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Monitoring of Nuclear Abnormality Frequencies as Indicators of Environmental Pollution in Peripheral Erythrocytes of *Labeo rohita* Reared in Lakes of Bangalore

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Abstract

The present study was aimed to evaluate genotoxicity in peripheral erythrocytes of *Labeo rohita* reared in two lakes viz., Vengaiah lake - sewage polluted (Lake A) and Yellamallappa Chetty lake - industrially polluted (Lake B) of Bangalore. To assess the micronuclei and nuclear abnormalities in such erythrocytes, blood samples were collected from heart of the freshwater fish, *L. rohita* anesthetized by MS222. The results were compared with the fish reared in the Hebbal fish farm (Control). The data revealed significantly high frequencies of erythrocytic abnormalities including nuclear as well as cytoplasmic in the fish blood sampled from lake B when compared to those of lake A. Such abnormalities in erythrocytes of fish varied seasonally also with summer exhibiting maximum deformities which can be attributed to the presence of genotoxic pollutants in the selected water bodies. The values were statistically significant at P<0.0001.

Keywords: Blood, Erythrocytes, Genotoxicity, *Labeo rohita,* Pollutants

1. Introduction

Surface waters, such as lakes, rivers, and seas contain complex mixtures of pollutants including genotoxic compounds due to the anthropogenic action, which cause adverse effects on public health and aquatic ecosystems[26]. Aquatic environment serves as convenient repositories for man's biological and technological wastes and current awareness of the potential hazards of pollutants in the aquatic environment has stimulated much interest in the use of fish as indicators due to their position in the trophic chain, their sensitivity to low concentrations of genotoxic substances and their ability to metabolize xenobiotics and accumulate pollutants[40]. Hematological study is important in toxicological research because a hematological alteration is a good method for rapid evaluation of the chronic toxicities of a compound. Blood parameters are useful for the measurement of physiological disturbances in stressed fish and thus provide information about the level of damage in the fish[28]. A thin epithelial membrane separates fish blood from the water and any unfavorable change in the water body is reflected in the blood[22]. The study of blood characteristics may corroborate important subsidies of diagnoses and prognoses of morbid conditions in fish populations and therefore, contribute to better comprehending comparative physiology, phylogenetic relations, feeding conditions and other ecological parameters[28].

Among the currently available procedures, micronuclei and nuclear abnormalities assays are the most widely applied methods due to its proven suitability for fish species[7,21]. Nuclear abnormalities, such as micronuclei and other nuclear malformations are considered good indicators of cytotoxicity and genotoxicity, respectively[21]. For the determination of genotoxic effect in fish, the micronucleus test as well as the study of the abnormal shape of nuclei is a suitable measure with which the

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presence or absence of genotoxins can be detected in water. The detection of MN and NAs in fish helps us to assess the status of water quality as well as the health of a particular species and any potential risk it might have after consumption[37].

Schroder (1966) studied the formation of micronuclei in mammalian bone marrow cells for the first time subsequently this assay was developed by Schmid (1975) in mammalian systems. The MN are also known as Howell-jolly bodies in mammals. Like mammalian species, MNT has also been adopted to study genotoxicity in fishes. The formation of morphological nuclear alterations (NAs), was first described in fish erythrocytes by Carrasco *et al.* (1990). The unique information offered by MNT as a bioindicator for chromosomal aberrations is not available from other methods such as the integrated effect of a variety of environmental stresses on the health of an organism and the population, community, and ecosystem; and the effectiveness of remediation efforts in decontaminating waterways[42]. NAs including blebbed, lobed and notched nuclei and binucleated cells, have been used by several authors as possible indicators of genotoxicity[9].

The present investigation was conducted to analyse the blood samples for erythrocytic abnormalities in edible fish *Labeo rohita* reared in fresh water lakes of Bangalore as a consequence of variation in physico chemical parameters of water including detected trace metals.

2. Materials and Methods

2.1 Study Area

Bangalore also called as Bengaluru is the capital of Karnataka state in South India (Figure 1). It is located at 12.97°N 77.56°E and covers an area of 741 km^2. The two lakes, Vengaiah lake (Lake A - Area 65 acres; depth 8-10 feet) and Yellamallappa Chetty lake (Lake B - Area 110 ha; depth 10-12 feet) situated near Krishnarajpuram - Hoskote taluk, Bangalore District, Karnataka were selected for the study (Figure 2). Lake A received domestic sewage from an adjacent storm-water drain and lake B those of effluents from pharma-industry and other sources. Hebbal fish farm, which is maintained by the fisheries department was taken as reference site (Control).

2.2 Analysis of Water Samples

Water along with the test fish were sampled from Control site, Lake A and Lake B during early morning hours (6:30-7:30 a.m.). The physico-chemical parameters like temperature, pH, BOD, COD, DO, TDS, conductivity, acidity, alkalinity, phosphates, sulphates, nitrates and trace metals such as mercury, lead, aluminium, cadmium, etc of the water samples collected from control, lake A and B were determined by following standard methods by APHA *et al.*, 2005 and atomic absorption spectrophotometry (USEPA, 1983). The values obtained were compared with the Bureau of Indian Standards BIS:10500-1991 (Revised 2012) for lakes. Water quality index was calculated by following Brown *et al.*, 1972; Chatterjee and Razi uddin, 2000.

2.3 Analysis of Fish Blood Samples

Labeo rohita was selected as animal model for the present study. Blood was drawn out by Cardiac puncture with a heparinized syringe of test fish previously anesthetized in MS222 by stabbing body wall exactly in midline from the posterior margin of opercular cover and directed dorso-caudally at an angle of 45^0[,24]. A thin blood smear was spread on a clean dry slide (2x4x1mm), fixed in methanol and was left to air-dry. It was then stained with Leishman's stain[32]. The sample's smear was inspected using Zeiss Axidskop Plus microscope connected with camera and computer, equipped with image viewing analysis system. 1,000 erythrocytes of blood smear sampled from fish of lake A, lake B and control site were viewed and classified. For the scoring of micronuclei, the criterion was adopted from Fenech *et al.* (2003). MN if present should have similar staining as the main nucleus. They should be separated from or marginally overlap with main nucleus as long as there is clear identification of the nuclear boundary. Cellular and nuclear anomalies observed were registered and photographed, and their frequency was calculated.

3. Statistical Analysis

Statistical analysis was carried by using MS Excel and statistical software - Graphpad prism 6.05 to evaluate the physico chemical parameters with respect to three water bodies and erythrocytes of fish. Mean of the water and frequency of erythrocytic abnormalities [size (n = 6)] and standard deviation (mean ± SD) were calculated to quantify their variability which was followed by one way ANOVA to compare significant mean differences of the above mentioned groups. This was followed by Tukey's post-hoc test to compare pair of groups mean of water parameter in water and with erythrocytic abnormalities in fish blood of control group, lake A and lake B. p value at a

Figure 1. Map of India showing location of Karnataka state and the capital city, Bangalore.

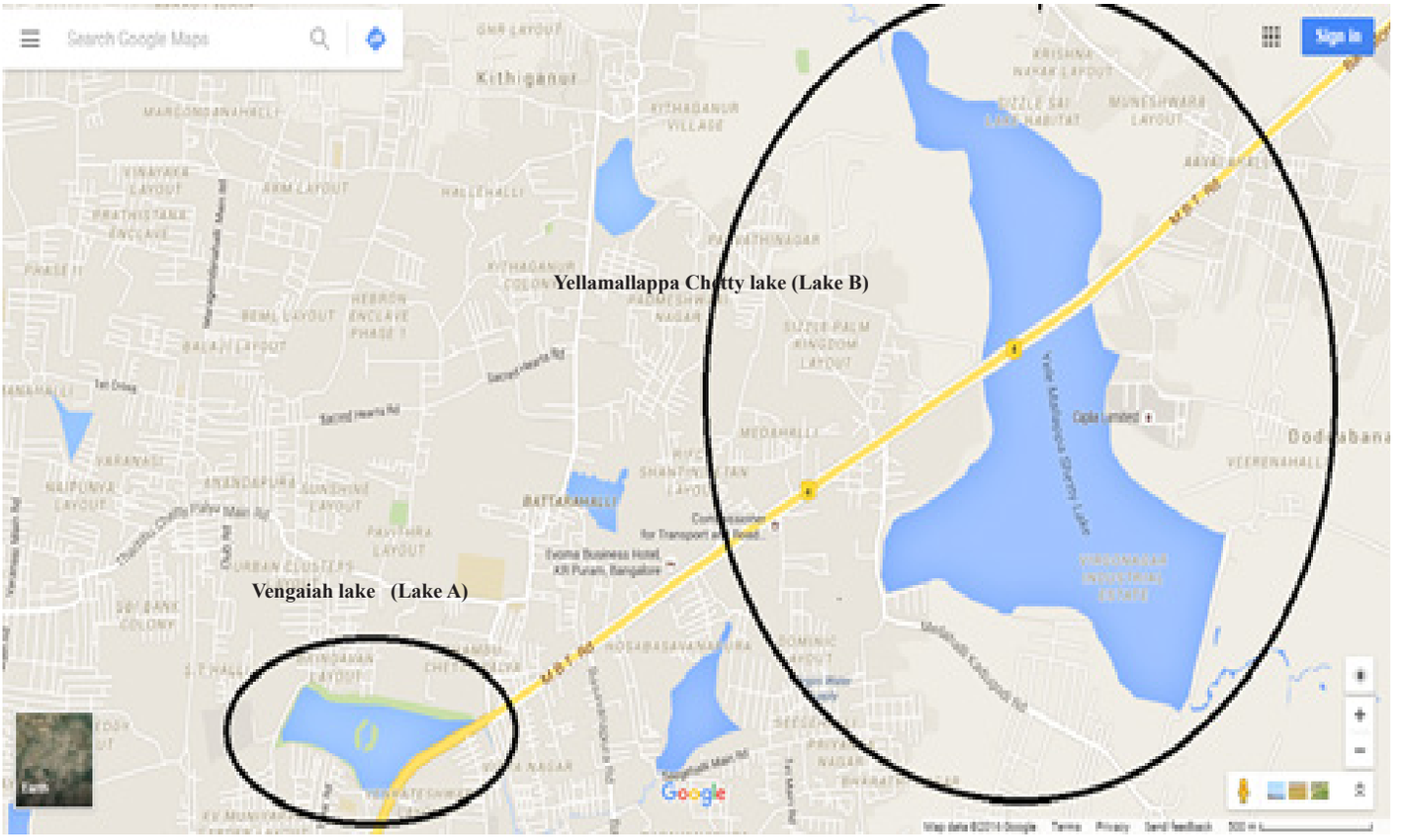


Figure 2. Representation of sampling location: Vengaiah lake (Lake A) and Yellamallappa Chetty lake (Lake B).

significant level of $p<0.05$ or less indicated significant relationship within variables. Pearson's correlation coefficient between physico chemical parameters of water sampled from control site and lakes (A & B) and frequency of erythrocytic abnormalities in blood samples of fish collected from these water bodies was also studied.

4. Results and Discussion

Physico-chemical parameters are the basic parameters to determine whether a lake is polluted or non polluted. Physico-chemical parameters of water sampled from control site were compared with lake A and lake B and inturn with the standard BIS: 10500-1991 (Revised 2012) taking into consideration the three different seasons viz., winter, summer and rainy season. The data was statistically analyzed and is represented in table I & II showing the significant mean differences at $p<0.001$ and 0.01. The data revealed high level of pollution in lake B (during all seasons) when compared to lake A, control site and BIS standard. The high levels of temperature, total suspended solids, chemical oxygen demand, biological oxygen demand, conductivity, turbidity, alkalinity and of trace metal content such as, aluminium, cadmium, copper, iron, lead and mercury was recorded in water samples of lake B. These results might be due to the discharge of industrial effluents from the pharma industry present on the banks of lake B and the agricultural runoff, idol immersion during festival season and discharge of domestic sewage and solid waste through various sources into the water body. The water analysis of lake B indicated significantly high level of trace metals along with other water parameters which were recorded above the BIS limits. This rendered the water quality to be very poor as per water quality index (WQI) (Table III) suggested by Chatterji and Raziuddin (2002) (Table IV) and unsuitable for drinking purpose. Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and on

Table 1. Physico-chemical parameters of water sampled from Hebbal fishfarm (Control site), Vengaiah lake (Lake A) and Yellamallappa Chetty lake (Lake B) during winter, summer and rainy season

Parameters	Standards BIS: 10500-1991 **(Revised 2012)**	**Control site**	**Winter season**		**Summer season**		**Rainy season**	
			Lake A	**Lake B**	**Lake A**	**Lake B**	**Lake A**	**Lake B**
Temperature	22 - 28	26 ± 0.63	21 ± 0.89 (-19.23)	22 ± 1.26 (-15.38)	26 ± 0.63 (0.00)	28 ± 1.26 (7.69)	22 ± 1.26 (-15.38)	24 ± 2 (-7.69)
Colour	5 - 25	3.1 ± 0.63	4.8 ± 0.63 (54.05)	7.3 ± 0.84 (136.22)	4.3 ± 0.63 (39.46)	6.1 ± 0.49 (98.38)	5.1 ± 0.45 (63.78)	8.6 ± 0.39[b] (178.38)
Odour	UOB	UOB	UOB	Fishy	UOB	Fishy	UOB	Fishy
pH Value	6.5-8.5	7.87 ± 0.08	7.09 ± 0.33 (-9.89)	7.22 ± 0.69 (-8.26)	7.65 ± 0.16 (-2.75)	6.85 ± 0.74 (-12.92)	7.65 ± 0.30 (-2.75)	7.34 ± 0.54 (-6.67)
Turbidity	5 - 20	7.8 ± 0.15	24 ± 2 (207.69)	33 ± 2.62 (322.01)	21 ± 0.89 (169.23)	34.2 ± 2.14 (338.03)	31 ± 1.26 (297.44)	42.7 ± 2.73[b] (447.01)
Conductivity	300	644 ± 64.70	654 ± 12.61 (1.63)	988 ± 1.64[b] (53.42)	837 ± 42.80 (29.98)	1207 ± 35.15[b] (87.57)	704 ± 5.47 (9.43)	1087 ± 2.07[b] (68.80)
TDS	500 - 2000	420 ± 7.69	523 ± 32.86 (24.62)	793 ± 2.42[b] (89.04)	750 ± 1.41 (78.71)	985 ± 2.93[b] (134.75)	570 ± 3.56 (35.90)	724 ± 3.25[b] (72.48)

Table 1 Continued

TSS	100	92 ± 0.82	162 ± 0.55 (76.18)	287 ± 1.64[b] (212.55)	150 ± 0.82 (64.00)	260 ± 4.73[b] (183.64)	180 ± 2.10 (96.36)	376 ± 2.88[b] (310.55)
Acidity	20	32 ± 0.52	31 ± 0.82 (-1.05)	76 ± 2.34[b] (138.95)	48 ± 1.21 (50.53)	95.7 ± 1.86[b] (202.11)	33.2 ± 1.17 (4.74)	70.4 ± 1.02[b] (122.37)
Total alkalinity	200 - 600	202 ±1.38	221 ± 0.82 (9.84)	470 ± 6.79[b] (133.17)	290 ± 1.60 (44.00)	544 ± 11.07[b] (174.86)	215 ± 0.84 (6.45)	464 ± 2.32[b] (130.36)
Nitrates	45 - 100	2.13 ± 0.28	3.17 ± 0.17 (48.94)	5.2 ± 0.30 (145.89)	2.25 ± 0.20 (5.87)	3.87 ± 0.10 (81.68)	2.58 ± 0.16 (21.38)	4.8 ± 0.10 (123.18)
Sulphates	200 - 400	62 ± 0.52	128 ± 0.55 (106.76)	253 ± 1.75[b] (310.81)	103 ± 1.21 (66.49)	210 ± 0.52[b] (241.08)	154 ± 0.55 (148.92)	276 ± 0.55[b] (346.76)
Total phosphorus	-	0.35 ± 0.01	2.08 ± 0.01 (500.00)	3.98 ± 0.01 (1046.63)	1.02 ± 0.01 (192.79)	2.42 ± 0.01 (598.56)	1.75 ± 0.01 (403.85)	3.52 ± 0.02 (915.87)
DO	4 - 6	3.7 ± 0.05	3.8 ± 0.08 (3.20)	1.7 ± 0.10 (-54.34)	3.7 ± 0.05 (0.00)	1.2 ± 0.08 (-68.04)	4.3 ± 0.09 (17.81)	2.8 ± 0.08 (-24.20)
BOD	2 - 6	6 ± 0.76	21 ± 1.17 (262.32)	97 ± 2.07[b] (1578.26)	24 ± 1.21 (323.19)	113 ± 1.33[b] (1868.12)	18 ± 0.98 (210.14)	92 ± 1.21[b] (1505.80)
COD	200	76 ± 1.72	153 ± 1.51 (102.20)	377 ± 1.37[b] (396.70)	126 ± 2.25 (66.59)	374.7 ± 2.88[b] (394.07)	164 ± 0.89 (116.26)	484 ± 1.67[b] (538.24)

Values are expressed in mg/l except -Temperature - (° C), Colour - (Pt-Co scale), Odour, pH, Turbidity - (NTU) and Conductivity - (mmho / cm). Values are expressed as mean ± SD where, n = 6. Values in parenthesis represent percent change (%). UOB - Unobjectionable. The superscripts a, b, c and d indicate statistical mean differences at $p < 0.0001$, 0.001, 0.01 and 0.05 respectively.

Table 2. Trace metals in water sampled from Hebbal fishfarm (Control site), Vengaiah lake (Lake A) and Yellamallappa Chetty lake (Lake B) during winter, summer and rainy season.

Parameters	Standards BIS: 10500-1991 **(Revised 2012)**	**Control site**	**Winter season**		**Summer season**		**Rainy season**	
			Lake A	**Lake B**	**Lake A**	**Lake B**	**Lake A**	**Lake B**
Arsenic	0.05	**0**	0	0.008 ± 0.001	0	0.003 ± 0.001	0.001	0.0027
Copper	0.05 – 1.5	0.013	0.03 (133.77)	0.39 ± 0.01 (2912.99)	0.03 (133.77)	0.32 ± 0.01 (2419.48)	0.028 (118.18)	0.25 ± 0.01 (1874.03)
Zinc	5 - 15	0.54 ± 0.02	2.88 (438.32)	3.34 ± 0.03 (523.99)	1.88 ± 0.01 (251.09)	2.68 ± 0.01 (401.56)	1.67 ± 0.01 (212.46)	3.12 ± 0.01 (482.87)
Aluminium	0.03 – 0.2	**0**	0.043 ± 0.001	3.9 ± 0.055	0.067 ± 0.002	3.7 ± 0.089 [b]	0.067 ± 0.001	3.7 ± 0.089[b]
Cadmium	0.01	0.001	0.04 ± 0.01 (5150.00)	0.184 (27550.00)	0.04 ± 0.01 (5150.00)	0.124 (18450.00)	0.04 ± 0.01 (5150.00)	0.103 (15375.00)
Iron	0.3 - 1	0.04 ± 0.008	0.14 ± 0.024 (269.57)	3.92 ± 0.056 (10113.04)	0.13 ± 0.022 (243.48)	3.68 ± 0.004 (9504.35)	0.11 ± 0.008 (191.30)	3.02 ± 0.006 (7778.26)
Lead	0.05	0.004 ± 0.001	0.04 ± 0.012 (947.62)	0.51 (14471.43)	0.04 ± 0.012 (947.62)	0.37 ± 0.020 (10376.19)	0.04 ± 0.008 (995.24)	0.23 (6471.43)
Mercury	0.001	**0**	0	0.03 ± 0.01	0	0.028	0	0.023

Values are expressed in mg/l except -Temperature - (° C), Colour - (Pt-Co scale), Odour, pH, Turbidity - (NTU) and Conductivity - (mmho /cm). Values are expressed as mean ± SD where, n = 6. Values in parenthesis represent percent change (%). UOB - Unobjectionable. The superscripts a, b, c and d indicate statistical mean differences at $p < 0.0001$, 0.001, 0.01 and 0.05 respectively.

Table 3. Water quality index (WQI) of control site, lake A and lake B during three seasons

Water body	Seasons		
	Winter	Summer	Rainy
Control	1.07		
Lake A	36.45	36.52	36.53
Lake B	2802.19	2570.12	2110.99

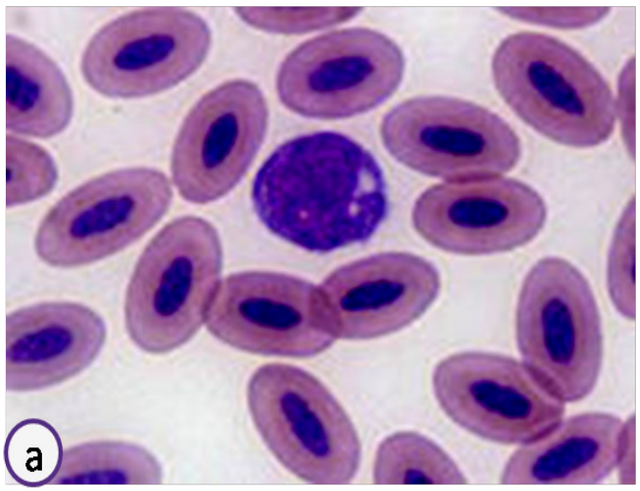
Table 4. Water Quality Index (WQI) and status of water quality (Chatterji and Raziuddin 2002)

Water Quality Index level	Water quality status
0 – 25	Excellent water quality
26 – 50	Good water quality
51 – 75	Poor water quality
76 – 100	Very poor water quality
>100	Unsuitable for drinking

the diversity of aquatic organisms[11] and are detrimental to the aquatic inhabitants, including fishes[27].

Micro nuclei test is recommended to be conducted as part of the monitoring protocols in aquatic toxicological assessment programs[26, 39]. The *in vivo* micronuclei frequency assay has been widely used as a technique for genotoxicity monitoring of polluted aquatic media and in the screening for the presence of toxic compounds suspected to be genotoxic[29, 45]. Morphological alterations in the nuclear envelope in erythrocytes of fish as described by Carrasco *et al.* (1990) are considered indicators of genotoxic damage and constitutes a complementary analysis to the scoring of micronuclei.

Change in the general structure of erythrocytes such as deformation and swelling along with few nuclear abnormalities was observed in the blood samples of fish from lake A (Figure 1a - 1b). Large number of nuclear abnormalities which can be classified as blebbed, notched, lobed[6], eight shaped[14] and pear shaped nucleus along with ruptured nuclear membrane and oozed out nuclear mass (Figure 1a - 1c) were noted from fish of lake B and are in similar lines with Moharram *et al.* (2011). They recorded the high percentage of deformed erythrocytes, pear and tear shaped erythrocytes, swelling erythrocytes with fading cytoplasm and indicated a decrease in haemoglobin content of *Siganus rivulatus* due to polluted water from Egyptian Eastern Mediterranean coast. Cytoplasmic abnormalities were also observed and classified into granulated, ruptured and vacuolated cytoplasm and irregularities in cell membrane of erythrocytes during the present piece of work. Deformed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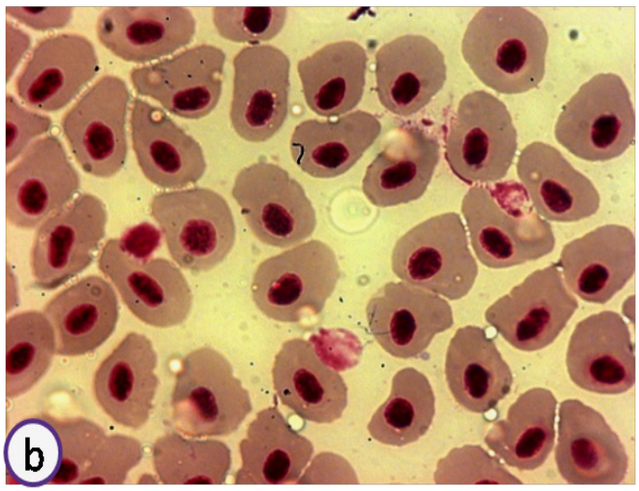
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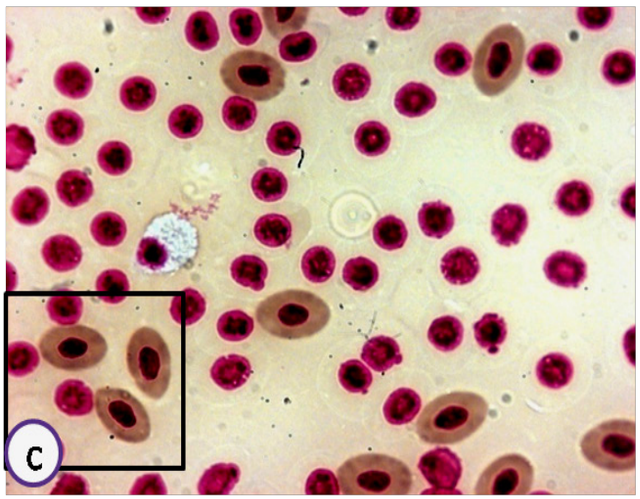
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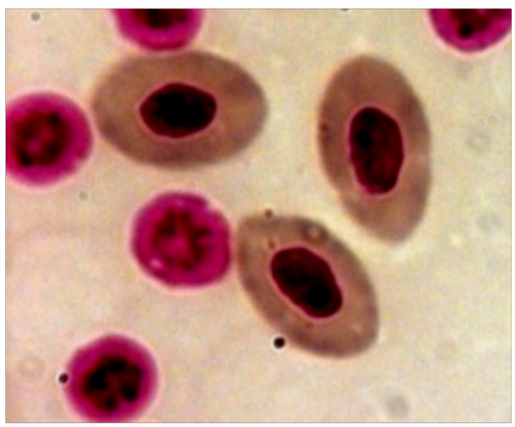

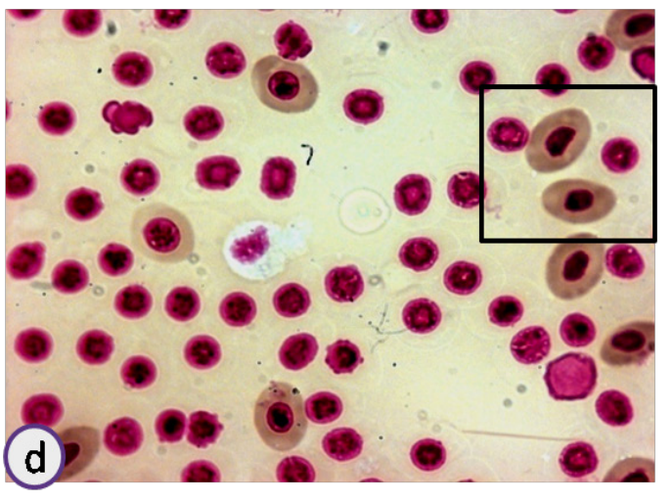
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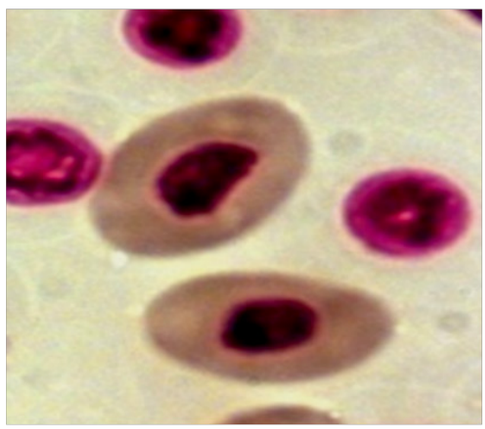


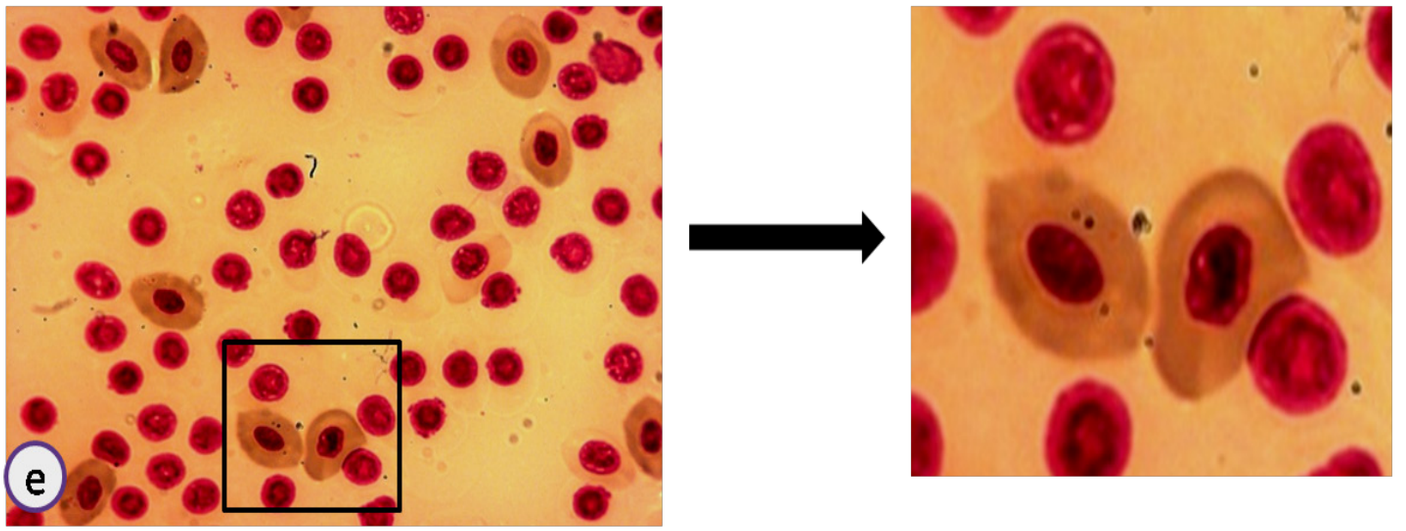
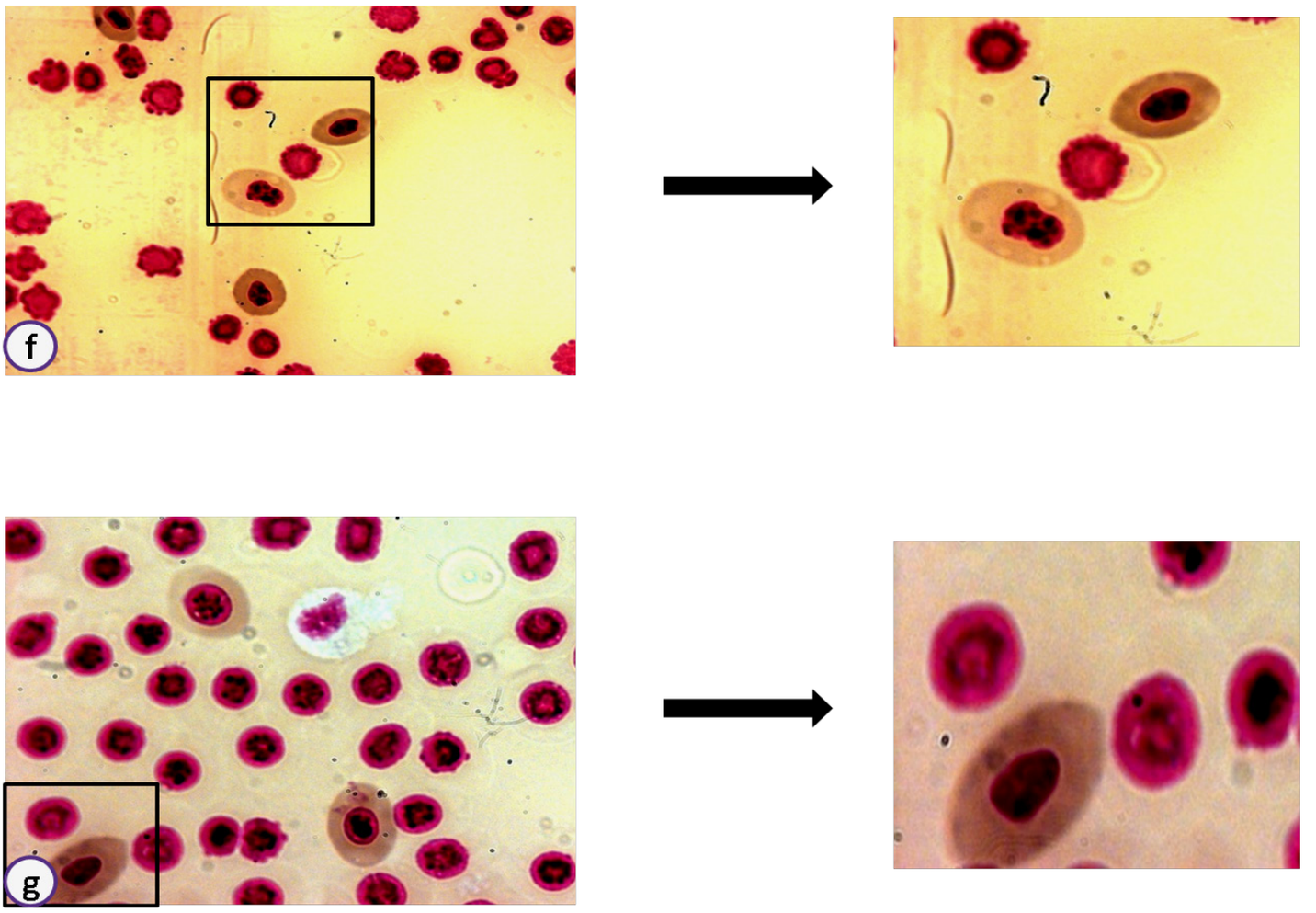


Figure 3a. Erythrocytic abnormalities : a- Normal RBC, b- Deformed RBC, c- blebbed nucleus, d- Notched nucleus and e- Lobed nucleus of *Labeo rohita* sampled from Hebbal fishfarm (control site), Vengaiah lake (lake A) and Yellamallappa Chetty lake (lake B).



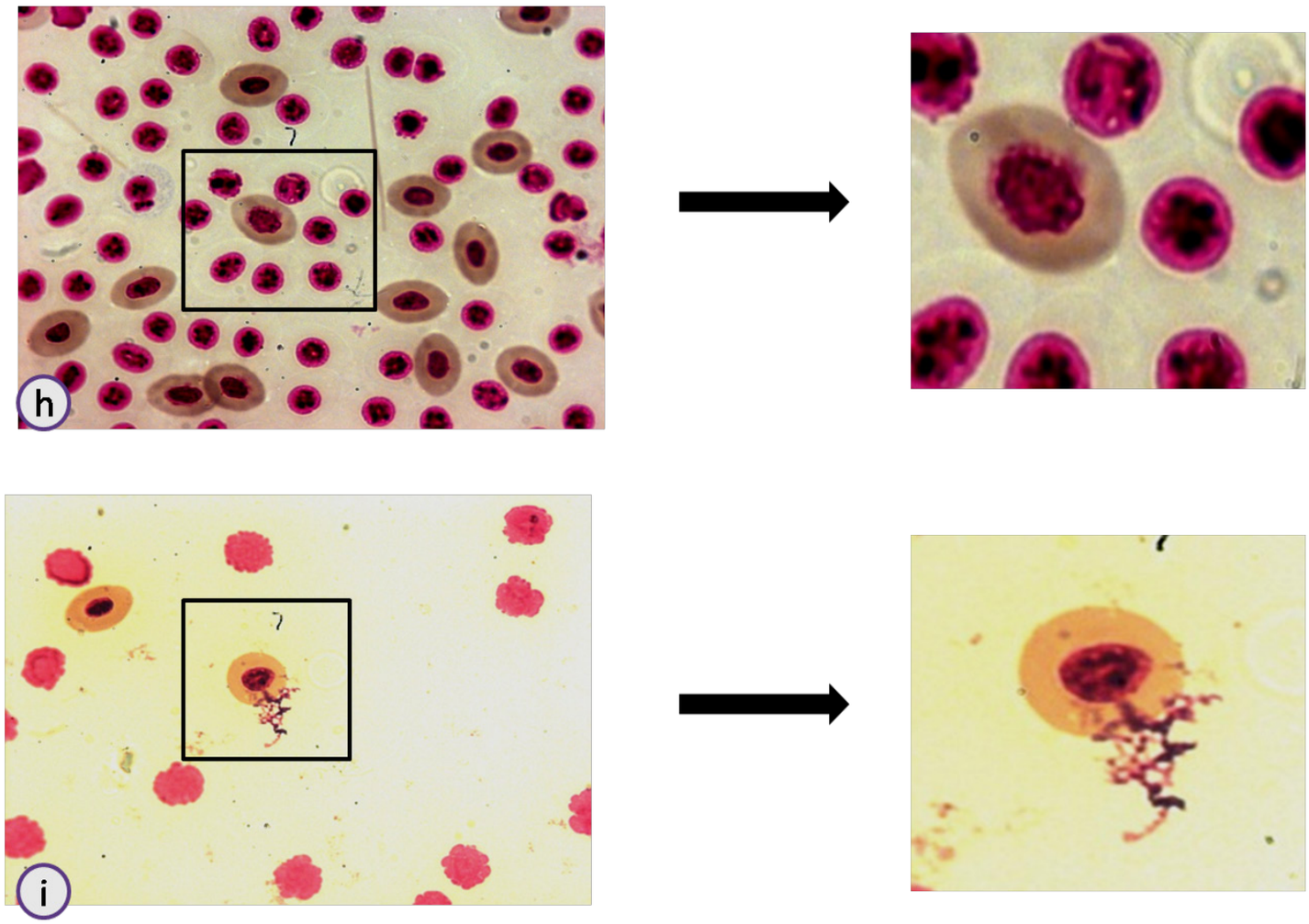
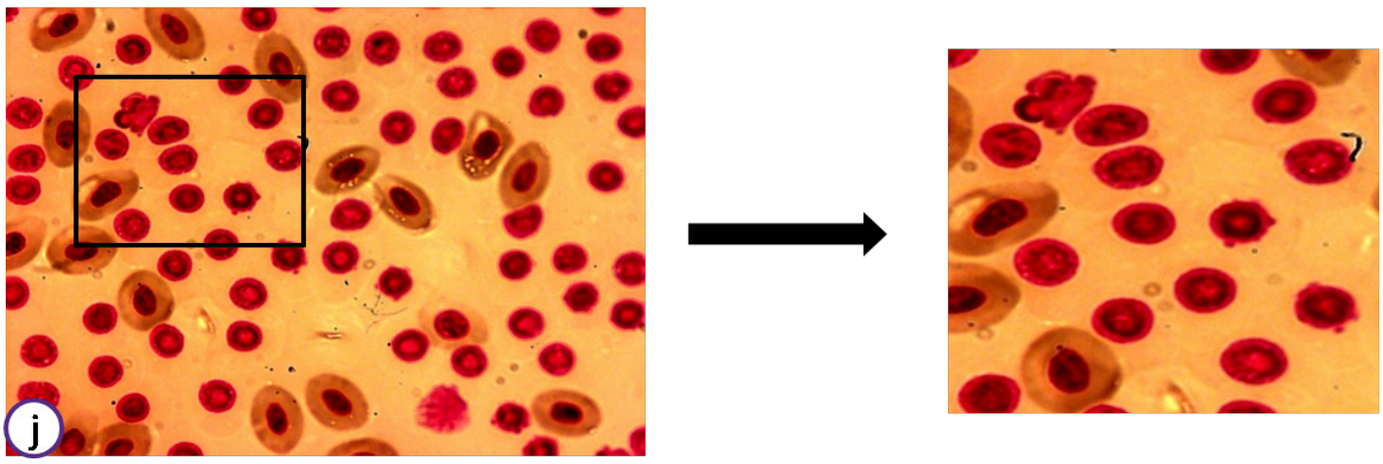


Figure 3b. b. Erythrocytic abnormalities : f- Eight shaped nucleus, g- Pear shaped nucleus, h- Ruptured nuclear membrane and i-oozing of nuclear mass of Labeo rohita sampled from Hebbal fishfarm (control site), Vengaiah lake (lakeA) and Yellamallappa Chetty lake (lake B).



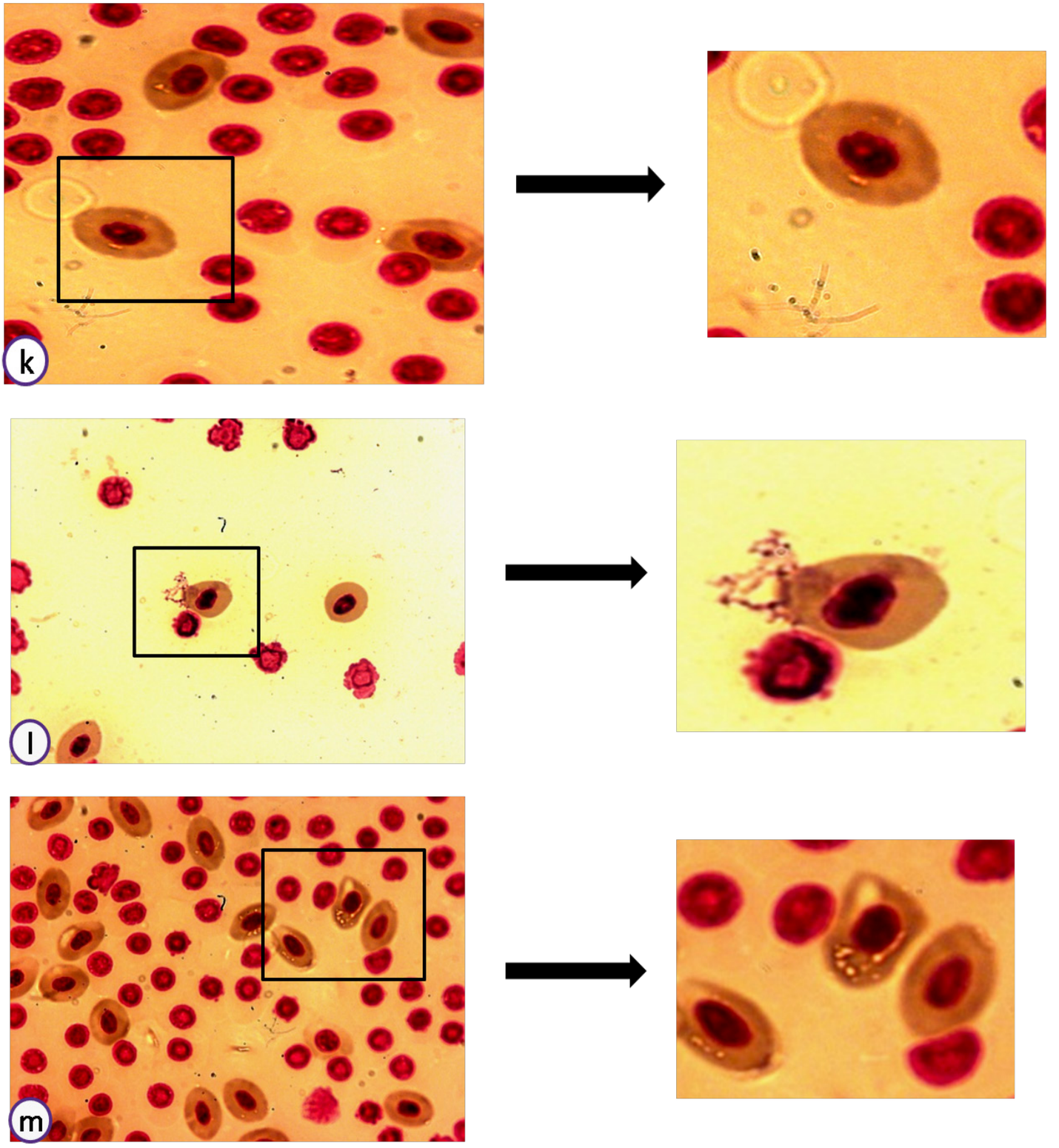


Figure 3c. Erythrocytic abnormalities : j- Disintegrated nucleus and nuclear material, k- Granulated cytoplasm, l- Ruptured cytoplasm and m-Vacuolated cytoplasm of Labeo rohita sampled from Hebbal fishfarm (control site), Vengaiah lake (lake A)and Yellamallappa Chetty lake (lake B).

RBCs were also detected in tench on short term exposure to cadmium by Witeska *et al.* (2006) and due to environmental pollution by Pacheco and Santos (2002). NAs recorded in the present investigation were in conformity with the reports by Furnus *et al.* (2014) on native fish from Parana river, Argentina. Juliana de Souza et al. (2012) observed two other NA in catfish *Cathorops spixii* (Ariidae) sampled from different sites on the south-eastern Brazilian coast. They named these NAs as "others" (OT); "heart" or "clover-leaf" shaped which were not classified by Carrasco et al. (1990). Such Nuclear abnormalities (NAs) are a consequence of exposure to environmental and chemical contaminants of genotoxic action[7].

In the present investigation, erythrocytes of fish sampled from control site were in good condition as the water parameters were within BIS limits. Erythrocytic abnormalities were frequent in samples from lake B (winter -51.87 ± 1.9, summer - 58.34 ± 2.3 and rainy 43.08 ± 2.2) when compared to control site (0.495 ± 0.11) and lake A (winter - 11.61 ± 1.65, summer - 8.62 ± 1.24 and rainy 6.19 ± 0.80) (Table V). Maximum abnormalities were observed in blood of fish during summer season in lake B where as such abnormalities were observed during winter followed by summer and rainy season in lake A. Such variations in frequency of erythrocytic abnormalities within the three water bodies are clearly attributed to the type and level of pollution during all the seasons throughout the year.

Heavy metals induced such changes in fishes which are not reversed and caused cytotoxic damage resulting in death of fishes[44]. Erythrocyte swelling, poikilocytosis, vacuolation, amitosis, deformation, and deterioration of cell membranes in *Barbus conchonius* exposed to chromium were reported by Gill and Pant (1985). Nuclear aberrations such as chromatin condensation, nuclear puffs, and chromatin leakage in the same fish species subjected to cadmium intoxication was again reported by Gill and Pant (1987). Karuppasamy *et al.* (2005) also reported similar observations as increased fragility, rupture of erythrocyte membrane, and hemolysis and increase in the frequency of nuclear and cytoplasmic abnormalities in Channa punctatus when exposed to sublethal concentration of cadmium. Nuclear abnormalities (NA) in erythrocytes are considered a useful parameter for assessing the genotoxic effects of environmental pollutants in fish, and have been applied successfully in various species such as *Anguilla Anguilla, Dicentrarchus labrax, Oncorhynchus mykiss* and *Centropomus parellelus* when exposed to polycyclic aromatic hydrocarbons and resin acids[30], β-naphthoflavone[17], metals[3] and from aquatic polluted environment[21] respectively.

In fish, both micronuclei and erythrocytic nuclear abnormalities appear spontaneously and their frequency may be seasonally dependent[36]. The present study also showed similar results. Variation was observed in the frequency of nuclear abnormalities from summer to winter and rainy since, excess evaporation during summer might cause the pollutants to concentrate in water followed by winter which had a post rain effect (Figure 1a - 1c and Table V). During rainy season influx of rain water and outpouring of lakes resulted in lowering of concentration of pollutants. These results were in agreement with Ergene *et al.* (2007) who reported increase in frequencies of nuclear anomalies such as irregular nucleus shape, vacuolation, binuclei and micronuclei indicating increase in genotoxic effects in fish exposed to water pollution; fluctuation of such abnormalities was reported by Strunjak-Perovic *et al.* (2009).

Nuclear abnormalities to be considered as precursors of micronuclei was suggested by Walia *et al.* (2013). Guner and Muranh (2011) reported differences in the erythrocyte micronucleus frequencies to be related to cell kinetics and replacement. They explained that individual and combination exposure of fishes to heavy metals led to an accumulation in the body but this accumulation did not assure an increase in MN frequency in peripheral blood erythrocytes. Since Cu and Cd induced only NAs when used alone and in combination but did not induce MN in *Gambusia affinis*. Similar results were observed in the present study in peripheral blood erythrocytes of rohu fish with a high frequency of nuclear and cytoplasmic abnormalities but absence of micronuclei in lake B which was polluted due to the presence of metals like aluminium, cadmium, copper, iron, lead and mercury.

Further, Von Sonntag (1987) and Steenken (1989) hypothesized that these abnormalities arise due to damage caused to the genetic material by free radical produced under oxidative stress due to the toxicants. Ventura *et al.* (2008) and Fernandes *et al.* (2007) reported aneuploidy, an abnormality caused by aneugenic actions of toxicants resulting in formation of binucleated cells and notched nuclei due to tubulin failure and mitotic fuses as was also seen frequent in the rohu fish in the present work. Elaborate sequence of cellular degradation under the impact of toxicants caused hypoxic conditions which resulted in depression of ATP may lead to abnormal shape of erythrocytes was reported by Ateeq *et al.* (2002). Various erythrocytic abnormalities, Heinz bodies, poikilocytosis, clumped

Table 5. Frequency (%) of erythrocytic abnormalities of *Labeo rohita* sampled from Hebbal fish farm (Control site),

Vengaiah lake (Lake A) and Yellamallappa Chetty lake (Lake B) during winter, summer and rainy season.							
Erythrocytic abnormalities (Nuclear and cytoplasmic)	**Control site**	**Winter season**		**Summer season**		Rainy season	
		Lake A	Lake B	Lake A	Lake B	Lake A	Lake B
Blebbed nucleus	0.285 ± 0.05	2.33 ± 0.30 [a]	8.2 ± 0.07 [a]	2.33 ± 0.13 [a]	9.85 ± 0.18 [a]	1.83 ± 0.04 [a]	7.53 ± 0.43 [a]
Notched nucleus	0.21 ± 0.02	2.29 ± 0.07 [a]	8.87 ± 0.05 [a]	3.5 ± 0.06 [a]	9.2 ± 0.14 [a]	2.05 ± 0.08 [a]	8.58 ± 0.04 [a]
Lobed nuleus	0	1.03 ± 0.05 [a]	4.8 ± 0.11 [a]	1.05 ± 0.05 [a]	5.305 ± 0.21 [a]	0.8 ± 0.09 [a]	3.5 ± 0.06 [a]
Eight shaped nucleus	0	1.06 ± 0.05 [a]	5.48 ± 0.04 [a]	1.24 ± 0.16 [a]	6.2 ± 0.10 [a]	1.03 ± 0.05 [a]	5.55 ± 0.10 [a]
Pear shaped nucleus	0	4.9 ± 0.08 [a]	3.45 ± 0.05 [a]	0.5 ± 0.03 [a]	3.6 ± 0.07 [a]	0.48 ± 0.07 [a]	3.38 ± 0.07 [a]
Ruptured nuclear membrane	0	0	4.75 ± 0.10 [a]	0	7 ± 0.14 [a]	0	3.57 ± 0.05 [a]
Oozing out of nuclear mass	0	0	7.35 ± 0.12 [a]	0	8.33 ± 0.18 [a]	0	5.87 ± 0.05 [a]
Cytoplasmic granules and rupture	0	0	5.45 ± 0.05 [a]	0	5.38 ± 0.07 [a]	0	2.57 ± 0.05 [a]
Cytoplasmic vacuoles	0	0	3.52 ± 0.07 [a]	0	3.47 ± 0.05 [a]	0	2.53 ± 0.05 [a]
Total abnormalities	0.495 ± 0.11	11.61 ± 1.65 [a]	51.87 ± 1.9 [a]	8.62 ± 1.24 [a]	58.34 ± 2.3 [a]	6.19 ± 0.80 [a]	43.08 ± 2.2 [a]
Values are expressed in % and as mean ± SD where, n = 6. The superscripts a, b, c and d indicate statistical mean differences at $p < 0.0001$, 0.001, 0.01 and 0.05 respectively.							

chromatin, ragged cell membranes, altered staining properties, and hemolysis in erythrocytes of *Oncorhynchus kisutch* from water contaminated with chlorinated sewage was reported by Buckley (1976). Zeni *et al*. (2002) reported erythrocyte echinocytosis in *Ictalurus melas* sub-lethally exposed to anionic detergent. It is well known that blood sampling, laboratory techniques, seasonal variations, size, genetic properties, sex, population density, lack of food supply, environmental stress and transportation could affect hematological data[22].

5. Conclusion

To conclude our study on water parameters and Micronuclei test in fish sampled from lake A, lake B and control site during winter, summer and rainy season, it can be stated that variation in levels of physico-chemical parameters due to environmental contaminants including trace metals interfered with fish physiology, disrupted normal processes, induced stress, toxic effects which inturn caused cyto- and geno-toxicity. Thus keeping in view health of these water bodies remedial measures should be undertaken to combat water contamination and its management.

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