

Haematological, Serological and Genotoxic Findings in the African Catfish *Clarias gariepinus* after the Administration of Copper Nanoparticles and Penconazole

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Abstract

This work was carried out to investigate the toxic potential of recent application of two fungicides, penconazole and copper nanoparticles, on the African catfish *Clarias gariepinus*. Haematological and serological indices of catfish blood were evaluated after three months of exposure to sub-lethal doses of penconazole and copper nanoparticles. During the experiment, *C. gariepinus* was exposed to three different concentrations (1.8, 3 and 4.2 µg/L) of the two fungicides. Haematological and biochemical parameters and the induction of micronucleated red blood cells (RBCs) and nuclear lesions were evaluated as biomarkers for the toxicity of the two fungicides. Our results revealed that both chemicals could potentially cause haemato-serological and genotoxic alterations in the experimental catfish. The deteriorations in the investigated parameters were dependent on the concentrations of the administered chemicals. On the other hand, least significant difference (LSD) tests indicated that the tested parameters were more adversely influenced by penconazole than by copper nanoparticles. The mean RBC counts and haemoglobin (Hb) and haematocrit (Hct) levels decreased with increasing concentrations of both pesticides (1.8, 3, 4.2 µg/L). In relation to the levels of the control fish, glucose, glycerol, protein, alanine amino transferase (ALT) and aspartic amino transferase (AST) were also altered in the blood of fish exposed to both pesticides. The induction of micro nucleated RBCs and nuclear lesions was determined to be related to the exposure concentrations of the selected fungicides.

Keywords: *Clarias gariepinus*; Genotoxicity; Copper Nanoparticles; Penconazole; Biomarker; MN Test

Introduction

Management of water quality, control of water pollution and environmental protection are major issues for preserving suitable living conditions for the future. Egypt has been listed among the ten countries that will be threatened by water deficiency by the year 2025 due to the rapidly increasing population [1]. Approximately 97% of Egypt's water resources are from the Nile River [2], which has become polluted due to discharge of untreated waste, dumping of industrial effluent and runoff from agricultural fields [3,4]. One of the most severe impacts of such pollution is biodiversity loss. The African catfish *Clarias gariepinus* is the most common fish in Egypt. The African catfish has been used in fundamental research and toxicological studies [5], since it has a well-documented biology and is easy to rear in captivity [6]. During recent decades, the use of insecticides has increased dramatically, contaminating soil, water and biota.

These pesticides are generally used for controlling agricultural pests [7] and may reach aquatic ecosystems, leading to toxic effects and altered haematology and biochemistry in fishes [3,8]. The potential genotoxicity of pesticides in aquatic organisms is poorly understood. Penconazole and copper nanoparticle fungicides have economic roles, being used as sprays in crop production; despite this, they have the potential to deteriorate the environment if applied in high doses, causing necrosis, specific structural changes or even death in animals [9,10]. Although penconazole is widely found in the aquatic environment, little is known about its acute toxicity in fish [11]. Despite the potential advantages of copper nanoparticles, many issues surrounding these compounds have still not been addressed. The concept of monitoring aquatic pollution using biomarkers has become important for modern environmental assessment as biomarkers can help to predict the presence of pollutants involved in monitoring programmes [12]. Blood parameters are considered good physiological biomarkers of the whole body and, therefore, they are important in diagnosing the structural and functional status of fish exposed to environmental pollutants [13]. Haematological and serological parameters have become promising biomarkers for measuring the impacts of water pollution in fish, because blood variables respond to low doses of pollutants [4]. Micronuclei (MN) and nuclear lesion (NL) tests are the most widely applied of these methods since they detect the genotoxicity of a wide range of compounds, especially in fishes. Assays of MN and NLs are sensitive tools for detecting genotoxicity resulting from surface runoff of agricultural fungicides [14]. According to the available literature, there are no available data regarding the potential haematological, serological and genotoxic consequences of penconazole and copper nanoparticles on the African catfish *C. gariepinus*. Therefore, this study was carried out to determine the toxicity of penconazole and copper nanoparticles in African catfish using haematological, biochemical and genotoxic biomarkers.

Materials and Methods

Experimental design

African catfish, *C. gariepinus*, with a mean weight of 175 ± 7 gm and mean length of 25 ± 5 cm from the Egyptian Nile River were purchased at Assiut City. The fish were transported to the Laboratory of Fish Biology, Zoology Department, Faculty of Science, Al-Azhar University, Assiut, Egypt for acclimatization for two weeks. During this period, the fish were kept in 120-litre rectangular tanks and fed 3% of their body weight with commercial feed pellets (40% crude protein, 4.22% fat, 5.88% crude fibre, 10.30% ash and 10.03% moisture). Laboratory conditions were maintained and adjusted as necessary (conductivity 2000 $\mu\text{S}/\text{cm}$; pH ≈ 7.5 ; oxygen 90 - 95% saturation; temperature 25°C; photoperiod 12:12 light:dark). The selected fungicides (penconazole and copper nanoparticles) were administered at sub-lethal concentrations (1.8, 3, 4.2 $\mu\text{g}/\text{L}$) for three months. Seven groups were set up in replicates as follows: one for the control group, three for the penconazole doses (1.8, 3, 4.2 $\mu\text{g}/\text{L}$) and three for the copper nanoparticle doses (1.8, 3, 4.2 $\mu\text{g}/\text{L}$). These serial concentrations were selected according to the previously determined LC_{50} values for both fungicides. At the end of the experiment, 5 fish from each tank were randomly taken for blood sampling. Blood samples were taken from the caudal region for haematological, biochemical and genotoxic investigations.

Haematological parameters

Whole blood was used for the estimation of red blood cell (RBC) counts and haemoglobin (Hb) and haematocrit (Hct) concentrations by using an automated technical analyser (Celltac α MEK- 6400J/K). White blood cell (WBC) counts and WBC differentials were calculated according to [15]. Neutrophils, lymphocytes, eosinophils and monocytes were counted using an Olympus oil immersion light microscope at 1000 \times magnification.

Biochemical parameters

Plasma samples were analysed for total protein, glucose, cholesterol, triglyceride, alanine amino transferase (ALT), aspartic amino transferase (AST), calcium and urea contents. Colorimetric determinations of the selected biochemical parameters were performed using a spectrophotometer (Jasco-V530). The absorbency of the detected sample was examined at an appropriate wavelength ranging from 340 to 546 nm according to the parameter tested. Commercial diagnostic kits from Bio-Merieux chemicals were used for the following biochemical assays:

- Serum total protein (g/dl) was determined using a biuret test according to [16]. Quantitative estimation of serum glucose (mg/dl) was estimated by enzymatic colorimetric methods as described by [17].
- Serum cholesterol (mg/dl) was estimated by enzymatic colorimetric methods as described by [18].
- Serum triglycerides (mg/dl) were estimated according to the method of [19].
- Activities of alanine amino transferase (ALT, U/I) and aspartate amino transferase (AST, U/I) were determined calorimetrically according to [20].
- Quantitative estimation of urea (mg/dl) was estimated according to the method of [16].

Genotoxicity and DNA damage using micronuclei tests

To assess DNA damage, MN and NL formation were analysed as described by [21,22]. A blood sample was smeared on a clean microscope slide. After fixation in pure methanol for 20 minutes, slides were air-dried and then stained with 5% Giemsa solution for 30 minutes. Ten slides per treatment were prepared from each fish and 1000 cells were scored under 1000× magnification to determine the frequencies of MN and NLs in RBCs. Coded and randomized slides were scored blindly by a single observer.

The frequency of distribution of MN and NLs were calculated as follows:

$$\text{RBC (MN/NL) \%} = \frac{\text{Number of cells containing micronuclei}}{\text{Total number of cells counted}} \times 1000$$

Results

Haematological analysis

The mean and standard deviation (SD) values of the haematological parameters of the African catfish *C. gariepinus* from the control and fungicide-exposed groups are presented in table 1. Compared to the mean values of RBCs (5.56 ± .19), Hb (13.25 ± .41) and Hct (44.11 ± 2.27) recorded from control catfish, values were significantly decreased in the treated groups with the increasing concentrations (1.8 µg/L, 3 µg/L, 4.2 µg/L) of copper nanoparticle and penconazole fungicides. LSD tests (Table 2) showed that the mean differences between RBCs, Hb and Hct in the control group and copper nanoparticle-treated fish were (2.111^{aa}), (6.529^{aa}) and (17.050^{aa}) respectively (p < 0.01). In addition, the difference in mean values of the same parameters namely, RBCs (2.881^{aa}), Hb (7.696^{aa}) and Hct (17.489^{aa}), between control and penconazole-treated fish achieved the highest statistical significance (p < 0.01). On the other hand and from a statistical point of view, penconazole could potentially have a more severe adverse effect on these parameters than copper nanoparticles. This effect was evident from the LSD results for copper nanoparticles vs. penconazole: RBCs (0.770^{aa}), Hb (1.167^{aa}) and Hct (7.439^{aa}), p < 0.01. Furthermore, the immune parameters, namely, WBCs and their differentials, were also altered in the fungicide-exposed groups compared to those of the control group. The mean value of WBCs counted from the blood of the control catfish was (8.57 ± .26), while this level increased in the blood of fish exposed to the experimental fungicides. WBCs exhibited a remarkable elevation paralleling the increase in fungicide concentrations, with values of 9.20 ± .76, 11.44 ± .33 and 15.52 ± 1.13 in fish exposed to 1.8, 3 and 4.2 µg/L copper nanoparticles, respectively and 9.67 ± .290, 12.64 ± .32 and 17.488 ± .79 in fish exposed to 1.8, 3 and 4.2 µg/L penconazole, respectively (Table 1). The mean percentage distribution of lymphocytes, monocytes and eosinophils increased dramatically at the low concentration of 1.8 µg/L for both fungicides, declined at 3 µg/L and increased again at 4.2 µg/L, except for eosinophils in penconazole-treated groups. LSD tests (Table 2) showed that WBCs and WBC differentials differed significantly between control and fungicide-treated catfish (0.05 ≥ p ≤ 0.01), except in the cases of monocytes and eosinophils between fish treated with copper nanoparticles or penconazole (p > 0.05).

Parameter (unit)	Treatments						
	Control	Copper nanoparticle concentrations			Penconazole concentrations		
		1.8 µg/L	3 µg/L	4.2 µg/L	1.8 µg/L	3 µg/L	4.2 µg/L
RBCs (×10 ⁶ /µl)	5.56 ± .19	4.77 ± .40	3.38 ± .66	2.81 ± .27	3.98 ± .68	2.42 ± .53	1.74 ± .17
Hb (g/dL)	13.25 ± .41	8.71 ± .56	6.68 ± .55	5.51 ± ± .63	7.71 ± .74	5.14 ± 1.20	3.93 ± .37
Hct (%)	44.11 ± 2.27	45.17 ± 6.48	23.37 ± 3.68	18.60 ± 4.56	41.95 ± 10.55	22.28 ± 7.53	20.20 ± 2.46
WBCs (×10 ³ /µl)	8.57 ± .26	9.20 ± .76	11.44 ± .33	15.52 ± 1.13	9.67 ± .290	12.64 ± .32	17.488 ± .79
Lymphocytes (%)	33.66 ± 2	63.4 ± 4.09	21.33 ± 7.19	41.00 ± 3.77	64.88 ± 8.43	33.66 ± 9.11	45.22 ± 3.80
Monocytes (%)	6.50 ± 1.09	9.00 ± 2.0	7.22 ± 3.30	11.00 ± 2.92	9.11 ± 3.40	7.22 ± 6.07	8.77 ± 2.27
Eosinophils (%)	3.77 ± .87	9.44 ± 3.24	4.44 ± 3.12	5.75 ± 1.75	10.22 ± 4.32	5.22 ± 2.53	4.55 ± 1.66

Table 1: Means ± SDs of blood parameters of *Clarias gariepinus* exposed to different concentrations of Cu nanoparticles and penconazole compared to control values.

Parameters (unit)	Control and Cu-NP	Control and PEN	Cu-NP and PEN
RBCs (×10 ⁶ /µl)	2.111 ^{aa}	2.881 ^{aa}	0.770 ^{aa}
Hb (g/dL)	6.529 ^{aa}	7.696 ^{aa}	1.167 ^{aa}
Hct (%)	17.050 ^{aa}	17.489 ^{aa}	7.439 ^{aa}
WBCs (×10 ³ /µl)	-3.855 ^{aa}	-4.715 ^{aa}	-0.859 ^{NS}
Lymphocytes (%)	-8.714 ^{aa}	-14.806 ^{aa}	-6.092 ^{aa}
Monocytes (%)	-3.245 ^{aa}	-2.241 ^a	-1.388 ^{NS}
Eosinophils (%)	-3.045 ^{aa}	-3.933 ^{aa}	-0.111 ^{NS}

Table 2: Generalized LSD multiple comparison of the total means of blood parameters of *Clarias gariepinus* exposed to Cu nanoparticles (Cu-NP) and penconazole (PEN) with those of control groups.

^a: The mean difference is significant at the 0.05 level.

^{aa}: The mean difference is significant at the 0.01 level.

^{NS}: The mean difference is not significant.

To confirm the toxic potential of the selected fungicides, Pearson correlation analysis was applied at a confidence level of 0.05. Table 3 shows that RBCs, Hb and Hct were negatively and strongly correlated with the increasing concentrations of Cu nanoparticles (0.05 > p = 0.01) and penconazole (p ≤ 0.01). On the other hand, WBCs and WBC differentials increased with the increasing concentrations of Cu nanoparticles and penconazole (0.05 = p < 0.01).

Biochemical analysis

African catfish treated with fungicides exhibited lower protein contents than did control fish (Table 4). The decline was dose-dependent in the case of Cu nanoparticle exposure, for which protein content declined from 6.66 ± .76 in the control fish to 5.23 ± .22 in the fish exposed to 1.8 µg/L Cu nanoparticles, to 4.0 ± .56 in the fish exposed to 3 µg/L Cu nanoparticles and finally to 2.55 ± .59 in the fish exposed to 4.2 µg/L Cu nanoparticles (Table 4). The same trend of a dose-dependent decline was recorded for the serum protein contents of African catfish exposed to penconazole (Table 4). Serum glucose slightly decreased in the blood of fish exposed to the lower concentration of both fungicides (1.8 µg/L). Then, the values increased with increasing fungicide concentrations (Table 4). The mean values of cholesterol and triglycerides increased in the blood of fish exposed to the lower fungicide concentration (1.8 µg/L) and then decreased with the increase in both fungicide concentrations. In contrast, ALT and AST significantly decreased in the blood of fish exposed to the lower fungicide concentration (1.8 µg/L) and then increased with the increase of both fungicide concentrations. The mean values of urea were

significantly increased with the increasing fungicide concentrations compared to those of the control group (Table 4). Penconazole was found to have stronger impact than copper nanoparticles on the biochemical variables. From the toxicological point of view, the impact of fungicides on the serum biochemistry of the African catfish could be ranked as follows: control < Cu nanoparticles < penconazole. In total, the generalized LSD tests (Table 5) showed that most biochemical parameters investigated were potentially adversely affected by the fungicides, with significance levels ranging from ($0.05 \geq p \leq 0.01$). Biochemical variables were strongly correlated ($0.05 \geq p < 0.01$) with the increasing fungicide concentrations (Table 6). Some variables were negatively correlated with the fungicide concentrations (protein, cholesterol and triglycerides), while others were positively correlated ($0.05 \geq p \leq 0.01$) with fungicide concentration (glucose, ALT, AST and urea) (Table 6).

Correlation	Cu nanoparticle concentrations						
	RBCs	Hb	Hct	WBCs	Lymphocytes	Monocytes	Eosinophil
(r)	-.97 ^{aa}	-.97 ^{aa}	-.85 ^a	.99 ^{aa}	.87 ^a	.88 ^a	.97 ^a
(Sig.)	.014	.013	.037	.004	.054	.056	.051
Penconazole							
(r)	-.99 ^{aa}	-.100 ^{aa}	-.99 ^{aa}	1.00 ^{aa}	.97 ^{aa}	.89 ^a	.98 ^a
(Sig.)	.004	.013	.017	.000	.002	.055	.051

Table 3: Correlation coefficients between haematological parameters of *Clarias gariepinus* and the exposure concentrations of Cu nanoparticles and penconazole.

^a: The mean difference is significant at the 0.05 level.

^{aa}: The mean difference is significant at the 0.01 level.

Copper nanoparticle concentrations				
Parameter (unit)	Control	1.8 µg/L	3 µg/L	4.2 µg/L
Protein (g/dl)	6.66 ± .76	5.23 ± .22	4.0 ± .56	2.55 ± .59
Glucose (mg/dl)	151.21 ± 8.06	130.39 ± 7.51	141.70 ± 7.040	175.84 ± 9.04
Cholesterol (mg/dl)	159.06 ± 13.78	169.2 ± 6.91	152.45 ± 9.29	127.83 ± 11.78
Triglyceride (mg/dl)	507.08 ± 134.12	616.2 ± 29.12	518.73 ± 57.09	211.37 ± 78.5
ALT (U/l)	53.27 ± 18.11	30.44 ± .98	56.61 ± 4.78	43.35 ± 6.83
AST (U/l)	67.57 ± 22.78	33.92 ± .77	83.31 ± 9.80	53.71 ± 9.29
Urea (mg/dl)	10.03 ± 5.18	10 ± .10	10.88 ± .75	11.55 ± 1.33
Penconazole concentrations				
Parameter (unit)	Control	1.8 µg/L	3 µg/L	4.2 µg/L
Protein (g/dl)	6.66 ± .76	4.03 ± .070	3.14 ± .18	2.15 ± .200
Glucose (mg/dl)	151.21 ± 8.06	150.66 ± 5.93	156.63 ± 7.48	187.42 ± 8.12
Cholesterol (mg/dl)	159.06 ± 13.78	178.25 ± 11.49	166.36 ± 18.61	149.82 ± 30.29
Triglyceride (mg/dl)	507.08 ± 134.12	641.98 ± 36.74	582.60 ± 85.95	378.72 ± 228.03
ALT (U/l)	53.27 ± 18.11	32.18 ± 2.57	39.87 ± 5.36	71.62 ± 9.82
AST (U/l)	67.57 ± 22.78	36.40 ± 3.00	52.33 ± 8.94	87.33 ± 10.77
Urea (mg/dl)	10.03 ± 5.18	13.66 ± .13	14.24 ± .38	16.28 ± 4.75

Table 4: Means ± SDs of biochemical indices of *Clarias gariepinus* exposed to different concentrations of Cu nanoparticles and penconazole in relation to control values.

Parameters (unit)	Control and Cu-NP	Control and PEN	Cu-NP and PEN
Protein (g/dl)	2.571 ^{aa}	3.25 ^{aa}	0.821 ^{NS}
Glucose (mg/dl)	3.77 ^a	-13.58 ^{aa}	-15.81 ^{aa}
Cholesterol (mg/dl)	9.76 ^{aa}	-5.94 ^a	-15.18 ^{aa}
Triglyceride (mg/dl)	59.84 ^{aa}	-26.68 ^{aa}	-85.25 ^{aa}
ALT (U/I)	10.25 ^{aa}	7.52 ^{aa}	-5.26 ^{aa}
AST (U/I)	10.63 ^{aa}	9.86 ^{aa}	-2.22 ^{aa}
Urea (mg/dl)	0.38 ^{NS}	-1.26 ^a	-4.26 ^{aa}

Table 5: Generalized LSD multiple comparisons of the total means of biochemical parameters of *Clarias gariepinus* exposed to Cu nanoparticles (Cu-NP) and penconazole (PEN) with those of control groups.

^a: The mean difference is significant at the 0.05 level.

^{aa}: The mean difference is significant at the 0.01 level.

^{NS}: The mean difference is not significant.

Correlation	Cu nanoparticle concentrations						
	Protein	Glucose	Cholesterol	Triglyceride	ALT	AST	Urea
(r)	-.99 ^{aa}	.98 ^{aa}	-1.00 ^{aa}	-.92 ^a	.98 ^{aa}	.89 ^a	.80 ^a
(Sig.)	.005	.014	.006	.058	.0172	.0539	.051
	Penconazole						
	Protein	Glucose	Cholesterol	Triglyceride	ALT	AST	Urea
(r)	-.98 ^{aa}	.97 [*]	-.99 ^{aa}	-.95 ^a	.94 ^a	.97 ^a	.87 ^a
(Sig.)	.008	.051	.008	.025	.056	.036	.021

Table 6: Correlation coefficients between serological indices of *Clarias gariepinus* and the exposure concentrations of Cu nanoparticles and penconazole.

^a: The mean difference is significant at the 0.05 level.

^{aa}: The mean difference is significant at the 0.01 level.

Parameter (unit)	Control	Exposure concentrations					
		Copper nanoparticles			Penconazole		
		(1.8 µg/L)	(3 µg/L)	(4.2 µg/L)	(1.8 µg/L)	(3 µg/L)	(4.2 µg/L)
MN%	3.33 ± 1.52	9 ± 2	15.11 ± 5.37	20.66 ± 1.5	17 ± 2	22.88 ± 5.62	29 ± 3.3
BN%	1.33 ± 1.52	3.66 ± .57	8.77 ± 4.63	14 ± 2	15 ± 2	17.66 ± 2.50	20 ± 1
Lobed%	000	1.33 ± .57	3.33 ± 2.23	3.33 ± 2.3	7.33 ± 1.52	10.55 ± 3.35	14.33 ± 2.08
Heart-shaped%	0.33 ± 0.57	2.33 ± 2.08	2.66 ± 1.41	3.33 ± 1.53	5.33 ± .57	6.22 ± 1.20	7.77 ± 1.15
Kidney-shaped%	000	.667 ± .57	1.11 ± 0.92	1.66 ± 1.15	4.33 ± .57	4.55 ± 1.23	5.66 ± 1.15
Irregularly shaped%	000	.33 ± .57	1.22 ± 1.09	2.33 ± .57	2 ± 1	3.22 ± 1.56	3.66 ± 2.08
Notched N%	000	000	0.55 ± 0.72	1 ± .00	1.66 ± .57	2.44 ± 1.23	3.33 ± 1.15

Table 7: Means ± SDs of micronuclei and nuclear lesions (%) from the blood of *Clarias gariepinus* exposed to different concentrations of Cu nanoparticles and enconazole in relation to control values.

Parameter (unit)	Control and Cu-NP	Control and PEN	Cu-NP and PEN
MN%	- 11.778 ^{aa}	- 19.556 ^{aa}	- 7.778 ^{aa}
BN%	- 7.444 ^{aa}	- 16.333 ^{aa}	- 8.888 ^{aa}
Lobed%	- 3.333 ^{NS}	- 10.555 ^{aa}	- 7.222 ^{aa}
Heart-shaped%	- 2.333 ^{aa}	- 5.888 ^{aa}	- 3.555 ^{aa}
Kidney-shaped%	- 1.111 ^{NS}	- 4.555 ^{aa}	- 3.444 ^{aa}
Irregularly shaped%	- 1.222 ^{NS}	- 3.222 ^{aa}	- 2.000 ^{aa}
Notched N%	- 0.555 ^{NS}	- 2.44 ^{aa}	- 1.888 ^{aa}

Table 8: Generalized LSD multiple comparisons of the total means of micronuclei and nuclear lesions in the blood of *Clarias gariepinus* exposed to Cu nanoparticles (Cu-NP) and penconazole (PEN) with those of control groups.

^{aa}: The mean difference is significant at the 0.01 level.

^{NS}: The mean difference is not significant.

Correlation	Cu nanoparticles concentrations						
	MN	BN	Lobed	H.-shaped	K.-shaped	Ir.- shaped	Notched N
(r)	1.00 ^{aa}	1.00 ^{aa}	1.00 ^{aa}	.86 ^a	1.00 ^{aa}	.99 ^{aa}	.99 ^a
(Sig.)	.017	.002	.004	.033	.002	.004	.041
	Penconazole						
(r)	1.00 ^a	1.00 ^{aa}	.99 ^a	1.00 ^{aa}	.99 ^a	.94 ^a	.95 ^a
(Sig.)	.008	.051	.008	.025	.056	.036	.021

Table 9: Correlation coefficients between MN and NLs in RBCs of *Clarias gariepinus* and the exposure concentrations of Cu nanoparticles and penconazole.

^a: The mean difference is significant at the 0.05 level.

^{aa}: The mean difference is significant at the 0.01 level.

L.-shaped = lobed shaped, K.-shaped = kidney shaped, Ir.- shape = irregularly shaped.

Genotoxicity studies

MN and six types of NLs were assessed in the blood of control and fungicide-treated African catfish. These abnormalities included binucleated (BN), heart-shaped, kidney-shaped, notched, irregularly shaped and lobed nuclei (Figure 1). The per thousand (‰) distribution of MN RBCs and NLs scored from control groups were lower than those scored from groups exposed to different concentrations of Cu nanoparticles and penconazole (Table 7 and Figure 1). The mean% value for MN scored from RBCs of the control catfish group was $3.33 \pm 1.52\%$. This value increased dramatically to $9 \pm 2\%$ in fish treated with $1.8 \mu\text{g/L}$ copper nanoparticles, to $15.11 \pm 5.37\%$ with exposure to $3 \mu\text{g/L}$ copper nanoparticle and reached a mean value of $20.66 \pm 1.5\%$ in the blood of catfish exposed to $4.2 \mu\text{g/L}$ Cu nanoparticles. In accordance with the MN results, the % value of BN cells increased significantly from $1.33 \pm 1.52\%$ in the control fish to $14 \pm 2\%$ in the blood of fish exposed to the highest Cu nanoparticle concentration ($4.2 \mu\text{g/L}$). Additionally, the distributions of the heart-shaped nuclei ($0.33 \pm 0.57\%$) showed the same increasing trend between the control group and the group exposed to $4.2 \mu\text{g/L}$ Cu nanoparticles ($3.33 \pm 1.53\%$). Similarly, significant increases were recorded for % values in MN [(17 ± 2), (22.88 ± 5.62), (29 ± 3.3)], BN cells [(15 ± 2), (17.66 ± 2.50), (20 ± 1)] and heart-shaped nuclei [(5.33 ± .57), (6.22 ± 1.20), (7.77 ± 1.15)] (Table 7) in the blood of catfish exposed to

increasing concentrations (1.8, 3 $\mu\text{g/L}$ and 4.2 $\mu\text{g/L}$) of penconazole, respectively, when compared to those of control fish. Lobed, kidney-shaped, irregularly shaped and notched nuclei were completely absent from the blood of African catfish in the control group. These four biomarkers of genotoxicity were recorded only in the exposed groups and increased proportionally with increasing concentrations of the tested fungicides. The generalized multiple comparison LSD test revealed the presence of significant differences ($0.05 \geq p \leq 0.01$) between the mean values of MN and NLs in the blood of the control fish and those in the blood of African catfish exposed to Cu nanoparticles and penconazole (Table 8). The mean values of MN, BN cells and heart-shaped nuclei recorded from the control group compared to those from fungicide-exposed fish could be ranked as follows: control < Cu nanoparticles (1.8 $\mu\text{g/L}$) < Cu nanoparticles (3 $\mu\text{g/L}$) < Cu nanoparticles (4.2 $\mu\text{g/L}$) < penconazole (1.8 $\mu\text{g/L}$) < penconazole (3 $\mu\text{g/L}$) < penconazole (4.2 $\mu\text{g/L}$). The correlation (Table 9) between the fungicide concentrations and blood variables was strong at significance levels ranging between ($0.05 \geq p \leq 0.01$). The correlation of MN and NLs was strong (MN ($r = 1, p = 0.01$); BN cells ($r = 1, p < 0.01$); lobed nuclei ($r = 1, p < 0.01$); heart-shaped nuclei ($r = 0.8, p < 0.05$); kidney-shaped nuclei ($r = 1, p < 0.01$); irregularly shaped nuclei ($r = 9, p < 0.01$); and notched nuclei ($r = 9, p < 0.05$)) and increased positively with the increasing dose (Figure 1). The same trend was recorded for penconazole, being positively correlated with the MN and NL values ($0.05 \geq p \leq 0.01$).

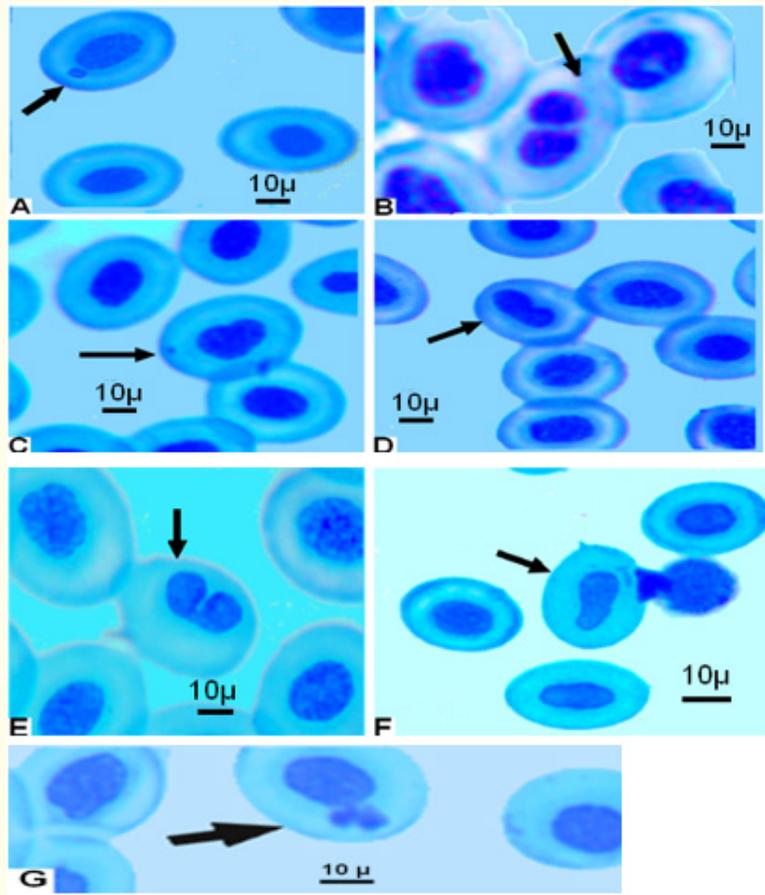


Figure 1: Photomicrographs of RBCs of *Clarias gariepinus* exposed to Cu nanoparticles and penconazole: micronuclei (A), binuclei (B), heart shaped nucleus (C), kidney shaped nucleus (D), notched nuclei (E), irregularly shaped nucleus (F) and lobed nucleus (G).

Discussion

Freshwater fauna suffer from the intensive use of pesticides for spraying crops. New pesticides are added daily. Fungicides are pesticides that specifically inhibit or kill fungi underlying diseases important to man. In agriculture, fungicides are used to protect tubers, fruits and vegetables during storage or are applied directly to ornamental plants, trees, field crops, cereals and turfgrasses [23]. Despite their benefits, Cu nanoparticles and penconazole have the potential to deteriorate the environment if applied in high amounts. These compounds, for instance, distribute in organs and tissues of animals and cause specific structural changes [9]. Their increase in organisms up to the toxic threshold (maximum tolerated dose) results in dystrophy and tissue necrosis. According to the available literature, this study is the first assessment of the effects of penconazole and Cu nanoparticle fungicides on the haematological, biochemical and toxicological parameters of African catfish. Clearly evidenced impacts of penconazole and Cu nanoparticles were recorded here in the values of the selected biomarkers. According to the results of the present work, the detected alterations in the haematological, serological and genotoxic variables could be used as potential biomarkers to evaluate the ecotoxicity of the examined fungicides in aquatic organisms.

Fungicides and haematology

Since haematological parameters are influenced by a variety of environmental stressors, they have the potential to be used as biomarkers of aquatic pollution. Their evaluation in fish has become an important means of understanding the toxicological impacts of exposure hazards [3,24-27]. In the present work, exposure of *C. gariepinus* to sub-lethal concentrations of copper nanoparticles and penconazole elicited changes in some haematological parameters. The decrease in the haematological parameters could be attributed to the toxo-chemical effect of increasing concentrations of both fungicides. The RBC, Hb and Hct values were appreciably reduced. This reduction may be attributed to haemolysis caused by the fungicidal action in the fish. In addition, the decrease may also be attributed to limited RBC synthesis due to impaired osmoregulation across the gill epithelium and accumulation of the toxicant in the gill region [28,29]. On the other hand, RBCs affected by toxicity may be disintegrated, causing a reduction in the Hb and Hct concentrations [30]. In the present study, the mean values of Hb under increasing copper nanoparticle and penconazole concentrations (1.8, 3, 4.2 µg/L) decreased dramatically compared to those of control fish.

Severe decreases in Hb have been shown to impair the oxygen supply to various tissues, resulting in a slow metabolic rate and low energy production [31]. Similarly, [32] found that Hb (%) decreased in freshwater catfish (*Heteropneustes fossilis*) after exposure to the insecticide deltamethrin. Regarding the immune system response to toxicity, the WBCs showed a significant increase when fish were exposed to fungicides. This result agrees with those of several previous studies, for example [33] and [30] indicated the presence of a defensive response of fish against toxic invasions, indicated by elevated WBC numbers. Furthermore, exposure to toxicants such as fungicides has the potential to stimulate the lymphocytes as a defence response against the stressor, hence the observed proliferation of WBCs in the peripheral blood [34]. Other leukocyte differentials such as those of monocytes and eosinophils were comparable throughout the experimental period. Similar observations have also been reported in other fishes and amphibians treated with different toxicants [35-37].

Fungicides and blood biochemistry

In toxicological studies using acute exposures, changes in concentrations and activities of biochemical variables may reflect alterations in the physiological status of an organism. Proteins are the most important compound in living organisms and play essential roles in the construction and physiology of cells and in cellular metabolism [38]. Exposure of aquatic animals to sub-lethal concentrations of toxic chemicals is usually associated with growth and biochemical consequences [39]. In the present investigation, the protein content decreased in the blood plasma of *C. gariepinus* exposed to different concentrations of Cu nanoparticles and penconazole compared to the control levels. The decrease in total protein may be due to the inhibition of RNA synthesis that controls protein metabolism [40]. Similar results were verified in *Oreochromis niloticus* after exposure to malathion [8] and *C. gariepinus* after exposure to carbofuran [41]. The

observed hypoproteinaemia could be caused by the reduction in protein from the inhibition of blood protein synthesis in the liver and damage to the subcellular structures responsible for protein synthesis [42] or by damage to renal tissue [43]. Blood glucose levels have long been used as indicators of stress in fish. Chemical pollutants modulate the metabolism of carbohydrates, causing hyperglycaemia by stimulating glycogenolysis in fish [44]. Compared to the control group, serum glucose slightly decreased in the blood of fish exposed to the lower concentration of both fungicides (1.8 µg/L). Then, the values increased with the increasing fungicide concentrations. Increased levels of glucose were previously recorded in blood of fishes exposed to heavy metals [44,45] and other pollutants [4,46-48]. This can be attributed to the alteration in the activity of glucose-6-phosphate dehydrogenase and lactate dehydrogenase previously detected by [49]. It has been reported that exposure of fish to pollutants [50] increased cholesterol and triglyceride concentrations in the blood. In the present work, the blood cholesterol and triglyceride levels increased in the blood of fish exposed to the lower fungicide concentration (1.8 µg/L) and then decreased with the increase of both fungicide concentrations. Such an alteration in cholesterol concentrations could be explained as a result of liver damage, leading to inhibition of enzymes that convert cholesterol into bile acid [51]. In the present study, ALT and AST significantly decreased in the blood of fish exposed to the lower fungicide concentration (1.8 µg/L) and then increased with the increase of both fungicide concentrations. The increase in the level of these enzymes was previously recorded in the blood of Nile tilapia and African catfish after exposure to pesticides [48], heavy metals [45,52], nonylphenols [46] and UV rays [22]. Increased levels of ALT and AST indicate an adaptive response to the leakage of these enzymes into the blood stream due to the presence of fungicides. Increased levels of urea indicate kidney damage [53,54]. Similar findings were reported by [55] who recorded a significant increase in urea in Nile tilapia due to cadmium exposure. In the present results, penconazole was found to have a stronger impact than copper nanoparticles on the biochemical variables.

Fungicides and genotoxicity

MN tests in fish have potential for detecting clastogenic substances in aquatic environments. Since teleost RBCs are nucleated, MN have been scored in fish RBCs as a measure of clastogenic activity. In addition to MN formation, many different nuclear abnormalities have been reported for fish species [4,12,22], but there is not a consensus about the type of aberrations to be included in MN tests. According to [56], BN cells have a similar origin to MN and are established genotoxic indicators. NL formation is a bioindicator of abnormal cell division due to blocking of cytokinesis. Such abnormal cell division results in genetic imbalance of the cells, which may also be involved in carcinogenesis [57]. Overuse of the fungicide penconazole in agricultural production could induce a potential risk for damage to genetic material [58]. showed that, after 9 days, penconazole impaired the genetic base and caused the induction of micro nucleated RBCs in the blood of the fish *Carassius auratus*. In our study, significantly higher frequencies of MN and NLs were observed in the blood of fish exposed to Cu nanoparticles and penconazole than in the blood of the control fish. Such increases were significantly dose dependent. The present results are in agreement with [58] who tabulated the mean value of MN as 25%, which was higher than the 2% in the control fish that he studied. The results of this study confirmed the usefulness of the RBC MN and NLs as powerful monitoring tools for detecting genotoxic agents in the freshwater environment [22]. Although the mechanism responsible for nuclear abnormalities has not been fully explained, these abnormalities are considered to be indicators of genotoxic damage and therefore, they may complement the scoring of MN in routine genotoxicity surveys [59]. The frequencies of MN and NLs detected in the present study were significantly ($0.05 \geq p \leq 0.01$) higher in the blood of catfish exposed to penconazole than in that of fish exposed to Cu nanoparticles, indicating that penconazole is more toxic than Cu nanoparticles in terms of genotoxic potential.

Conclusion

Penconazole and copper nanoparticles fungicides could potentially cause haemato-serological and genotoxic alterations in the experimental catfish, depending on the concentrations of the administered chemicals. In the present work, exposure of *C. gariepinus* to sub-lethal concentrations of copper nanoparticles and penconazole elicited reduction in RBC, Hb and Hct values, attributing to the toxic effect of increasing concentrations of both fungicides. The result of this work indicated the presence of a defensive response of

fish against toxic invasions by elevated WBC numbers. In the present investigation, the protein content decreased in the blood plasma of *C. gariepinus* exposed to different concentrations of Cu nanoparticles and penconazole compared to the control levels. In contrast, the levels of ALT and AST increased with the increase of both fungicide concentrations, indicating an adaptive response to the leakage of these enzymes into the blood stream due to the presence of fungicides. The induction of micro nucleated RBCs and nuclear lesions was determined to be related to the exposure concentrations of the selected fungicides. According to the present results, the tested parameters were more adversely influenced by penconazole than by copper nanoparticles.

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Bibliography

1. Engelman R and Le Roy P. "Sustaining water, population and the future of renewable water supplies". Population Action International, Population and Environment Program, Washington, D. C (1993): 30-318.
2. Abdel-Shafy HI and Aly RO. "Water Issue in Egypt: resources, pollution and protection endeavors, review article". *Central European Journal of Occupational and Environmental Medicine* 8.1 (2002): 3-21.
3. Osman A. "Biomarkers in Nile Tilapia *Oreochromis niloticus niloticus* (Linnaeus, 1758) to assess the impacts of river Nile pollution: Bioaccumulation, Biochemical and Tissues Biomarkers". *Journal of Environmental Protection* 03.8 (2012).
4. Osman A., et al. "Blood Biomarkers in Nile tilapia *Oreochromis niloticus niloticus* and African Catfish *Clarias gariepinus* to evaluate water quality of the river Nile". *Journal of Fisheries Sciences.com* 12 (2018).
5. Nguyen L and Janssen C. "Embryo-Larval toxicity tests with the African Catfish (*Clarias gariepinus*): Comparative sensitivity of endpoints". *Archives of Environmental Contamination and Toxicology* 42.2 (2002): 256-262.
6. Osman A., et al. "Lead induced malformations in embryos of the African catfish *Clarias gariepinus* (Burchell, 1822)". *Environmental Toxicology* 22.4 (2007): 375-389.
7. Kumar Yadav S. "Pesticide Applications-Threat to Ecosystems". *Journal of Human Ecology* 32.1 (2010): 37-45.
8. Hamed HS. "Impact of a short – term malathion exposure of Nile Tilapia, (*Oreochromis niloticus*): The protective role of selenium". *International Journal of Environmental Monitoring and Analysis* 3 (2015): 30-37.
9. Sizova E., et al. "Copper Nanoparticles as Modulators of Apoptosis and Structural Changes in Tissues". *Journal of Biomaterials and Nanobiotechnology* (2012): 03.
10. Warheit D., et al. "Comparative Pulmonary Toxicity Assessment of Single Wall Carbon Nanotubes in Rats". *Toxicological sciences: an official journal of the Society of Toxicology* 77.1 (2004): 117-125.
11. Husak VV., et al. "Acute exposure to the penconazole-containing fungicide Topas partially augments antioxidant potential in goldfish tissues". *Comparative Biochemistry and Physiology Toxicology and Pharmacology* 193 (2017): 1-8.
12. Osman A., et al. "In situ evaluation of the genotoxic potential of the river Nile: II. Detection of DNA strand-breakage and apoptosis in *Oreochromis niloticus niloticus* (Linnaeus, 1758) and *Clarias gariepinus* (Burchell, 1822)". *Mutation Research* 747 (2012): 14-21.
13. Seriani R., et al. "Relationship between water toxicity and hematological changes on *Oreochromis niloticus*". *Brazilian Journal of Aquatic Science and Technology* 15 (2011): 47-53.

14. Srivastava P, *et al.* "Pesticides toxicity in fishes: Biochemical, Physiological and Genotoxic Aspects, 16 (2016): 199-218.
15. Shaw AF. "A direct method for counting the leucocytes, thrombocytes and erythrocytes of birds blood". *Journal of Pathology and Bacteriology* 33.3 (1930): 833-835.
16. Henry RJ. "Clinical chemistry: principles and technics". Harper & Row, New York (1964).
17. Trinder P. "Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen". *Journal of Clinical Pathology* 22.2 (1969): 158-161.
18. Thomas L. "Enzymatic colorimetric method to determine the cholesterol". Lab. And Diagnose, Textbook of hematology 2nd edition, William and welcome company (1992): 415.
19. Friedewald WT, *et al.* "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge". *Clinical Chemistry* 18.6 (1972): 499-502.
20. Reitman S and Frankel S. "A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases". *American Journal of Clinical Pathology* 28.1 (1957): 56-63.
21. Cavas T and Ergene S. "Induction of micronuclei and nuclear abnormalities in *Oreochromis niloticus* following exposure to petroleum refinery and chromium processing plant effluents". *Aquatic toxicology* 74.3 (2005): 264-271.
22. Osman A and Harabawy A. "Hematotoxic and Genotoxic Potential of Ultraviolet-A Radiation on the African Catfish *Clarias gariepinus* (Burchell, 1822)". *Journal of Fisheries International* 5 (2010): 44-45.
23. Gupta PK and Aggarwal M. "Toxicity of fungicides". In: Veterinary Toxicology, Gupta, RC (Ed.), 1st edition (2007): 587-601.
24. Borges K, *et al.* "Endophytic fungi as models for the stereoselective biotransformation of thioridazine". *Applied Microbiology and Biotechnology* 77.3 (2008): 669-674.
25. Li ZH, *et al.* "Antioxidant responses and plasma biochemical characteristics in the freshwater rainbow trout, *Oncorhynchus mykiss*, after acute exposure to the fungicide propiconazole". *Czech Journal of Animal Science* 56.2 (2011).
26. Sudová E, *et al.* "The effect of praziquantel applied per os on selected haematological and biochemical indices in common carp (*Cyprinus carpio* L.)". *Fish Physiology and Biochemistry* 35.4 (2008): 599-605.
27. Said RE, *et al.* "Haemotoxic and genotoxic potential of lead on the Egyptian toad *Ametophrynus regularis*". *International Journal of Ecotoxicology and Ecobiology* 1 (2016): 94-102.
28. Pereira L, *et al.* "Hematological and biochemical alterations in the fish *Prochilodus lineatus* caused by the herbicide clomazone". *Environmental Toxicology and Pharmacology* 36.1 (2013): 1-8.
29. Saravanan M, *et al.* "Ecotoxicological impacts of clofibric acid and diclofenac in common carp (*Cyprinus carpio*) fingerlings: hematological, biochemical, ion regulatory and enzymological responses". *Journal of Hazardous Materials* 195 (2011): 188-194.
30. Shahi J and Singh A. "Genotoxic and haematological effect of commonly used fungicide on fish *Clarias batracus*". *Journal of Biology and Earth Sciences* (2014): 4.

31. Shakoori A., *et al.* "Sublethal Effects of Danitol (Fenprothrin), a Synthetic Pyrethroid, on Chinese Grass Carp, *Ctenopharyngodon Idella*". *Folia Biologica* 43.3-4 (1995).
32. Kumar S., *et al.* "Deltamethrin induced physiological changes in freshwater catfish *Heteropneustes fossilis*". *Bulletin of Environmental Contamination and Toxicology* 62.3 (1999): 254-258.
33. Davis M., *et al.* "Breathing: The relaxation and stress reduction workbook chapter singles". Oakland: New Harbinger Publications (2008)
34. Campbell SA. "The science and engineering of microelectronic fabrication". Oxford University Press, New York; Oxford (1996)
35. Said RE., *et al.* "Hepatic alteration of the Egyptian toad *Amietophrynus regularis*, as biomarker to environmental deterioration". *International Journal of Environmental Monitoring and Analysis* 3 (2015): 22-29.
36. Velisek J., *et al.* "Comparison of the effects of four anaesthetics on biochemical blood profiles of perch". *Aquaculture Research* 40.3 (2008): 354-361.
37. Mohammad Nejad Shamoushaki M., *et al.* "Effects of organophosphate, diazinon on some haematological and biochemical changes in *Rutilus frisii kutum* (Kamensky, 1901) male brood stocks". *Iranian Journal of Fish Science* 11.1 (2012).
38. Mommsen T and Walsh JP. "Biochemical and environmental perspectives on nitrogen metabolism in fishes". *Experientia* 48.6 (1992): 583-593.
39. Abdou KA and Zaky ZM. "Toxic effects of the fungicide malachite green on catfish (*Clarias gariepinus*)". *Bulletin of Environmental Research* 3 (2000).
40. Thoker MA. "Comparative study of biochemical alterations induced by carbofuran and malathion on *Channa punctatus* (Bloch.)". *International Research Journal of Biological Sciences* 4.9 (2015): 61-65.
41. Harabawy A and Ibrahim A. "Sublethal toxicity of carbofuran pesticide on the African catfish *Clarias gariepinus* (Burchell, 1822): Hematological, biochemical and cytogenetic response". *Ecotoxicology and Environmental Safety* (2014): 103.
42. Fontana L., *et al.* "Serum amino acid changes in rats with thioacetamide-induced liver cirrhosis". *Toxicology* 106.1-3 (1996): 197-206.
43. Gad NS. "Impact of environmental pollution in the southern region of lake Manzalah Egypt on some biochemical parameters of *Tilapia zillii*". *Journal of the Egyptian German Society of Zoology* 48.A (2005):279.
44. Levesque H., *et al.* "Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field". *Aquatic Toxicology* 60.3-4 (2002): 257-267.
45. Mekkawy IA., *et al.* "Effects of cadmium on some haematological and biochemical characteristics of *Oreochromis niloticus* (Linnaeus, 1758) dietary supplemented with tomato paste and vitamin E". *Fish Physiology and Biochemistry* 37.1 (2011b): 71-84.
46. Mekkawy I., *et al.* "Effects of 4-nonylphenol on blood cells of the African catfish *Clarias gariepinus* (Burchell, 1822)". *Tissue and Cell* 43.4 (2011a): 223-229.
47. Poléo A and Hytterød S. "The effect of aluminium in Atlantic salmon (*Salmo salar*) with special emphasis on alkaline water". *Journal of Inorganic Biochemistry* 97.1 (2003): 89-96.

48. Adedeji BO and Agbede O. "Effects of diazinon on blood parameters in the African catfish (*Clarias gariepinus*)". *African Journal of Biotechnology* 8 (2009): 3940-3946.
49. Osman A., et al. "Enzymatic and histopathologic biomarkers as indicators of aquatic pollution in fishes". *Natural Science* 2 (2010): 1302-1311.
50. Rajamanickam V and Narayanan M. "The impact of toxic heavy metals on the hematological parameters in common carp (*Cyprinus carpio* L.)". *Iranian Journal of Environmental Health Science and Engineering* 6.1 (2009): 23-28.
51. Murray RK., et al. "Harpers Biochemistry". 22nd edition. Lange Medical Books/McGraw-Hill: New York (1991).
52. Oner M., et al. "Changes in serum biochemical parameters of fresh water fish *Oreochromis Niloticus* following prolonged Metal (Ag Cd Cr Cu Zn) Exposures". *Environmental Toxicology and Chemistry* 27.2 (2008): 360-366.
53. Abu OMG., et al. "Evaluation of biochemical changes associated with replacement of maize with whole cassava root meal in the diet of hybrid catfish". *Journal of Aquaculture Feed Science and Nutrition* 1.3 (2009): 68-72.
54. Kamal SM and Omar WA. "Effect of different stocking densities on hematological and biochemical parameters of silver Carp, *Hypophthalmichthys molitrix* fingerlings". *Life Science Journal* 8.4 (2011): 580-586.
55. Zaki M., et al. "Clinicopathological, biochemical and microbiological change on grey mullet exposed to cadmium chloride". *American-Eurasian Journal of Agricultural and Environmental Science* (2009): 5.
56. Serrano L and Montero R. "Micronuclei and chromatid buds are the result of related genotoxic events". *Environmental and Molecular Mutagenesis* 38.1 (2001): 38-45.
57. Rodilla B. "Los términos relacionados con la medicina en el Diccionario de Autoridades". *Boletín de la Real Academia Española* 73 (1993): 463-512.
58. Kurteshi K., et al. "Algalenosis of river Përlepnica during spring season 2010". *Journal of Mountain Agriculture on the Balkans* 18 (2015): 239-247.
59. Guner U and Gökalp F. "Micronucleus test, nuclear abnormalities and accumulation of Cu and Cd on *Gambusia affinis* (Baird & Girard, 1853)". *Turkish Journal of Fisheries and Aquatic Sciences* 11 (2011): 615-622.

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