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RESEARCH ARTICLE

# Erythrocytic nuclear abnormalities in *Cyprinus carpio* L. cultivated in water bodies of agroecosystems

**Masarooru V. Chethanakumara, Ranjana R. Hegade, Sannanegunda V. Krishnamurthy**

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**Abstract**. Agrochemicals contaminate water bodies in agroecosystems, which affects the health of fishes. We assessed erythrocytic nuclear abnormalities (ENA) in *Cyprinus carpio* reared in uncontaminated and contaminated water bodies. We recorded micronucleus, notched nucleus, lobed nucleus, kidney bean-shaped nucleus, bi-nucleated, blebbed nucleus, karyopyknosis, nuclear shift, ooze out nucleus, and elongated nucleus at higher incidences in fish reared in the contaminated site. We observed a high percent incidence of ENA and micronucleus in fish from the contaminated site. The analysis of heavy metals with atomic absorption spectroscopy revealed that all heavy metals (Mn, Zn, Ni, Pb, Cd, Fe) were high in the contaminated site except for Cu in water and Cu and Cr in sediments. Correlation matrix analysis showed that total ENA increased with Cu ($r = 0.996$, $p < 0.0001$), Mn ($r = 0.942$, $p < 0.0001$), Zn ($r = 0.517$, $p = 0.07$), and Cr ($r = 0.997$, $p = 0.0001$). Compared to the uncontaminated site, the incidence of micronuclei was 20 times higher in the fish from the contaminated site, and this was influenced by the Cu, Mn, Zn, and Cr contents in the sediments. This study indicated that the heavy metal contamination of a water body located in an agroecosystem contributed to nuclear abnormalities in *C. carpio*.

**Keywords**: abnormalities, common carp, contamination, heavy metals, micronucleus, sediments

M.V. Chethanakumara, R.R. Hegade, S.V. Krishnamurthy [✉]
Department of Environmental Science, Kuvempu University, Jnana Sahyadri, Shankaraghatta Shimoga, Karnataka, India
E-mail: svkrishnamurthy@gmail.com

## Introduction

Common carp (*Cyprinus carpio*, L.) is an important fish cultivated in many parts of the world. These fish are exposed to various water contaminants. Exposure to contaminants and to their metabolites directly in water and through the food chain results in physiological, hematological, cellular, and nuclear changes (Llorente et al. 2002, Luskova et al. 2002, Velkova-Jordanoska et al. 2008, Saglam and Yonar 2009, Lopez-Lopez et al. 2011, Karadag et al. 2014, Ogueji et al. 2017, Shahjahan et al. 2020). *C. carpio* showed specific physiological, cellular, and hematological responses after being exposed to contaminants such as agrochemicals, heavy metals, pharmaceuticals, and other compounds present in sewage (Llorente et al. 2002, Grisolia and Starling 2001, Cavas et al. 2005, Da Rocha et al. 2009, Mitkovska and Chassovnikarova 2020). Hematological and biochemical biomarkers are used to assess the health status of organisms that are affected by stress induced by environmental contaminants (Adhikari et al. 2004, Georgieva et al. 2014, Iheanacho et al. 2017). Contaminants induce a specific response in *C. carpio*, and this species is considered a bioindicator for ecotoxicological investigations in aquatic environments (OECD, 1992).

In India, *C. carpio* is the most culturable species in inland waters. A total of 3.15 million hectares of reservoirs, 2.36 million hectares of ponds and tanks, and 0.19 million hectares of lotic water are used to cultivate this fish (Anonymous 2017). As *C. carpio* is a bottom feeder, it is cultivated in polyculture systems with *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton). In India, nearly 70% of surface water and groundwater reserves are contaminated by biological, toxic, organic and inorganic pollutants discharged with industrial effluents, municipal wastes, and agricultural pollutants (Mali et al. 2015). In the study area, most of the water bodies used for fish cultivation are in agricultural landscapes. These water bodies are sources of crop irrigation, and because of their location, they receive a variety of agrochemicals and residues (Gurushankara et al. 2007, Hegde and Krishnamurthy 2014). However, in India, a total of 0.981 million tons of exotic carp are produced annually (Anonymous 2020), and information on the effect of contaminants such as heavy metals have on this fish is scant.

Biological responses such as nuclear abnormalities of possibly affected organisms are used as biomarkers for biomonitoring aquatic environments (Da Rocha et al. 2009, Ramsdorf et al. 2009). Exposure of natural populations to contaminants can result in serious health implications and ecological consequences (Llorente et al. 2002). Therefore, the present study attempted to explain the nuclear abnormalities in *C. carpio* cultivated in water bodies located in agroecosystems.

## Methods

### Study area and sampling site

The study was conducted in two lentic water bodies (≈1.2 – 2 ha.) located in the Western Ghats in Karnataka State. Both water bodies are in the same landscape and have no industrial effluent discharge. Of these two, the water body located at the edge of the forest, which has no human intervention or agricultural activities, was considered the reference site (14°01'40" N, 75°18'33" E alt: 696 m asl, Fig. 1). The annual production of carp from the reference site was reported to be between 790 kg and 865 kg $ha^{-1}$. The other lentic water body (Loc: 14°01'05" N, 75°18'43" E alt 694 m asl), which is surrounded by 13.56 ha. of agricultural crop cultivation area and has surface flow from adjacent agricultural fields, was considered the contaminated site. This site has an annual production of carp between 1,235 kg $ha^{-1}$ and 1,285 kg $ha^{-1}$. Fish farmers used the same stock of fish fingerlings for both sites for cultivation.

During the study, concurrent with each sampling, the water pH, water temperature, and dissolved oxygen were recorded in triplicate on the spot. pH was determined at three locations in the water bodies with a portable Hanna pH meter (model HI 98107), and water temperature was recorded using a mercury bulb thermometer (G H Zeal Ltd, London). The dissolved oxygen content (mg $l^{-1}$) of the water was estimated with the Winkler Iodometric method as described in

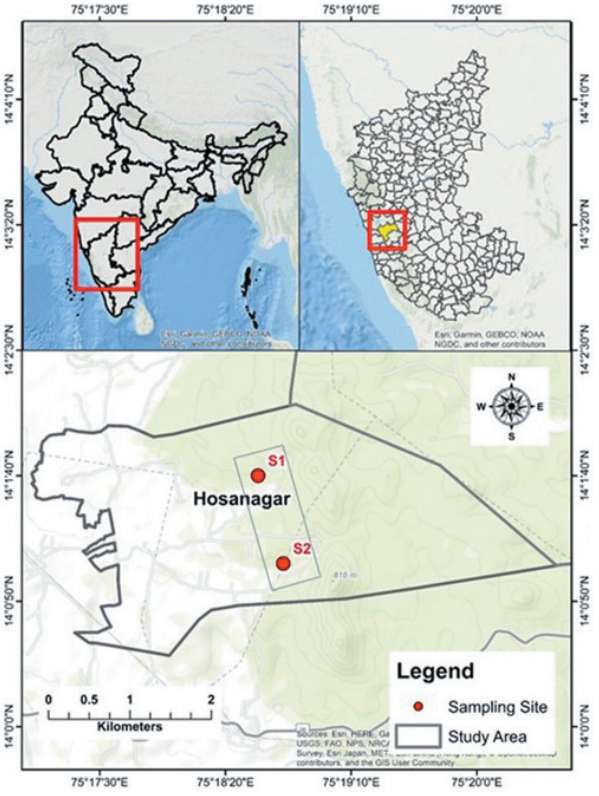


Figure 1. Map showing the study area and location of the water bodies used for the current study. Note: S1 = reference site and S2 = contaminated site.

APHA/AWWA/WEF (2017). The water temperature of the reference site was 19°C–29°C (23 ± 4.4°C), the water pH was 5.8–7.5, and the dissolved oxygen content of the water was 4 mg $l^{-1}$–8.6 mg $l^{-1}$ (6.8 ± 2.04 mg $l^{-1}$). At the contaminated site, the water temperature was 21°–30°C (25 ± 4.2°C), the pH was 5–7.6, and the dissolved oxygen content was 4 mg $l^{-1}$–7.7 mg $l^{-1}$ (5.1 ± 1.75 mg $l^{-1}$).

## Heavy metal analysis of water and sediments

Concurrent with fish sampling, water and sediment samples were collected following the standard methods of APHA/AWWA/WEF (2017). The water samples and the shade-dried-powdered sediment samples were acid-extracted following the methods of APHA/AWWA/WEF (2017) and 3050 B of U.S. EPA (1996), respectively. Then, the heavy metal contents (copper [Cu], manganese [Mn], zinc [Zn], nickel [Ni], lead [Pb], cadmium [Cd], iron [Fe], and chromium [Cr]) of the sediments and water were analyzed simultaneously with every fish sampling using atomic absorption spectroscopy (PinAAcle 900F series).

## Fish sampling

Samples of *C. carpio* were collected from both water bodies every 30 days during the harvest periods of January-April 2021, and January-April 2022, using a drag net. This study was based on a total of four samplings per site/year. Further, each sampling was conducted in three to four attempts in different parts of the water body. In these waterbodies, as composite fish culture is being practiced, in every catch, we chose at random five to nine *C. carpio* specimens. Then, of these, any two of the fish were used for blood sampling. Blood samples were collected by puncturing the gill (Nepomuceno et al. 1997) and caudal vein (Kumar et al. 2010). Then, a thin peripheral blood smear was made on clean glass microscopic slides and air-dried for further observations. The average total length of the fish in the sample (n= 24) from the reference and contaminated sites was 19.6 ± 3.65 cm and 23.6 ± 4.52 cm, respectively; and body mass (g) was 109.3 ± 50.17 g and 253 ± 168.1 g, respectively.

## Evaluation of nuclear abnormalities

In the laboratory, the air-dried slides were fixed with absolute methanol for 10 min, followed by staining with 10% Giemsa for 30 min. Excess stain was washed off with tap water (Ergene et al. 2007, Re et al. 2021), and the slides were left to air dry overnight. Then, the slides were observed under an optical microscope (Olympus CX21) using 40X and 100X objective magnification lenses. The size of the erythrocytes was measured using micrometry. Then, a minimum of 1,000 random erythrocytes were scored from each slide to identify erythrocytic nuclear abnormalities (ENA). Different types of nuclear abnormalities were recorded based on the literature and the following abnormalities were considered. A micro nucleus (MN) (Carrasco et al. (1990) is smaller than one-third of the main nucleus and displays the same staining, is separated from the main nucleus, and is located on the same plane of the focus. The other ENAs identified were as follows: binucleates (BN) – cells with two nuclei of the same size and a common cytoplasm(Sharmin et al. 2021); blebbed nuclei (BL) – cells containing euchromatin with small tubular outgrowth of the nuclear membrane (Guilherme et al. 2008, Omar et al. 2012, Furnus et al. 2014); lobed nuclei (LN) – nuclei with an outgrowth larger than that in BL with a lobe (Omar et al. 2012, Furnus et al. 2014, Re et al. 2021); notched nuclei (NN) – nuclei with vacuoles with a notch into the nucleus and without nuclear material (Omar et al. 2012, Furnus et al. 2014, Ashaf-Ud-Doulah 2019, Re et al. 2021, Sharmin et al. 2021); elongated nuclei (EN) – nuclei with an unusual length compared to normal nuclei (Islam et al. 2019); karyopyknosis (KP) – irregular nuclear membrane and cells with condensation and clumping of the chromatin material at the edge of the nuclei (Ashaf-Ud-Doulah 2019, Sharmin et al. 2021);

nuclear shift (NS) – the nucleus shifted from its actual position compared to a normal nucleus (Shah et al. 2021); kidney-bean-shaped nucleus (KB) – nuclear invagination resembling a kidney (Guilherme et al. 2008, Re et al. 2021); nuclear ooze-out (ON) – nucleus with small thread-like structure coming out of the cell.

The nuclear abnormality of each type was calculated as a percent incidence with the following formula:

$$\%\ Abnormality = \frac{Number\ of\ cells\ contain\ in\ abnormalities}{Total\ number\ of\ cells\ counted} \times 100$$

### Data Analysis

The data were tabulated in MS Excel. The incidence rate of nuclear abnormality was calculated as the average incidence. Two-way ANOVA was used to explain the significance ($p < 0.05$) of differences in the incidence of abnormalities between control and contaminated sites. The Karl Pearson correlation coefficient was used to explain the direct correlation between heavy metals and incidences of abnormalities. Matrix intercorrelation was used to determine the relationship between the incidence of ENA and MN and individual heavy metals. We used SPSS (ver.20) for all statistical calculations.

## Results

The erythrocytes were 16.16 ± 2.625 μm in length and 11.5 ± 1.702 μm in width. Fig. 2 illustrates the nuclear abnormalities recorded in the present study. Table 1 presents the percent incidence of nuclear abnormalities of erythrocytes in *C. carpio* at the reference and contaminated sites. Of a total of ten recorded nuclear abnormalities, we found eight types in specimens from the reference site and nine types in those from the contaminated site. Nearly 70% of the types of abnormalities were common in both sites. However, the percent incidence of individual abnormalities differed between the sites ($F = 19.64$, $p = 0.001$). The incidence of abnormalities was high in the contaminated site. The total percent incidence of abnormality was 2.297 in the contaminated site against 0.527 for the reference site. NS predominated at the reference site, followed by BL and EN abnormalities, while at the contaminated site, BN predominated, followed by NS and MN. MN represented 10.54% of the total abnormality in the contaminated site, which was 2.25% at the reference site. Further, we found no significant relationship between fish total length or body mass on the abnormalities (control site – total length v/s abnormalities, $r = 0.034$, $p = 0.886$ and body mass v/s abnormalities, $r = 0.103$, $p = 0.663$; contaminated site – total

**Table 1**
Average incidence (%) of nuclear abnormalities recorded in *C. carpio* at reference and contaminated sites. The total number of erythrocytes screened for the reference site was 42,126 cells, and for the contaminated site, it was 23,550 cells

| | Reference site | | Contaminated site | |
|---|---|---|---|---|
| Abnormalities | abnormal cells | % incidence | abnormal cells | % incidence |
| Micro Nucleus | 5 ± 0.91 | 0.012 | 57 ± 3.42 | 0.242 |
| Notched Nucleus | - | - | 10 ± 0.96 | 0.042 |
| Lobed Nucleus | 20 ± 1.83 | 0.047 | 29 ±3.33 | 0.123 |
| Kidney bean shape | 12 ± 1.23 | 0.028 | 37 ±5.07 | 0.157 |
| Bi Nucleated | 2 ± 0.37 | 0.005 | 3 ± 0.48 | 0.013 |
| Blebbed Nucleus | 66 ± 7.48 | 0.157 | 189 ± 21.90 | 0.803 |
| Karyopyknosis | - | - | 2 ± 0.29 | 0.001 |
| Nuclear shift | 75 ± 6.43 | 0.178 | 169 ± 13.21 | 0.718 |
| Ooze out Nucleus | 7 ± 1.28 | 0.017 | - | - |
| Elongated Nucleus | 35 ± 6.39 | 0.083 | 45 ± 7.11 | 0.191 |

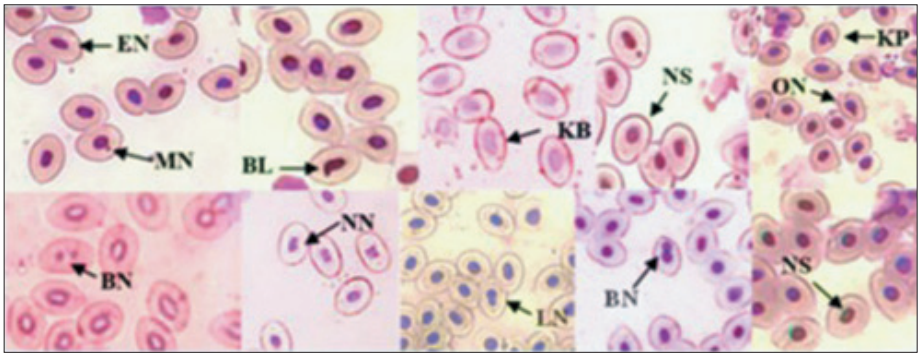


Figure 2. Nuclear abnormalities recorded in erythrocytes of *C. carpio*. Each abnormality is marked with letters: MN = micronucleus; NN = notched nucleus; LN = lobed nucleus; KB = kidney bean-shaped nucleus; BN = binucleated; BL = blebbed nucleus, KP = karyopyknosis; NS = nuclear shift; ON = ooze-out nucleus; EN = elongated nucleus.

length v/s abnormalities, r = 0.115, p = 0.737; body mass v/s abnormalities, r = 0.336, p = 0.312).

## Heavy metal concentrations

Table 2 presents the heavy metal concentrations recorded for the water and sediments of the reference and contaminated sites. In general, sediments had higher concentrations of metals than did water (reference site – total heavy metals in sediments = 23,080.80 mg $kg^{-1}$ and total heavy metals in water = 5.47 mg $l^{-1}$; contaminated site – total heavy metals in sediments = 46022.87 mg $kg^{-1}$ and total heavy metals in water = 6.02 mg $l^{-1}$). The total heavy metal concentration in sediments was double in the contaminated site. No difference was found in the total heavy metal content of water between the reference and contaminated sites. In water, the concentration of Cu was high at the reference site, while Pb did not show any difference between the reference and contaminated sites. The remaining heavy metals were recorded to be high in the contaminated site. However, except for Mn ($p < 0.0001$), no other heavy metals showed any statistically significant differences between the sites. Except for Cu and Cr contents, all other heavy metals were higher in the sediments at the contaminated site than at the reference site. Also with the exception of Cu ($p = 0.524$), the differences in concentrations of all other heavy metals were statistically significant.

## Correlation between heavy metals, ENA, and MN

The results of correlation analyses among various heavy metal contents and ENA and MN are presented in Table 3. The sum of ENA did not correlate with total heavy metals in the water ($r = 0.353$, $p = 0.379$) or sediments ($r = 0.723$, $p = 0.0837$) of the reference site. Similarly, the total ENA did not correlate with the sum of heavy metals in the water ($r = 0.365$, $p = 0.381$) and sediments ($r = 0.05$, $p = 0.48$) of the contaminated site. However, correlation analysis between different heavy metals and corresponding total ENAs revealed a positive correlation between Cu ($r = 0.996$, $p < 0.0001$), Mn ($r = 0.942$, $p < 0.0001$), Zn ($r = 0.517$, $p = 0.07$) and Cr ($r = 0.997$, $p = 0.0001$) in the sediments of the contaminated site.

The MN represented the lowest (next to BN) incidence at the reference site. However, at the contaminated site, MN had a high incidence (0.242), which was nearly 20 times higher than that at the reference site. Furthermore, MN was the top third most common abnormality at the contaminated site. The incidence of MN did not exhibit any correlation with heavy metals in the water or sediments of the reference site. However, the Cu, Mn, Zn, and Cr in the sediments showed a strong influence on the incidence of MN at the contaminated site. The incidence of MN increased with increasing concentrations of Cu ($r = 0.97$, $p < 0.0001$), Mn ($r = 0.99$, $p < 0.0001$),

**Table 2**

Heavy metal content in sediments ($\bar{x}$ mg kg$^{-1}$ sed. ± SE) and water ($\frac{\bar{x}}{L} \pm SE$) of the reference and contaminated sites with the significance of differences between the sites

| | Sediment | | | Water | | |
|---|---|---|---|---|---|---|
| Heavy metal | reference site | contaminated site | significance | reference site | contaminated site | significance |
| Cu | 174.2 ± 36.52 | 148.1 ± 21.63 | $F_{1,13}$ = 0.43, p = 0.524 | 2.32 ± 0.507 | 1.89 ± 0.692 | $F_{1,6}$ = 0.26, p = 0.6258 |
| Mn | 88.4 ± 14.18 | 387.8 ± 19.71 | $F_{1,13}$ = 123.3, p < 0.0001 | 0.05 ± 0.022 | 0.53 ± 0.026 | $F_{1,6}$ = 182.48, p < 0.0001 |
| Zn | 45.0 ± 2.93 | 80.8 ± 2.78 | $F_{1,13}$ = 73.93, p < 0.0001 | 0.28 ± 0.033 | 0.25 ± 0.037 | $F_{1,6}$ = 0.26, p = 0.626 |
| Ni | 22.9 ± 3.29 | 59.3 ± 2.46 | $F_{1,13}$ = 81.74, p < 0.0001 | 0.02 ± 0.004 | 0.03 ± 0.003 | $F_{1,6}$ = 2.8, p = 0.1452 |
| Pb | 17.5 ± 1.67 | 29.0 ± 0.95 | $F_{1,13}$ = 41.7, p < 0.001 | 0.09 ± 0.024 | 0.09 ± 0.046 | $F_{1,6}$ = 0.35, p = 0.487 |
| Cd | 0.9 ± 0.22 | 1.4 ± 0.08 | $F_{1,13}$ = 6.08, p = 0.028 | 0.005 ± 0.0012 | 0.01 ± 0.001 | $F_{1,6}$ = 0.039, p = 0.555 |
| Fe | 22579.9 ± 3823.95 | 45202.8 ± 2767.80 | $F_{1,13}$ = 24.18, p = 0.0003 | 2.46 ± 0.949 | 2.86 ± 1.374 | $F_{1,6}$ = 0.06, p = 0.814 |
| Cr | 152.1 ± 9.35 | 113.7 ± 6.19 | $F_{1,13}$ = 12.8, p = 0.0033 | 0.23 ± 0.148 | 0.35 ± 0.183 | $F_{1,6}$ = 0.28, p = 0.46 |

**Table 3**

Results of correlation analyses among the various heavy metal contents and ENA and MN

| | ENA | | | | MN | | | |
|---|---|---|---|---|---|---|---|---|
| | Water | | Sediment | | Water | | Sediment | |
| Heavy metal | reference site | contaminated site | reference site | contaminated site | reference site | contaminated site | reference site | contaminated site |
| Cu | r = 0.30 | r = 0.68 | r = -0.20 | r = 0.99 | r = 0.18 | r = 0.98 | r = -0.65 | r = 0.97 |
| | p = 0.622 | p = 0.500 | p = 0.974 | p < 0.0001 | p = 0.902 | p = 0.121 | p = 0.240 | p < 0.0001 |
| Mn | r = 0.10 | r = -0.70 | r = 0.88 | r = 0.94 | r = 0.40 | r = -0.81 | r = -0.08 | r = 0.99 |
| | p = 0.871 | p = 0.510 | p = 0.641 | p < 0.0001 | p = 0.630 | p = 0.399 | p = 0894 | p < 0.0001 |
| Zn | r = 0.38 | r = -0.97 | r = -0.51 | r = 0.52 | r = 0.47 | r = -0.79 | r = -0.72 | r = 0.66 |
| | p = 0.528 | p = 0.148 | p = 0.377 | p = 0.07 | p = 0.428 | p = 0.370 | p = 0.174 | p = 0.025 |
| Mn | r = -0.452 | r = -0.35 | r = -0.66 | r = 0.16 | r = -0.11 | r = -0.18 | r = -0.39 | r = 0.32 |
| | p = 0.445 | p = 0.775 | p = 0.227 | p = 0.901 | p = 0.866 | p = 0.886 | p = 0.517 | p = 0.790 |
| Pb | r = -0.26 | r = -0.99 | r = -0.25 | r = 0.15 | r = 0.441 | r = -0.96 | r = -0.77 | r = 0.32 |
| | p = 0.667 | p = 0.084 | p = 0.968 | p = 0.906 | p = 0.458 | p = 0.174 | p = 0.132 | p = 0.795 |
| Cd | r = 0.35 | r = -0.88 | r = 0.80 | r = 0.11 | r = -0.42 | r = -0.79 | r = -0.39 | r = 0.28 |
| | p = 0.566 | p = 0.304 | p = 0.104 | p = 0.933 | p = 0.487 | p = 0.415 | p = 0.522 | p = 0.822 |
| Fe | r = 0.09 | r = 0.29 | r = -0.66 | r = -0.10 | r = 0.47 | r = 0.12 | r = -0.14 | r = 0.08 |
| | p = 0.889 | p = 0.812 | p = 0.221 | p = 0.058 | p = 0.860 | p = 0.923 | p = 0.983 | p = 0.951 |
| Cr | r = 0.07 | r = 0.37 | r = 0.62 | r = 0.99 | r = 0.50 | r = 0.20 | r = 0.02 | r = 0.99 |
| | p = 0.917 | p = 0.762 | p = 0.267 | p = 0.0001 | p = 0.091 | p = 0.872 | p = 0.971 | p < 0.0001 |
| Total | r = 0.35 | r = 0.37 | r = 0.72 | r = 0.05 | r = 0.42 | r = 0.42 | r = -0.65 | r = 0.96 |
| | p = 0.379 | p = 0.381 | p = 0.0837 | p = 0.48 | p = 0.487 | p = 0.923 | p = 0.240 | p = 0.169 |

Zn ($r = 0.663$, $p = 0.025$), and Cr ($r = 0.99$, $p < 0.0001$) in the sediments of the contaminated site.

## Discussion

The results of the present study represent the occurrence of a high incidence of ENA in fish cultivated in a contaminated water body. Many studies have revealed that freshwater fish can serve as reliable bioindicators of metal-induced genotoxicity (Gomes et al. 2015, Jindal and Verma 2015, Pavlaki et al. 2016, Singh et al. 2019). The micronucleus assay is considered an indicator to assess exposure to genotoxic and mutagenic contaminants in aquatic ecosystems (Braham et al. 2017). Chemical and physical agents cause genetic defects and nuclear abnormalities (Llorente et al. 2002, Furnus et al. 2014).

The fish *C. carpio* is better adapted to temperatures ranging between 27 and 31°C. At high temperatures (> 35°C), this fish exhibits nuclear and cellular abnormalities (Shahjahan et al. 2020). Similarly, in other fishes, extreme temperature and pH are known to cause nuclear and cellular abnormalities (Ashaf-Ud-Doulah et al. 2019, Pinheiro et al. 2019). In the present study, we did not find any correlation between pH and temperature with nuclear abnormalities. The pH ranged between 5.8 and 7.6, and the water temperature was low (range 19-30°C) at both the reference and contaminated sites.

Heavy metals ingested through the food chain are known to accumulate in fishes and cause damage at various biological levels (Furnus et al. 2014). Exposure to various chemicals individually or in combination causes morphological nuclear abnormalities in fishes (Cavas et al. 2005, Talapatra and Banerjee 2007). Although the formation mechanism of these abnormalities is not fully understood, the presence of abnormalities is considered an indication of genetic damage in fishes (Ergene et al. 2007). In the present study, the fish showed low nuclear abnormalities at the reference site, which had low concentrations of heavy metals in the water and sediments. In contrast, the contaminated site had a high concentration of heavy metals, and the fish exhibited high ENA that showed a strong correlation with heavy metals. Therefore, the fish grown in the contaminated site were profoundly affected at the genetic level by heavy metals.

The concentrations of Cu, Mn, Zn, and Cr exhibited a strong influence on the occurrence of total nuclear abnormalities and MN. *Labeo bata* (Hamilton) cultivated in sewage water exhibited nuclear abnormalities and MN stemming from the presence of the heavy metals Cr, Zn, Cu, Pb, and Mn (Talapatra and Banerjee 2007). The induction of MN in fish populations by Cr, Pb, Cd, and Ni is well documented (Poongothai et al. 1996, Corduk, et al. 2018, Shah et al. 2021). We found high concentrations of these heavy metals in the water and sediments of the contaminated site; therefore, these heavy metals were the reason for the high ENA recorded in the blood cells of *C. carpio*.

## Conclusions

Fish cultivated in the contaminated water body exhibited more ENA than those cultivated in the uncontaminated water body. The contaminated water body had high concentrations of heavy metals in water and sediments compared to the reference site. Individual heavy metal concentrations exhibited a strong correlation with nuclear abnormalities and micronuclei. Heavy metal concentrations in the water and sediments of the contaminated water body caused the high occurrence of nuclear abnormalities in *C. carpio*.

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**Authors' contribution**. All authors contributed to the study's conception and design. Fieldwork, data collection, formal analysis, and investigation was performed by M. V. Chethanakumara, and Ranjana R. Hegade. Conceptualization, methodology, software, validation, formal analysis, resources, data curation, review, editing, visualization, and supervision were performed by S. V. Krishnamurthy.

**Data availability**. The data generated during this study are available from the corresponding author upon reasonable request.

**Conflict of interest**. The authors declare no competing interests.

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**ORCID iD**

Ranjana Ramesha Hegade:

https://orcid.org/0000-0003-3193-8918

Sannanegunda Venkatarama Bhatta Krishnamurthy:

https://orcid.org/0000-0002-0129-2709

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