



## Parasitological indicators, haemato-biochemical alternations, and environmental risks of heavy metals in cultivated and wild freshwater catfish, Egypt

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### ABSTRACT

This study aims to monitor physico-chemical parameters and heavy metal (HM) levels in the water, HM levels, and haemato-biochemical changes in parasite-infected and non-infected catfish, *Clarias gariepinus*, in wild and cultivated origins. The outcomes of physico-chemical properties and heavy metal (HM) levels in water revealed significant variations between the two selected sites, except for cadmium metal ( $p < 0.05$ ). HM concentrations in both water and organs of the two catfish origins decreased as the following decreased:  $Fe < Zn < Pb < Cu < As < Cd$ . Furthermore, the concentration of vital HM was significantly lower in wild catfish as compared to cultivated catfish, whereas non-vital HM was significantly higher in wild catfish. However, the HM accumulation in the different organs of catfish is ranked in ascending order: liver > gills > intestine > muscles. The hematological and biochemical investigations revealed a significant decline in the hemoglobin value, packed cell volume, number of red blood cells, albumin, total protein, and globulin while total aspartate aminotransferase activity, white blood cell count, alanine aminotransferase activity, glucose, and urea levels were all significantly higher in parasitic-infected catfish from two origins compared to uninfected catfish. Human health hazards, as evaluated by non-carcinogenic risk (hazard index and target hazard quotient), estimated daily intake, and carcinogenic risk, were all below the benchmark's allowable range. Nonetheless, the outcome of the health risk assessment suggests that consumption of the muscles of catfish from wild and cultivated origins cannot pose considerable health risks for children and adult consumers. Additionally, the prevalence of parasites can be used as a surrogate indicator to predict the possible impact of metal pollution and bioaccumulation. Consequently, this study suggests that Egypt's environmental management conducts routine HM monitoring in the studied sites to reduce potential health risks.

### INTRODUCTION

Water pollution due to HM levels is one of the most important concerns today, it directly threatens not just aquatic ecosystems but also human health impacts on organisms and humans (Abd El-Aziz *et al.*, 2022). HM levels have significant environmental impacts on all organisms. They can be introduced into food chains and can accumulate in aquatic organisms to levels that influence their physiological status (Abbas *et al.*, 2021). Metals, including Fe, Cu, Co, Mn, and Zn, are necessary for biological life, but they become poisonous at higher concentrations and other

metals, such as Cd, As, Pb, and Hg are not essential and, even at low concentrations, can be hazardous (Radwan *et al.*, 2022a). Although HMs are found naturally in the environment, industry, anthropogenic, and agricultural activities have all contributed to harmful levels that are undesirable (Bonanno and Vymazal, 2017). The level of metals in fish varies according to tropical level, locality, distribution, feeding habit, habitual preference, size, metal exposure period, age, homeostatic regulation activity, metabolic activity and can cause oxidative stress as well as biochemical, physiological, hematological, and morphological alterations (Milenkovic, *et al.*, 2019).

Parasites of fish are considered very sensitive to HM pollutants, as they not only accumulate metals in their organs but also have a physiological response to them, due to the varied methods by which parasites adapt to anthropogenic pollutants, they can be categorized as harm indicators or accumulation indicators (Radwan 2022).

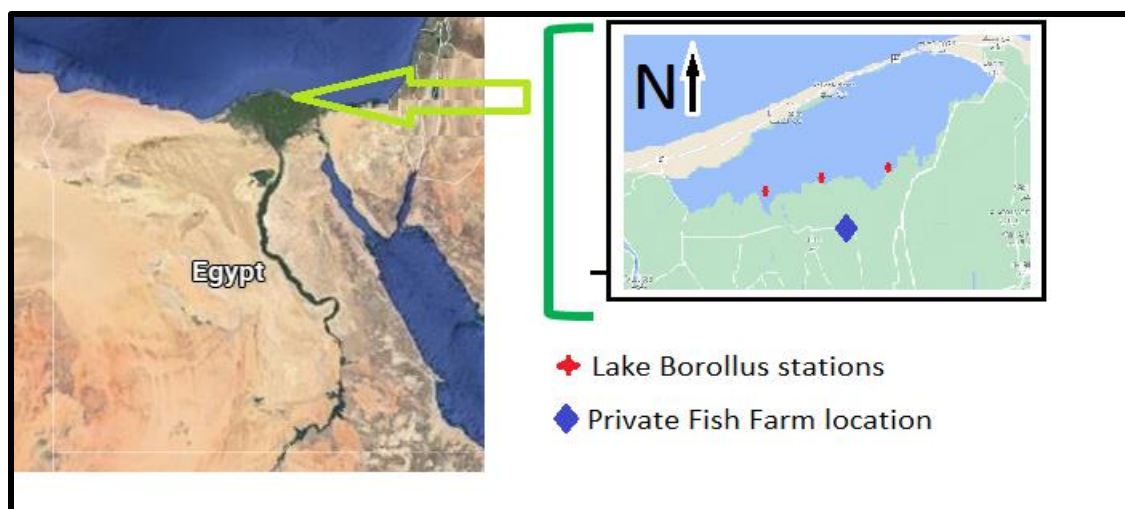
Haemato-biochemical markers have been used to efficiently monitor organisms' responses to stressors and thus their health status even under adverse environments. In general, hematological investigations are utilized in human and veterinary studies to assess normal vital signs and to diagnose disorders resulting from a variety of causes, including environmental stress, metal pollution, parasite infections, nutrition, and the genotoxic impact of pollutants (Esmaili, 2021). Fish blood is susceptible to pollution-induced damage, and alternations in hematological markers, including packed cell volume, erythrocyte count, and hemoglobin concentration, may be employed to detect damage induced by pollutants, including heavy metals (Radwan *et al.*, 2021). Biochemical markers in fish are also sensitive to possible adverse effects of metal accumulations. Changes in haemato-biochemical indices caused by various parasites build up a database that can be employed to aid in the diagnosis of diseases and guide the execution of therapy or protective methods (Hamouda and Abd Alkareem, 2021).

Pollutants and deteriorated biological environments may be promoting infections in aquatic species, particularly fish, while few studies have compared the accumulation of HM in cultivated and wild fish (Yipel *et al.*, 2016; Radwan *et al.*, 2022). There is limited evidence on the differences in HM content and parasite prevalence (PP) relationships among cultivated and wild catfish. Therefore, the objective of the present is to identify for the first time both HM content and PP in both cultivated and wild catfish. The present study also aims to monitor physic-chemical parameters and HM levels of water, HM levels, and haemato-biochemical alternations in parasite-infected and un-infected catfish in cultivated and wild origins, as well as the assessment of possible health risks for children and adult consumers when eating catfish muscles.

## MATERIALS AND METHODS

### A sampling of fish and water

Water samples were obtained from two different sites. The first site was Lake Borollus, and the other was a private fish farm in Kafr El-Sheikh governorate (Figure 1). A total of 382 catfish, *Clarias gariepinus*, were collected at the same time and from the same site as the water samples, the first collection from Lake Borollus (wild origin) and the second one from the private fish farm (cultivated origin). After collection, samples were transferred on the same day to the Marine Biology lab, Zoology Depart., Faculty of Science, Al-Azhar Univ., Egypt.



**Fig. 1.** A map of the private fish farm and Lake Borollus stations.

### Water Physico-chemical investigations

According to the methods discussed in the American Public Health Association (APHA, 2005), physico-chemical parameters were detected in the water of a private fish farm and Lake Borollus.

### Catfish parasitological examinations

In the laboratory, parasitological examinations, and isolated parasites were stained with identification according to those described by (El-Shahawy *et al.*, 2017, Radwan *et al.*, 2021 and Radwan (2022).

El-Shahawy *et al.* (2017) used the appropriate equation to determine fish PP:  $FPP (\%) = (HI \text{ No.} \div HE \text{ No.}) \times 100$ .

Where FPP stands for fish parasite prevalence, HI No. is the number of members of a host species who have been infected by a certain parasite species, and HE No. is the total number of hosts examined.

### The length-weight relationship of catfish

The following method was used to calculate the length-weight relation using the power formula or its logarithmic modification:  $\text{Log } W (\text{Lagler, 1956}) = \text{Log } a \pm b \text{ Log } L$ .

Where W is the fish's weight in grams. L is the standard length in centimeters. The least squares technique is used to estimate the values of the constants a and b. The fish were divided into groups of 10 mm in length, and weights were estimated and empirically determined.

### HM levels measurement

#### Digestion of samples

After parasitological examination, the investigated organs (gills, muscles, intestine, and liver) from infected and uninfected fish were assigned to HM analysis. Five milliliters of concentrated nitric acid were required to treat 0.5 g of organs. The mixture was warmed until completely digested on the hot plate. The digested tissue was then placed in a volumetric flask, in which the volume was diluted with deionized water to 50 ml. An acid digestion technique for total metals was used to estimate the levels of HM in water samples. The digested mixture was added to a volumetric flask and diluted with deionized water to a total volume of 100 ml. The diluted solutions were then tested. (AOAC, 2012).

## Analysis of ICP

Vital metals (copper Cu, iron Fe, and zinc Zn) and non-vital metals (arsenic As, lead Pb, and cadmium Cd) concentrations were detected based on those described by (Abbas *et al.*, 2021). To determine the strategy detection limit, duplicate blank specimens from each analytical group were performed in a randomized order. On a wet weight basis, the contents of metals in the fish samples were assessed in mg/kg, while those in the water samples were assessed in mg/L (Abbas *et al.*, 2022).

## Catfish physiological investigation

### Blood samples collection

After catfish collection, using a 2 ml plastic syringe, blood was obtained from the caudal peduncle of the catfish samples that had been carefully sacrificed (Lucky, 1977). One portion was allowed to coagulate at room temperature to obtain serum inside a normal centrifuge tube, while the other portion was mixed with EDTA as an anticoagulant, into Eppendorf vials. The blood tube was centrifuged at 4 °C for 10 min at 3000 rpm, and the serum was removed. It was then stored at -20 °C until tested (a maximum of 30 days). Hematological and biochemical studies were performed with the methods of Shah and Altindag (2004 and 2005).

### Human risk measurement

We employed a technique established by the USEPA (US Environmental Protection Agency, (USEPA, 2018) to evaluate the risk to human health of HM consumed by ingestion of the muscles of the investigated fish. The average daily dose (ADD; the daily average ingestion of a specific chemical over a lifetime) was used to calculate the exposure dose caused by oral human consumption of some metals found in edible fish tissues. The ADD was calculated using the following formula and represented as mg kg<sup>-1</sup> day<sup>-1</sup> (Mwakalapa *et al.*, 2019).  $ADD = (EP \times IR \times CF \times ER / BW \times AT) \times 10^{-3}$ .

Where the EP refers to the lifespan of exposure time, which is estimated to be 70 years old; the IR needs to account again for the daily ingestion of fish consumed (kg day<sup>-1</sup>), which in this case investigation was regarded to be 20.1 g/day for adults and 7.9 g/day for children. CF stands for the element levels in edible fish flesh (mg kg<sup>-1</sup> wet wt.); ER means standing for exposure rate (365 days year<sup>-1</sup>); BW refers to the body weight, which was previously defined as 70 per kg in adults and 52.5 per kg children 6 to 11 years old, which refers to the 95<sup>th</sup> percentile (USEPA, 2008; Mannzhi *et al.*, 2021). AT is the typical lifespan (70 years × 365 days year<sup>-1</sup>).

The target hazard quotient (THQ), a non-cancer estimate of bad health effects from ingesting certain HM pollutants in edible fish flesh, was established to assess human risk. THQ was computed from the ratio of ADD (average daily dose) to ORD (mg kg<sup>-1</sup> day<sup>-1</sup>, oral reference dose of HMs) using the formula  $THQ = ADD / ORD$  (USEPA, 2018).

Where ORD stands for oral reference doses of metal, the ORDs for Pb, Cu, Cd, As, Fe, and Zn are 0.00357, 0.001, 0.0003, 0.7, and 0.3 mg kg<sup>-1</sup> day<sup>-1</sup>, respectively (USEPA, 2018). The THQ levels < 1.0 suggest that negative health consequences for humans are unlikely to occur. In particular, if the computed THQ > 1.0, humans should expect negative impacts on health.

The HI (hazard index) is also a mathematical equation that reflects the effect of non-carcinogenic hazards by the total of the examined metals' THQ values as the following equation:  $HI$  (USEPA, 2018) = THQ (Pb) + THQ (Cd) + THQ (Cu) + THQ (Fe) + THQ (Zn) + THQ (As). When the HI value exceeds 10, the non-carcinogenic risk for those exposed increases.

The incremental likelihood of an individual acquiring cancer depending on the cancer slope factor (CSF) was defined as the lifelong CR of heavy metal exposure (CSF). The carcinogenic risk could have been calculated using the CR value, with  $CR > 1 \times 10^{-4}$  indicating carcinogenic danger,  $1 \times 10^{-4} > CR > 1 \times 10^{-6}$  indicating tolerable carcinogenic risk, and  $CR < 1 \times 10^{-6}$  indicating minimal carcinogenic risk (Liang *et al.*, 2018). This equation was applied to calculate the CR (Varol *et al.*, 2017):  $Cancer\ risk = (ER \times EP \times EDI \times CSF \times 10^{-3}) / AT$ . Where CSF refers to the factor of carcinogenic slope (CSF for As, Cd, and Pb were 1.5, 6.3, and 0.0042 mg kg<sup>-1</sup> day<sup>-1</sup>, respectively).

### Statistical analysis

A one-way ANOVA was performed to determine the statistical difference between the HM levels in various catfish tissues for each metal, and when differences were found, multivariate, post hoc Tukey analyses were applied. However, the relationships between physico-chemical parameters, HM levels in both water and catfish, and hemo-biochemical parameters with the parasite prevalence of both cultivated and wild origins were checked using Pearson's correlation coefficient. On the other hand, the independent-samples T test was used to investigate the statistical differences between the parasitic-infected and un-parasitic groups for both metal levels and hemato-biochemical parameters of catfish. At  $p < 0.05$ , statistical significance was recognized. The statistical analyses were performed using SPSS, a statistical program (software, Version 22; SPSS, USA). The data is given in the tables as means  $\pm$  standard deviation.

## RESULTS

### Physico-chemical parameters and HM levels in waters

**Table 1** shows the distribution of physico-chemical characteristics and HM levels in Lake Borollus and private fish farm waters. The concentrations of H<sub>2</sub>S, phosphate, total hardness, ammonia, TDS, turbidity, NO<sub>3</sub>, NO<sub>2</sub>, total alkalinity, and pH in the Lake Borollus waters were considerably lower compared to fish farm waters ( $p < 0.05$ ). However, dissolved oxygen in the lake waters was considerably higher compared to farm waters ( $p < 0.05$ ).

### Parasitological investigation

The prevalence of trematodes was higher in cultivated *C. gariepinus* catfish than in wild ones, with 20.18% in the former and 10.98% in the latter. On the other hand, Nematoda ranged from 23.17 % in cultivated fish to 35.78 % in wild fish. Cestoda was found in 25.23 % of cultivated fish and 15.85 % of wild fish. Moreover, the highest overall occurrence of parasite infestations in *C. gariepinus* was observed in fish cultivated (85.78 %) and the minimal value (50 %) was recorded in wild fish (**Table 2**). Moreover, **Figure (2)** revealed that five isolated parasites were found in the fish that were investigated. These helminths were classified into three higher taxa (viz., Platyhelminthes). One of these was a trematode (*Orientocreadium* sp.), two were cestodes (*Proteocephalus* sp. and *Polygonchobothrium clarias*), and two were nematodes (*Procamallanus* sp., and *Paracamallanus* sp.).

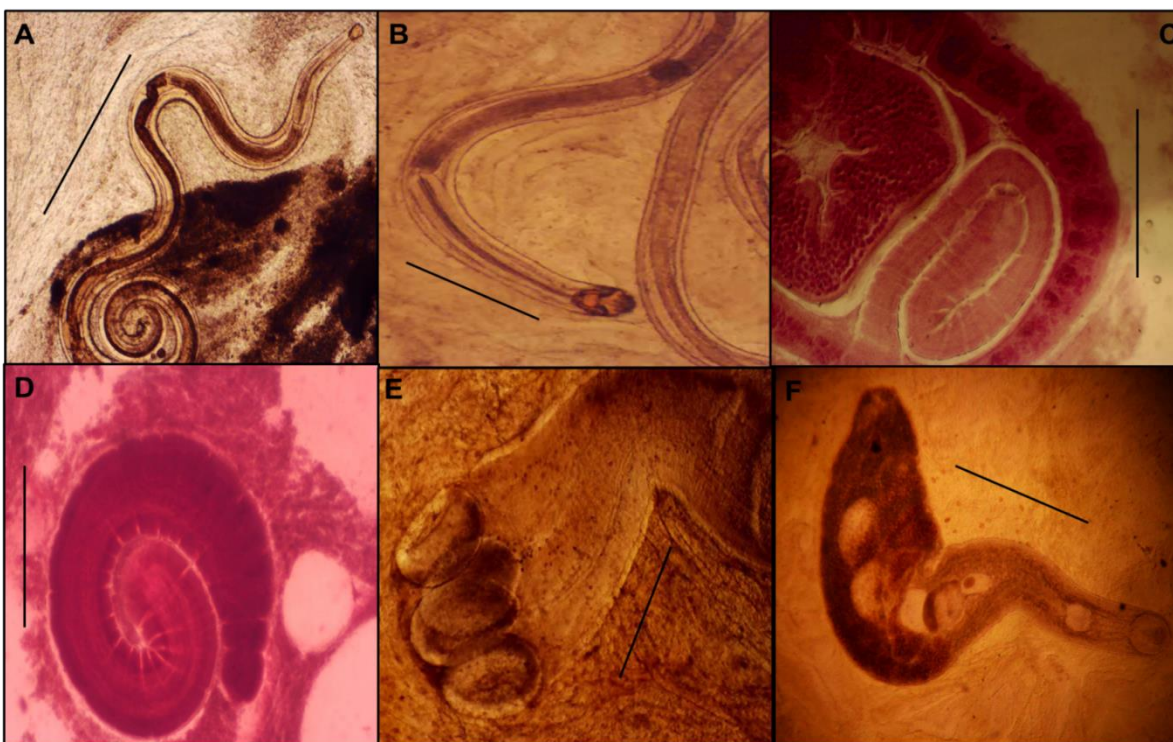
**Table 1.** Physico-chemical parameters and HM levels (mean±SD, n=5) of the water collected from Fish Farm and Lake Borollus.

Physico-chemical parameters	Lake Borollus	Fish Farm
	<b>pH</b>	8.15±0.16 <sup>b</sup>
<b>Turbidity (%)</b>	73.67±2.52 <sup>b</sup>	86.67±4.51 <sup>a</sup>
<b>TDS (mg/L)</b>	786.67±4.93 <sup>b</sup>	859.00±3.0 <sup>a</sup>
<b>Ammonia (mg/L)</b>	0.72±0.03 <sup>b</sup>	1.39±0.02 <sup>a</sup>
<b>NO<sub>2</sub> (mg/L)</b>	0.37±0.04 <sup>b</sup>	0.57±0.06 <sup>a</sup>
<b>NO<sub>3</sub> (mg/L)</b>	0.63±0.06 <sup>b</sup>	0.95±0.02 <sup>a</sup>
<b>Total alkalinity (mg/L)</b>	203.0±2.0 <sup>b</sup>	228.67±2.52
<b>Total hardness (mg/L)</b>	216.33±5.03 <sup>b</sup>	252.67±1.53 <sup>a</sup>
<b>Total phosphate (mg/L)</b>	0.29±0.04 <sup>b</sup>	0.40±0.04 <sup>a</sup>
<b>H<sub>2</sub>S (mg/L)</b>	0.79±0.02 <sup>b</sup>	1.12±0.09 <sup>a</sup>
<b>Dissolved Oxygen (mg/L)</b>	8.43±0.11 <sup>a</sup>	7.24±0.11 <sup>b</sup>
<b>Water HM levels</b>		
<b>Cu (PPm)</b>	0.062±0.010 <sup>b</sup>	0.080±0.015 <sup>a</sup>
<b>Zn (PPm)</b>	0.522±0.036 <sup>b</sup>	0.573±0.060 <sup>a</sup>
<b>Fe (PPm)</b>	0.611±0.072 <sup>b</sup>	0.918±0.061 <sup>a</sup>
<b>AS (PPm)</b>	0.049±0.007 <sup>b</sup>	0.054±0.004 <sup>a</sup>
<b>Cd (PPm)</b>	0.005±0.001	0.005±0.001
<b>Pb (PPm)</b>	0.076±0.008 <sup>b</sup>	0.100±0.008 <sup>a</sup>

-At P<0.05, results from the same row with different alphabetic letters are statistically different.

**Table 2.** Prevalence of different parasites among the examined fish, *Clarias gariepinus* collected from Fish Farm and Lake Borollus.

Parasites Family	Fish origin			
	Fish Farm (218)		Lake Borollus (164)	
	No. of infected	Prevalence %	No. of infected	Prevalence %
<b>Trematodes</b>	44	20.18	18	10.98
<b>Nematoda</b>	78	35.78	38	23.17
<b>Cestoda</b>	55	25.23	26	15.85
<b>Total</b>	187	85.78	82	50.00



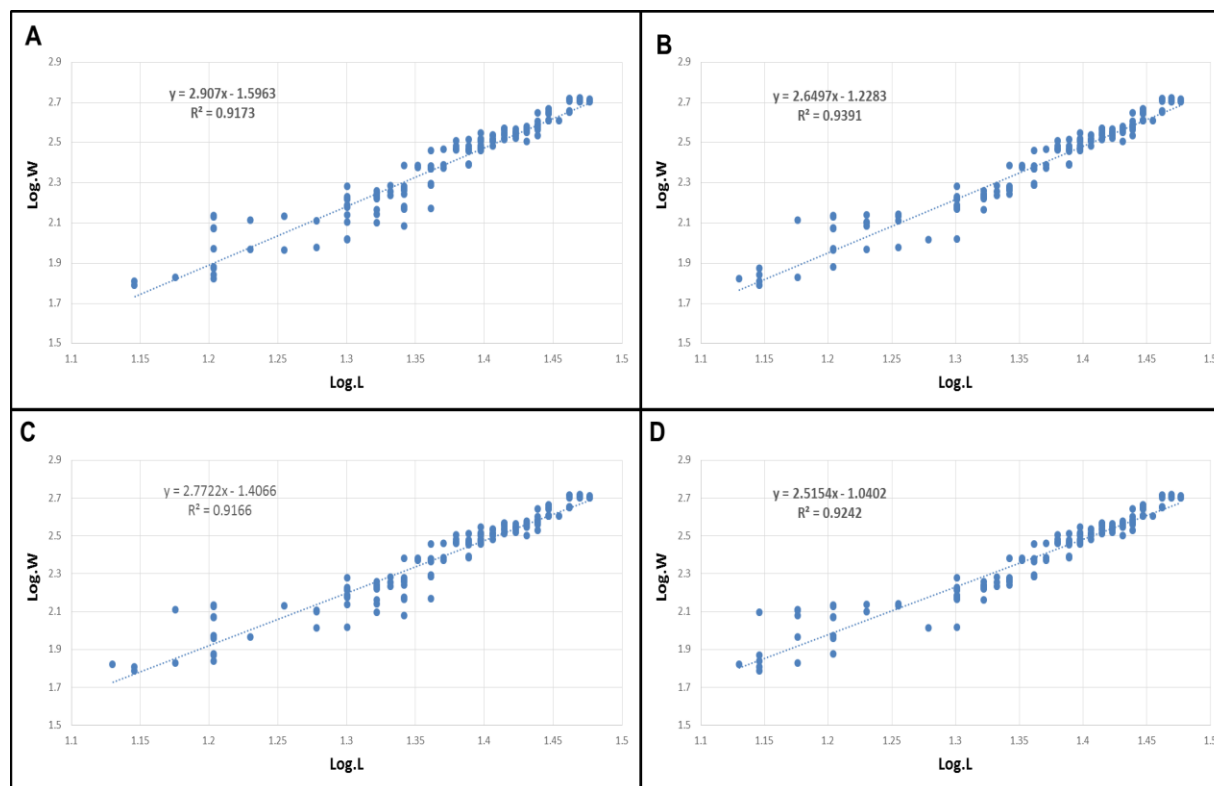
**Fig. 2.** Helminth species were reported through study (A): *Procammallanus* sp. (B): *Paracammallanus* sp. (C and D): *Polyonchobothrium clarias* stained with acetic acid alum carmine. (E) *Proteocephalus* sp. (F): *Orientocreadium* sp. (scale bar: 0.5 mm).

### Length-Weight Relationship

The length-weight relationship of catfish origins and its association with parasite infection are shown in **Figure 3**: A–D. The *b* values of the catfish specimens, both parasitic infected and uninfected, ranged from 2.51 to 2.90. In both parasitic-infected and uninfected catfish of wild and cultivated origins, the value of "b" was less than 3, demonstrating a negative allometric growth trend for fish independent of their parasitic infection. Nearly all species demonstrated a significant and crucially important correlation between weight and length ( $r > 0.91$ ).

### HM levels in fish organs

HM levels of wild and cultivated catfish, *Clarias gariepinus*, are illustrated in **Table 3**. The uninfected wild and cultured catfish had significantly greater levels of HM (vital heavy metals Zn, Cu, and Fe; and non-vital heavy metals Cd, As, and Pb) than the parasitic-infected ones ( $p < 0.05$ ). Vital HM levels in the wild catfish were significantly lower compared to cultivated catfish in both. Notably, non-vital HM levels in the wild catfish increased significantly compared to cultivated catfish in uninfected and parasitic-infected groups ( $p < 0.05$ ). Additionally, the order of vital and non-vital HMs in both groups of uninfected and parasitic-infected wild and cultivated catfish tissues was as follows: liver > gills > gut > muscles. Consistently, when comparing fish with and without parasite infections, the T-test revealed that the examined HMs were significantly decreased in catfish with parasite infections ( $p < 0.05$ ).



**Fig. 3.** Length-weight relationship of cultivated and wild *C. gariepinus*. (A): Un-infected cultivated fish (B): Parasitic-infected cultivated fish; (C): Un-infected wild fish (D): Parasitic-infected wild fish.

### Haemato-biochemical alternations

**Table 4** shows the findings of the haemato-biochemical alternations of wild and cultivated catfish, *Clarias gariepinus*, for both parasitic-infected and un-parasitic species. The findings show that for both examined origins (wild and cultivated), the infected fish's RBC, PCV, and Hb levels were lower than those of the non-infected catfish. However, compared to non-infected fish, infected fish exhibited higher WBC counts. The hematological indices of the infected and non-infected catfish for both of the investigated origins revealed statistically significant variations ( $p < 0.05$ ). However, the AST, glucose level, ALT, and urea were shown to be significantly higher ( $p < 0.05$ ) in the parasitic-infected catfish compared to uninfected ones for both investigated origins. For both of the studied origins, the levels of globulin, albumin, and total proteins were significantly lower ( $p < 0.05$ ) in the parasitic-infected catfish compared to the un-parasitic fish. The studied biochemical parameters of the infected and non-infected catfish in both of the examined origins showed statistically significant differences ( $p < 0.05$ ).



**Table 3.** Descriptive of HM levels (mean±SD, n=5, µg/g, wet weight basis) in different tissues of wild and cultivated catfish.

HM-Concentrations		Fish origin							
		Cultivated fish				Wild fish			
		Muscles	Intestine	Liver	Gills	Muscles	Intestine	Liver	Gills
<b>Vital metals</b>									
<b>Cu</b>	<b>Non-infected</b>	0.99±0.0 <sup>Ad</sup>	1.16±0.16 <sup>Ac</sup>	1.74±0.16 <sup>Aa</sup>	1.57±0.08 <sup>Ab</sup>	0.93±0.10 <sup>Ad</sup>	1.15±0.25 <sup>Ac</sup>	1.45±0.30 <sup>Aa</sup>	1.17±0.12 <sup>Ab</sup>
	<b>Infected</b>	0.95±0.20 <sup>Bd</sup>	1.11±0.18 <sup>Bc</sup>	1.69±0.18 <sup>Ba</sup>	1.53±0.20 <sup>Bb</sup>	0.88±0.16 <sup>Bd</sup>	1.06±0.33 <sup>Bc</sup>	1.39±0.14 <sup>Ba</sup>	1.12±0.13 <sup>Bb</sup>
<b>Zn</b>	<b>Non-infected</b>	6.07±0.24 <sup>Ad</sup>	6.95±0.56 <sup>Ac</sup>	8.18±0.24 <sup>Aa</sup>	7.16±0.44 <sup>Ab</sup>	5.33±0.28 <sup>Ad</sup>	5.55±1.13 <sup>Ac</sup>	7.41±0.25 <sup>Aa</sup>	6.63±0.25 <sup>Ab</sup>
	<b>Infected</b>	5.95±0.23 <sup>Bd</sup>	6.15±0.41 <sup>Bc</sup>	8.00±0.32 <sup>Ba</sup>	7.00±0.26 <sup>Bb</sup>	5.25±0.26 <sup>Bd</sup>	5.41±1.28 <sup>Bc</sup>	7.19±0.31 <sup>Ba</sup>	6.19±0.20 <sup>Bb</sup>
<b>Fe</b>	<b>Non-infected</b>	13.94±1.55 <sup>Ad</sup>	20.07±2.68 <sup>Ac</sup>	31.76±2.95 <sup>Aa</sup>	25.39±1.14 <sup>Ab</sup>	11.69±0.96 <sup>Ad</sup>	17.85±2.61 <sup>Ac</sup>	30.15±1.51 <sup>Aa</sup>	24.18±2.40 <sup>Ab</sup>
	<b>Infected</b>	12.55±0.66 <sup>Bd</sup>	19.94±1.10 <sup>Bc</sup>	30.87±1.52 <sup>Ba</sup>	24.43±1.67 <sup>Bb</sup>	10.30±0.72 <sup>Bd</sup>	17.72±1.03 <sup>Bc</sup>	29.26±1.43 <sup>Ba</sup>	23.88±1.56 <sup>Bb</sup>
<b>Non-vital metals</b>									
<b>As</b>	<b>Non-infected</b>	0.28±0.06 <sup>Ad</sup>	1.00±0.06 <sup>Ac</sup>	1.24±0.14 <sup>Aa</sup>	1.09±0.01 <sup>Ab</sup>	1.08±0.07 <sup>Ad</sup>	1.13±0.11 <sup>Ac</sup>	1.53±0.17 <sup>Aa</sup>	1.35±0.15 <sup>Ab</sup>
	<b>Infected</b>	0.23±0.03 <sup>Bd</sup>	0.92±0.08 <sup>Bc</sup>	1.06±0.14 <sup>Ba</sup>	0.96±0.07 <sup>Bb</sup>	1.03±0.07 <sup>Bd</sup>	1.08±0.21 <sup>Bc</sup>	1.49±0.29 <sup>Ba</sup>	1.27±0.21 <sup>Bb</sup>
<b>Cd</b>	<b>Non-infected</b>	0.04±0.01 <sup>Ad</sup>	0.39±0.09 <sup>Ac</sup>	0.63±0.03 <sup>Aa</sup>	0.45±0.01 <sup>Ab</sup>	0.57±0.02 <sup>Ad</sup>	0.77±0.06 <sup>Ac</sup>	1.13±0.18 <sup>Aa</sup>	1.01±0.06 <sup>Ab</sup>
	<b>Infected</b>	0.03±0.03 <sup>Bd</sup>	0.34±0.05 <sup>Bc</sup>	0.56±0.07 <sup>Ba</sup>	0.45±0.01 <sup>Bb</sup>	0.51±0.06 <sup>Bd</sup>	0.70±0.05 <sup>Bc</sup>	1.10±0.16 <sup>Ba</sup>	0.98±0.05 <sup>Bb</sup>
<b>Pb</b>	<b>Non-infected</b>	1.12±0.12 <sup>Ad</sup>	2.43±0.08 <sup>Ac</sup>	4.46±0.34 <sup>Aa</sup>	3.64±0.48 <sup>Ab</sup>	2.20±0.11 <sup>Ad</sup>	2.87±0.29 <sup>Ac</sup>	4.68±0.12 <sup>Aa</sup>	3.99±0.16 <sup>Ab</sup>
	<b>Infected</b>	1.07±0.18 <sup>Bd</sup>	2.21±0.05 <sup>Bc</sup>	4.09±0.20 <sup>Ba</sup>	3.45±0.15 <sup>Bb</sup>	2.06±0.08 <sup>Bd</sup>	2.34±0.36 <sup>Bc</sup>	4.27±0.08 <sup>Ba</sup>	3.59±0.15 <sup>Bb</sup>

\*Between non-infected and infected catfish, unique subscripts capital letters within the same column and metal display a significant difference (T-test,  $p < 0.05$ ). however, ANOVA,  $p < 0.05$ , indicates that findings from the same rows and origin (among tissues) having distinct alphabetic small letters are significantly different.

**Table 4.** Haemato-biochemical alternations (mean±SD, n=5) in wild and cultivated catfish.

Haemato-biochemical parameters	Fish origin			
	Cultivated fish		Wild fish	
	Non-infected fish	Infected fish	Non-infected fish	Infected fish
<b>Haematological</b>				
RBCs (x 10 <sup>6</sup> cell / mm <sup>3</sup> )	2.74 ± 0.14 <sup>a</sup>	2.06 ± 0.15 <sup>b</sup>	2.49 ± 0.09 <sup>a</sup>	1.95 ± 0.27 <sup>b</sup>
WBCs (x 10 <sup>3</sup> cell / mm <sup>3</sup> )	18.49 ± 0.29 <sup>b</sup>	22.77 ± 0.89 <sup>a</sup>	17.45 ± 0.78 <sup>b</sup>	20.61 ± 0.97 <sup>a</sup>
Hb (g/dl)	11.78 ± 0.39 <sup>a</sup>	9.07 ± 0.70 <sup>b</sup>	8.40 ± 1.52 <sup>a</sup>	7.36 ± 0.35 <sup>b</sup>
PCV (%)	32.37 ± 1.22 <sup>a</sup>	27.13 ± 1.44 <sup>b</sup>	28.08 ± 1.11 <sup>a</sup>	25.33 ± 1.17 <sup>b</sup>
<b>Biochemical</b>				
AST (U/ml)	82.36±2.36 <sup>b</sup>	113.81±3.18 <sup>a</sup>	71.53±6.52 <sup>b</sup>	94.07±8.42 <sup>a</sup>
ALT (U/ml)	47.11±1.12 <sup>b</sup>	69.16±4.82 <sup>a</sup>	46.17±3.52 <sup>b</sup>	54.39±3.64 <sup>a</sup>
Urea (mg/dl)	7.26±0.68 <sup>b</sup>	8.97±0.41 <sup>a</sup>	6.67±0.98 <sup>b</sup>	7.46±0.14 <sup>a</sup>
Glucose (mg/dl)	72.16±1.94 <sup>b</sup>	84.12±2.22 <sup>a</sup>	66.16±2.28 <sup>b</sup>	76.21±3.04 <sup>a</sup>
Total protein (g/dl)	4.38±0.16 <sup>a</sup>	2.98±0.91 <sup>b</sup>	3.45±0.18 <sup>a</sup>	2.73±0.43 <sup>b</sup>
Albumin (g/dl)	3.62±0.45 <sup>a</sup>	2.76±0.27 <sup>b</sup>	2.88±0.47 <sup>a</sup>	2.03±0.29 <sup>b</sup>
Globulin (g/dl)	2.91±0.34 <sup>a</sup>	2.17±0.15 <sup>b</sup>	2.43±0.51 <sup>a</sup>	2.19±0.29 <sup>b</sup>

\*Different small letters in the same row and origin (between non-infected and infected catfish) are significantly different (T-test,  $p < 0.05$ ).

### **Correlation rates**

**Table 5** reveals the Pearson correlation (r) calculated to determine if some of these metals were interrelated with each other and with PP, and the results displayed a positive correlation for Cu-Zn, Cu-As, Cu-Cd, Cu-Fe, Cu-Pb, Zn -As, Zn-Fe, Zn-Pb, Zn-Cd, Fe-Pb, Fe-As, Fe-Cd, As-Cd, As-Pb, and Cd-Pb in the wild and cultivated catfish for both groups (parasitic-infected and non-infected). On the other hand, a negative correlation was observed between the PP of wild and cultivated catfish with all studied metals (Cu, Zn, Fe, As, Cd, and Pb). In contrast, Pearson correlation coefficients between parasite prevalence and physico-chemical factors revealed that NO<sub>3</sub>, turbidity, NO<sub>2</sub>, total alkalinity, and phosphate had a positive correlation with PP ( $r = 0.75, 0.69, 0.65, \text{ and } 0.67$ ). However, pH and dissolved oxygen had a negative correlation with the PP of wild and cultivated catfish, *Clarias gariepinus* ( $r = -0.51$  and  $-0.66$ ). Furthermore, PP and water HM level relationships showed a negative correlation between the PP-Pb, PP-As, and PP-Zn in both fish from Lake Borollus and farm waters. However, a positive correlation was seen between PP-Cu and no correlation was seen between PP-Cd and PP-Fe. Moreover, RBCs, Hb, PCV, AST, ALT, urea, and glucose phosphate had a positive correlation with PP of cultivated and wild catfish ( $r = 0.50; 0.57, 0.49; 0.56, 0.52; 0.71, 0.81; 0.74, 0.75; 0.56, 0.45; 0.39$  and  $0.25; 0.34$ , respectively). However, there was a negative correlation between WBCs, total proteins, albumin, and globulin with PP in both wild and cultivated catfish origins ( $r = -0.56; -0.51, -0.51; -0.53, -0.77; -0.60$  and  $-0.36; -0.43$ , respectively).

**Table 5.** Pearson correlation coefficients between physico-chemical parameters, HMs levels, and haemato-biochemical alternations with parasites prevalence of *Clarias gariepinus* collected from Fish Farm and Lake Borollus sites.

<b>Pearson correlation coefficients</b>												
Physico-chemical parameters		pH	Turbid.	TDS	NH <sub>3</sub>	NO <sub>2</sub>	NO <sub>3</sub>	Alka.	T. hard	PO <sub>4</sub>	H <sub>2</sub> S	DO
Parasites prevalence	Fish Farm site	-0.61	0.50	-0.44	-0.33	0.95	0.94	0.98	-0.70	0.74	-0.35	-0.65
	Lake Borollus site	-0.51	0.66	-0.36	-0.37	0.86	0.83	0.80	-0.69	0.58	-0.33	-0.71
Water HMs levels		Cu	Zn	Fe	AS	Cd	Pb					
Parasites prevalence	Fish Farm site	0.88	-0.95	0.22	-0.55	-0.15	-0.61					
	Lake Borollus site	0.78	-0.99	0.14	-0.60	-0.17	-0.67					
Fish heavy metals		Cu	Zn	Fe	As	Cd	Pb					
Parasites prevalence	Fish Farm site	-0.72	-0.98	-0.98	-0.96	-0.95	-0.82					
	Lake Borollus site	-0.89	-0.79	-0.91	-0.92	-0.88	-0.79					
Haemato-biochemical alternations		RBCs	WBCs	Hb	PCV	AST	ALT	Urea	Gluc.	T.p.	Alb.	Glob.
Parasites prevalence	Fish Farm site	0.50	-0.56	0.49	0.52	0.81	0.75	0.45	0.25	0.51	-0.77	-0.36
	Lake Borollus site	0.57	-0.51	0.56	0.71	0.74	0.56	0.39	0.34	0.53	-0.60	-0.43

### Human health assessment

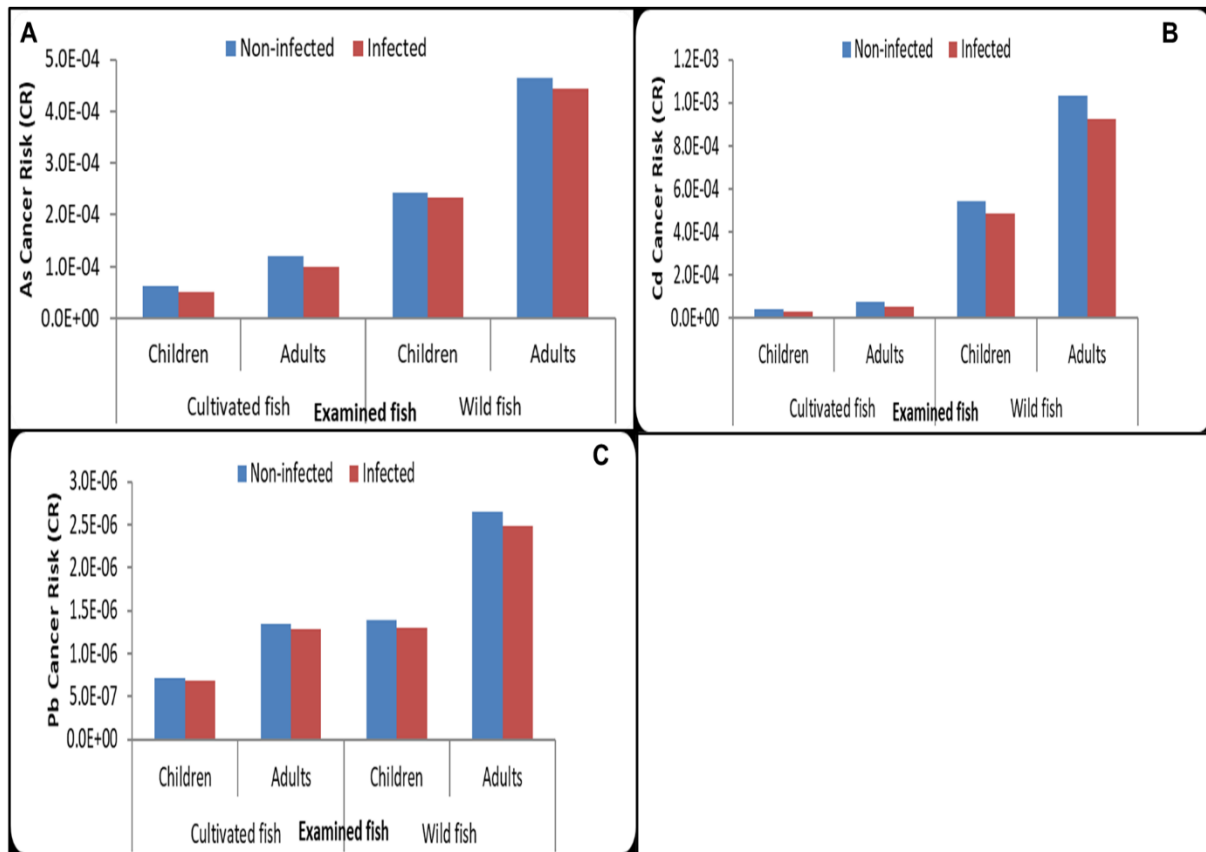
**ADD** ( $\text{mg}^{-1}\text{kg}^{-1}\text{day}^{-1}$ ) and **THQ** for elements in the muscles of both cultivated and wild catfish, *Clarias gariepinus*, are represented in **Table (6)**. **ADD** in wild fish was higher than in cultivated fish and varied from 0.005 to 0.682  $\text{mg kg}^{-1} \text{day}^{-1}$  for adult fish eaters, while for child fish eaters it fluctuated between 0.003 and 0.357  $\text{mg kg}^{-1} \text{day}^{-1}$ . However, for cultivated catfish, **ADD** for adult fish eaters ranged from 9E-06 to 0.027  $\text{mg kg}^{-1}\text{day}^{-1}$ , while for child fish eaters it ranged from 5E-06 to 0.014  $\text{mg kg}^{-1}\text{day}^{-1}$ . However, the higher values of **ADD** were recorded for adult fish eaters, and the lower values of **ADD** were recorded for child fish eaters in both parasitic-infected and uninfected fish. Furthermore, the values of **THQ** were all below 1, implying that consumption of cultivated catfish, *Clarias gariepinus*, will not impose any health implications for adults and young fish eaters. It was higher in wild catfish eaters compared to cultivated catfish for studied consumers. Additionally, the order of **HI** in the cultivated catfish was **HI**-non-infected fish > **HI**-infected fish for children and adult fish eaters, being 0.0033 and 0.0030, respectively, for the first fish eaters and 0.0063 and 0.0058 for the second one. For wild catfish eaters, it was higher in **HI** of uninfected fish compared to **HI** of infected fish, 0.241 and 0.223, respectively for children; 0.461 and 0.426, respectively for adult fish eaters (**Table 6**).

**Table 6.** Average daily dose (ADD, mg kg<sup>-1</sup>/day<sup>-1</sup>) and hazard quotients (THQ) for the studied metals in muscles of cultivated and wild catfish.

HRA-Heavy metals	Fish origin								HMs PTDI
	Cultivated fish				Wild fish				
	ADD		THQ		ADD (mg/kg/day)		THQ		
	Children	Adults	Children	Adults	Children	Adults	Children	Adults	
<b>Non-infected muscles</b>									
<b>Cu</b>	1E-04	3E-04	1E-04	3E-04	0.004	0.007	0.0035	0.0067	35
<b>Zn</b>	9E-04	0.002	8E-04	0.002	0.003	0.006	0.00267	0.0051	70
<b>Fe</b>	0.002	0.004	0.002	0.003	0.003	0.006	0.00251	0.0048	50
<b>As</b>	4E-05	8E-05	2E-04	3E-04	0.014	0.027	0.05417	0.1034	0.14
<b>Cd</b>	6E-06	1E-05	9E-05	2E-04	0.006	0.011	0.08577	0.1637	0.07
<b>Pb</b>	2E-04	3E-04	3E-04	6E-04	0.047	0.09	0.09273	0.177	0.25
<b>Infected muscles</b>									<b>HMs ORDs</b>
<b>Cu</b>	0.014	0.027	1E-04	3E-04	0.357	0.682	0.00331	0.0063	0.04
<b>Zn</b>	9E-04	0.002	8E-04	0.002	0.003	0.006	0.00263	0.005	0.30
<b>Fe</b>	0.002	0.004	0.002	0.003	0.003	0.005	0.00221	0.0042	0.70
<b>As</b>	3E-05	7E-05	2E-04	3E-04	0.012	0.022	0.05166	0.0986	0.003
<b>Cd</b>	5E-06	9E-06	8E-05	1E-04	0.005	0.009	0.07674	0.1464	0.001
<b>Pb</b>	2E-04	3E-04	3E-04	6E-04	0.045	0.086	0.08683	0.1657	0.0036
<b>Hazard index (HI)</b>									
<b>Non-infected muscles</b>	--	--	0.003	0.006	--	--	0.241	0.461	
<b>Infected muscles</b>	--	--	0.003	0.006	--	--	0.223	0.426	

- **PTDI** (mg day<sup>-1</sup> 70 kg<sup>-1</sup> body weight), or permissible tolerated daily ingestion **FAO/WHO (2004)**.
- Oral reference doses of HMs (**ORDs**), mg kg<sup>-1</sup> day<sup>-1</sup>, **USEPA (2018)**.

The CR values for As, Cd, and Pb in the catfish muscles were determined for both adult and children's eaters and the results are presented in **Figs.4 (A-C)**. The highest value of CR-As in cultivated fish was  $1\text{E-}04$  and the lowest value was  $5\text{E-}05$ . However, for wild catfish, it fluctuated between  $2\text{E-}04$  and  $5\text{E-}04$ . Moreover, CR-Cd in the muscles of crayfish varied from  $3\text{E-}05$  to  $7\text{E-}05$  for cultivated fish and  $5\text{E-}04$  to  $0.001$  for wild catfish. CR-Pb in the muscles of crayfish ranged from  $7\text{E-}07$  to  $1\text{E-}06$  for cultivated fish and  $1\text{E-}06$  to  $3\text{E-}06$  for wild catfish.



**Fig. 4.** Cancer Risk (CR) of arsenic (As) (A); cadmium (Cd) (B) and lead (Pb) (C) in the muscles of wild and cultivated catfish, *C. gariepinus*, collected from the studied sites.

## DISCUSSION

Water in ponds, lakes, and rivers has an equilibrium of physical, chemical, and biological characteristics for the successful production of fish (Mwakalapa *et al.*, 2019). Data showed, fluctuations in the values of several physico-chemical parameters during the study period could be linked to variations in climate and anthropogenic activities in and around the body of water within the nearly limit of guidelines for aquatic life survival (WHO, 2004, Yerima *et al.*, 2017). In addition, results determined, iron levels were the maximum and Cd levels were minimum in both Lake Borollus and fish farm waters, and estimated HM levels in Lake Borollus and fish farm

waters were within the baseline acceptable limit set by WHO (2011), and USEPA (2018) except for Fe, Pb, and As (Melegy et al., 2019).

The current study found the PP in cultivated catfish higher than in wild catfish. This result could be due to fish farm preparation procedures performed before fish stocking, as well as restricting fish feed to processed foods rather than poultry contaminates, variation in water quality properties and other natural aquatic contaminants (Enyidi and Eneje, 2015, Radwan et al., 2022). Correspondingly, data disagree with Afolabi et al. (2020), who revealed a higher PP (33.75%) in wild catfish compared to cultivated catfish (20%).

The findings of the study correlation of physicochemical variables and parasite prevalence suggest the effect of the monitored parameters on the PP. However, NO<sub>3</sub>, turbidity, NO<sub>2</sub>, phosphate, and total alkalinity were significantly associated with PP (Zargar et al., 2012; and Radwan, 2022). Also, pH and dissolved oxygen had a significantly negative relationship with the PP of wild and cultivated catfish, *Clarias gariepinus*. This finding is in agreement with Zargar et al., 2012; Radwan et al., 2022).

Higher concentrations, of heavy metals can become harmful to living organisms at larger concentrations (Abdel-Kader and Mourad, 2019). In the studied organs, however, copper and zinc accumulations were in the correct sequence: liver > gills > intestine > muscle for the wild and cultivated catfish. The levels of Cu in the fish organs were below the FAO permissible value (FAO, 2016). In this context, Fe accumulation in the catfish organs was below the FAO permissible value of 100 ppm (FAO, 2016). Conversely, Cadmium, arsenic, and lead accumulation in the studied organs were higher than the FAO permissible value of 0.5 ppm (FAO, 2016). This finding is in accordance with Murtala et al. (2012); and Abdel-Kader and Mourad (2019). Generally, the present study revealed that the organs of wild and cultivated catfish had more iron (Fe) than any other heavy metal studied, while cadmium was at the minimum end, and metal levels varied in the correct sequence; iron (Fe) > Zn > Pb > Cu > As > cadmium (Cd). This observation agrees with Al-Halani et al. (2021) who revealed that the HM levels in wild organs, *Dicentrarchus labrax*, are estimated in the correct sequence: iron (Fe) > Zn > Mn > Cu > Pb > Ni > Cr > cadmium (Cd).

The wild catfish had significantly lower levels of vital HM (Fe, Zn, Cu) than the cultivated catfish, which could be because these metals are required for different biological activities and thus supplied into fish diets and wild fish surviving over several years compared to cultivated fish, which are captured within six months. (Chatta et al., 2016; Yipel et al., 2016; Simukoko et al., 2022).

HM levels in the healthy (non-infected) wild and cultivated catfish were significantly higher than those of the parasitic-infected catfish ( $p < 0.05$ ). This could be due to parasites accumulating significantly increased levels of HM in their tissues, which serves as a metal sink for their host fish and aids in their survival when surrounded by toxins. These findings are consistent with Sures, 2007 and Eissa et al., 2012. The metals in different tissues were increased in unparasitic fish than in parasitic-infected ones (Hassan et al., 2016).

In the present study, the HM levels in the species-specific different fish organs showed that there was a significant possibility of HM bioaccumulation in the tissues of fish. These findings agree with **Maurya et al. (2019)**.

The liver and gill of each fish species showed a higher HM accumulation, while the intestine and muscle showed a lower HM accumulation. This indicated that the HM was accumulated from water into fish tissues and metabolically active of these tissues accumulate greater HM than other tissues like the skin and muscles (**Ali et al., 2019**).

Blood is a reliable bio-indicator of an organism's health and acts as a pathogenic reflection of the body's systems. Therefore, hemato-biochemical variables are crucial for understanding the clinical outcomes of a catfish (host) that is parasite-infested as well as assessing the physiological condition of fish (**Radwan et al., 2021**).

The results of the current study suggested that the parasite-infected farmed and wild catfish had significantly lower levels of **PCV**, **Hb**, and **RBC** count and higher **WBC** counts as compared to the un-parasitic specimens, which may be due to stressors, parasites influence hematocrit and activate the early stages of stress causes the production of catecholamine, which either mobilizes erythrocytes from the spleen or leads to erythrocyte expansion as a result of fluid migrating into the intracellular environment (**Radwan et al., 2023**). Low levels of these hematological parameters may suggest anemia or hemodilution brought on by osmoregulation failure (**Vivanco-Aranda et al., 2018**).

Data revealed that the **AST**, glucose, urea, and **ALT** were established to be significantly ( $p < 0.05$ ) increased in the infected catfish compared to un-infected ones for both studied origins. This might be brought on by the intoxicating inflammatory responses carried on by the parasites that are prevalent in this fish and the pathogenesis of hepatic diseases in fish is reported to be associated with viral hepatitis, parasitic infections, and/or mechanical injuries. Similar observations were recorded by **Nnabuchi et al. (2015)** and **Omeji et al. (2018)**.

For both of the studied origins, the levels of globulin, albumin, and total proteins were significantly ( $p < 0.05$ ) decreased in the parasite-infected catfish compared to the un-parasitic fish. This may be attributed to haemodilution which decreased nutritional deficit, nutrient absorption, starvation, and infectious disorders have all been related to the decline in protein levels (**Del Rio-Zaragoza et al., 2011**). Fish protein depletion may be caused by changes in water quality brought on by effluents from many sources, such as metal pollution in agricultural and industrial drainage (**Yacoub & Gad, 2012**). This is also explained as the accumulation of metals in the gills may cause structural destruction and a significant decline in oxygen consumption, which in turn causes a sharp decrease in the metabolism of fish and, as a result, a reduction in the amount of protein in their tissues. The significant changes demonstrated metal toxicity at the biochemical levels (glycogen, protein, and lipid contents) in the Indian major carp, *Cirrhinus mrigala* (**Bhilave et al., 2008**).

This study focused on the **ADD** ( $\text{mg}^{-1} \text{kg}^{-1} \text{day}^{-1}$ ), **THQ**, and **HI** for **HM** levels in the muscles of wild and cultivated catfish, *Clarias gariepinus*. The average daily dose or tolerable dose was used to describe the permissible levels of HM (**Keshavarzi et al., 2018**). **ADD** in wild

fish was higher than in cultivated fish and varied from 0.003 to 0.682 mg<sup>-1</sup> kg<sup>-1</sup> day<sup>-1</sup>. However, for cultivated catfish, it varied from 5E-06 to 0.027 mg<sup>-1</sup> kg<sup>-1</sup> day<sup>-1</sup>. However, the higher values of **ADD** were recorded for adult fish consumers, and the lower values of **ADD** were recorded for children fish consumers in both parasitic-infected and non-parasitic fish. The recorded **ADD** of both groups was compared to the **PTDI** (permissible tolerable daily intake) of 70 kg BW, provided by **FAO/WHO (2004)**, and showed that mean **ADD** values of the metals exceed the **PTDI** values.

**THQ** values calculated for this study were all below 1, implying that consumption of cultivated catfish, *Clarias gariepinus*, will not impose any health implications for adult and child consumers. According to **Lei et al. (2015)**, the hazard index (HI) is assessed based on the values of THQ; if the HI value was less than one, the impacts on humans would be detrimental; HI more than one would probably have a negative impact; and HI > 10 would likely have strong or chronic or acute consequences. The present study recorded that, the hazard index calculated for this work has all been less than one, showing that consuming cultivated or wild catfish, *Clarias gariepinus*, has no negative health consequences for adults or children. The estimated potential health non-carcinogenic risks (**THQ** and **HI**) associated with the consumption of wild and cultivated catfish suggest that children and adult catfish consumers indicate that there are no non-cancer risks. Hence, research on heavy metals found in the studied species in the researched region is critical in order to provide accurate data to protect the safety of residents and customers. Furthermore, the study underlines the importance of conducting regular monitoring of these HM and suggests that actions be taken to control them.

Cd, As, Cr, and Pb have been identified as carcinogens by the IARC (International Agency for Research on Cancer, **Bonsignore et al., 2018**). The findings indicate that oral intake is the predominant route of exposure to heavy metals. The CR values for cadmium and lead were determined in the catfish muscles for both adult and child consumers. Using the acceptable limit of E-4, the CR values for Pb in the muscles of catfish were less than the accepted limit for both children and adult consumers. However, As and Cd pose a cancer risk to children and adult consumers of catfish muscles as the CR values were higher than the set limit. These findings show that it is vital to monitor the concentrations of Cd and As metals in catfish from both locations (lake and farm waters) to minimize possible health hazards.

## CONCLUSIONS

The parasite prevalence in catfish considers a bioindicator of environmental changes, metals pollution, and their physiological status. The estimated non-cancer risks associated with the consumption of wild and cultivated catfish suggest that catfish consumers indicate that there are no non-cancer risks. In contrast, Cd and As posed a cancer risk to adult and child consumers of catfish muscles as the cancer risk values were higher than the set limit. These findings show that it is vital to monitor the concentrations of metals in catfish from both locations (lake and farm waters) to minimize possible health hazards. Therefore, a long-term management strategy and bio-monitoring of these HM in Lake Borollus and around fish farm waters are required.



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### Availability of data and materials

The data sets in this study are available from the corresponding author on reasonable request

### Competing interests

The authors declare that they have no competing interests.

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