJ, Means JC. Lack of development of pollutant – resistance or genetic differentiation of darter gobies (Gobionellus boleosoma) inhabiting a produced water-discharge site, Mar. Environ. Res 1997;44:377-395.
16. Lakra WS, Nagpure NS. Genotoxicological studies in fishes, Indian Journal of Animal Science 2009;79(1): 93-98.
17. Maccubin AE, Earsing N. Tumors in fish from Detroit river. Hydrobiologia 1990;219:301-306.
18. M. Abul Farah, Bushra Ateeq, M. Niamat Ali, Waseem Ahmad. Evaluation of genotoxicity of PCP and 2, 4-D by micronucleus test in freshwater fish *Channa punctatus*, Ecotoxicology and Environmental Safety 2003;54:25-29.
19. Nehl S, Senger H. Detection of DNA damage in two cell lines from rainbow trout RTG-2 and RTL-W1, using the comet assay, Environ. Toxicol 2001;16:321-329.
20. Nelson JS. Fishes of the World, John Wiley and Sons, Inc 2006.
21. Ram RN, Sathyanesan AG. Histopathological changes in liver and thyroid of the teleost fish, *Channa punctatus* (Bloch) in response to ammonium sulphate fertilizer treatment. Ecotoxicol. Environ. Safety 1987b; 13:185-190.
22. Sah NP, Mishra S, Sinha SK, Kumari P, Thakur GK. Effects of increasing time period of exposure of SSP (fertilizer) on corpuscular haematology of an air-breathing fish *Channa punctatus* (bloch.). J. Haematol. and Ecotoxicol 2006;1(1):6-12.
23. Saha N, Ratha BK. Ureogenesis in Indian air-breathing teleost: adaptation to environmental constrains, Comp. Biochem. Physiol. A 1998;120:195-208.
24. Saha TK, Arya MB, Das AB. Alterations in the patterns of nitrogenous excretion in a freshwater air-breathing teleost, *Channa punctatus* (Bloch), during ammonia stress, Ind. Jour. of. Expt. Biol 1994;32:196-199.
25. Sah TK, Das NP, Arya MB, Das AB. Effect of ammonia stress on the tissue levels of nitrogenous biomolecules of the air-breathing fish *Channa punctatus* (Bloch). Ind. Jour. of. Exp. Biol 1995;33:943-952.
26. Sanjay Pandey, Ravindra Kumar, Shilpi Sharma, Nagpure NS, Satish KS, Verma MS. Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish *Channa punctatus*, Ecotoxicology and Environmental Safety 2005;61:114-120.
27. Sanjay Pandey, Nagpure NS, Ravindra Kumar, Shilpi Sharma, Satish KS, Mahendra SV. Genotoxicity evaluation of acute doses of endosulfan to freshwater teleost *Channa punctatus* (Bloch) by alkaline single cell-gel electrophoresis, Ecotoxicology and Environmental Safety 2006;65:56-61.
28. Sarkar SK. Use of ammonium sulphate nitrate in rearing major carp spawn; Geobios 1991;18:177-181.
29. Shaw AJ. The evolution of heavy metal tolerance in plants: adaptations, limits and costs, in VE Forbes (Ed), Genetics and Ecotoxicology, Taylor and Francis, Philadelphia, PA 1999, 9-30.
30. Srivastava VMS, Srivastava DK. Adaptive response of the fish fingerlings under different urea radiants with their physical behaviour and metabolic changes, IInd All India Seminar on Ichthyology. Abs 1979;93:62.
31. Thakur GK, Sah NP. Impact of increasing time period of exposure of urea on the haematological changes in an air-breathing fish *Channa punctatus* (bloch) Environ

risk assessment (eds. Sahai YN, Deshmukh PB, Mathai TA, Pillai KS), The Academy of Environmental Biology, India 1989, 261-265

32. Thakur GK, Sah NP. Effect of increasing concentrations of Single Super Phosphate (SSP) on corpuscular haematology of an air-breathing fish *Channa punctatus* (bloch). Proc. Nat. Symp. Emerg. Tr. Anim. Haematol 1989, 1-9.
33. Varadachari C. Phosphoric acid, phosphates and fertilizers for the future, Proc. Indian. Natl. Sci. Acad. B 1992;58:119-126.
34. Weis JS, Mugue N, Weis P. Mercury tolerance population effects and population genetics in the mummichog, *Fundulus heteroclitus* in, VE Forbes (ed.), Genetics and Ecotoxicology, Taylor and Francis, Philadelphia 1999, 31-54.
35. Wirgin I, Waldman JR. Resistance to contaminants in North American fish populations, Mut. Res 2004;552:73-100.
36. Wood CM. Ammonia and urea metabolism and excretion in the physiology of fishes (ed.) DH Evans (Boca Raton: CRC Press) 1993, 379-425.