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The use of *Clinostomum complanatum* (Trematoda: Clinostomidae) in freshwater fish as a bio indicator of some heavy metals conatmination from Sarchnar, Sulaimanyah city, Iraq

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Abstract

The aim of this study is to investigate the role of *Clinostomum complanatum* in freshwater fish as a bio indicator for the presence and concentration of some heavy metals from Sarchnar in Sulaimanyah city, Iraq. The parasite *Clinostomum complanatum* (Rudolphi, 1814) is a digenean trematode in the family Clinostomidae. Total of 35 *Clinostomum* parasites were collected from the gills of 13 infected fish specimens two leuciscid species: *Squalius cephalus* and *Squalius lepidus* from Sarchnar in Sulaimanyah city. The prevalence value of infection fish *Squalius cephalus* and *Squalius lepidus* was %20 and %16.66, respectively and the mean intensity of the parasite showed 2.87 and 2.4, respectively. The morphology of this parasite was illustrated using compound microscope. Moreover, some heavy metals (Cd, Cu, Pb, Zn and Fe) were analyzed in water and (Cd, Cu, Pb and Zn) were analyzed in parasite and the results showed the concentrations of Cd, Cu, and Pb at four sites were below the (LOQ), Iron (Fe) levels were also below the (LOQ) in sites 1 and 3 but in site 2 and site 4 were above the (LOQ); however, zinc (Zn) concentrations exceeded the (LOQ) in all sites. Additionally, the concentration of Cd and Zn were deposited in parasite tissues more than water in all study sites. Cd was recorded its highest concentration 3174.7 mg/Kg above the (LOQ). The *Clinostomum complanatum parasite could be used as bio indicators of heavy metals pollution of water.*

Keywords: Clinostomum complanatum, Squalius cephalus, Squalius lepidus, Prevalence of parasite, Heavy metal

I. Introduction

The digenean trematode *Clinostomum complanatum* (Rudolphi, 1814) belongs to the Clinostomidae family. Due to intra- and inter-specific morphological diversity, Clinostomum has undergone many taxonomic revisions (Feizullaev and Mirzoeva, 1983). *C. complanatum* has a life cycle with other members of the Clinostomidae (Gustinelli et al., 2010). Adult forms can angulate in the buccal cavities of mammals, including humans, and usually live in the buccal cavities of fish-eating birds or reptiles. Fish and amphibians come after freshwater snails as the first intermediate host (Kanev et al., 2002). Miracidia in snails develop from sporocysts to rediae to brevifurcate cercariae after hatching from eggs and going through multiple rounds of asexual reproduction. Brevifurcate cercariae that swim freely pierce fish skin to develop into metacercariae, which are the infectious stage for final host like piscivorous birds (Dias et al., 2003).

Clinostomum sp. is well known as yellow grubs, identified a bulge that includes whitish yellow and elliptical or diamond-shaped metacercariae in the fish's branchial cavity (Wang et al., 2017). The parasite has an impact on zoonotic

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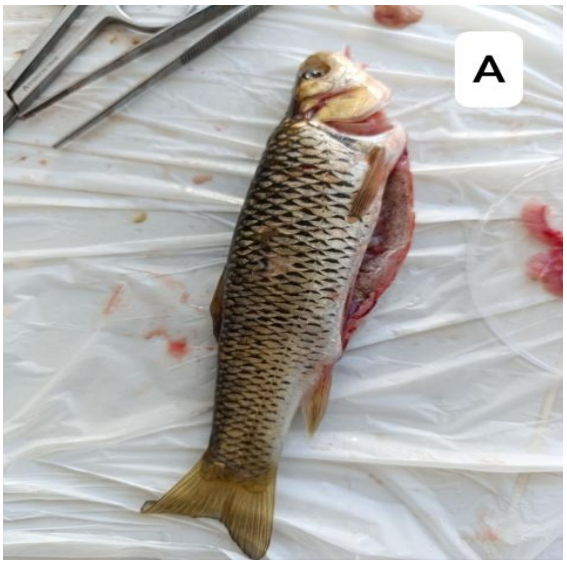
hazard in addition to fisheries. Peoples have repeatedly come into contact with *C. complanatum* across the world, primarily by eating uncooked or undercooked freshwater fish can causes laryngitis or pharyngitis (Park et al., 2009; Hara et al., 2014; Lee et al., 2017).

Heavy metals are the most dangerous contaminants because of their environmental durability and susceptibility to bioaccumulation in aquatic settings (Akpor and Muchie, 2011; Khidhir, 2022). Elements that weigh more than 5 grams per cubic centimeter are classified as heavy metals (HV). According to Mehana et al. (2020) and Othman (2023), there are two categories of heavy metals: those that are harmful at trace levels (like Cd, Pb, and Hg) and those that are physiologically useful in fish metabolism (like Zn, Cu, Ni, and Fe). Fish-related heavy metals that contribute to water contamination include lead, zinc, copper, iron, and manganese (Afshan et al., 2014; Othman et al., 2023; Khashroum, 2024). The objective of this research is to investigate *Clinostomum complanatum* potential as a bio indicator for the amount and presence of some heavy metals from Sarchnar in Sulaimanyah city.



II. Materials and Methods

2.1. Sample collection

Seventy samples of two fresh water fish (*Squalius cephalus* and *Squalius lepidus*) (Fig.1 A and B) respectively collected from Sarchnar stream in Sulaimanyah city, Iraq by gill net. The fish with water transported to the laboratory for analysis and parasitological examination for the presence of *Clinostomum complanatum*.

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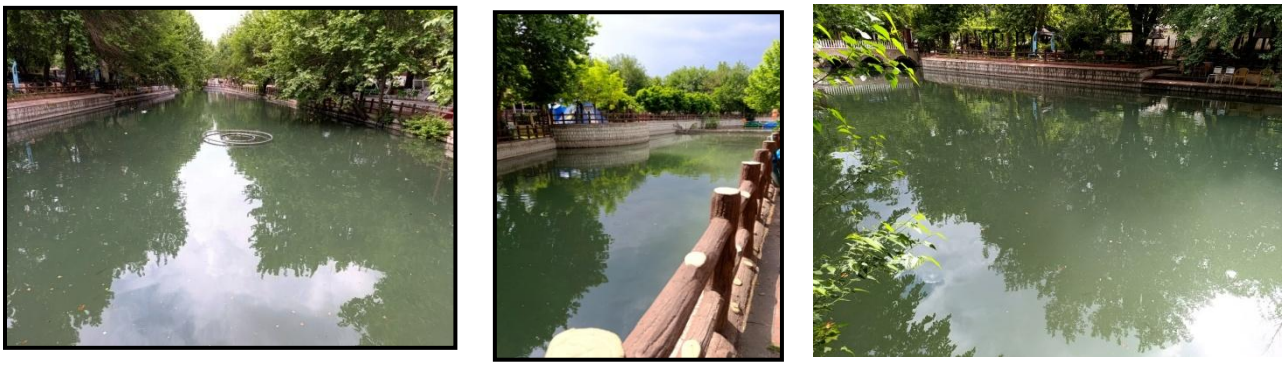

Figure -1- A- *Squalius cephalus*. B- *Squalius lepidus*

2.2. Isolation and Identification of *Clinostomum complanatum*

Fish were dissected and analyzed for internal Clinostomum metacercaria after being examined for external metacercaria; however, only metacercaria was detected from the gill. After having been flattened with a cover glass slide under moderate pressure, the collected specimens were stored in 70% ethanol and cleaned with normal saline. Two tiny needles were used to break apart the cysts under a dissecting microscope in order to remove the metacercaria. Five percent hot formalin was used for fixing the metacercariae.

2.3. Water samples collection and prepared

Water samples are collected from four sites of Sarchnar (Fig.2). The bottles 500 ml have been used for gathering water samples. Water samples were brought to the laboratory and add 2ml nitric acid of (%69) and complete by water to 50ml.

Figure -2: Sarchnar water locations

2.4. Analysis of heavy metals in water samples

Heavy metals in collected water samples were determined after preparation of samples using ICP-OES instrument for determination.

2.5. Analysis of heavy metals in *Clinostomum complanatum*

Put the sample on in oven in $60C^0$ for two hours after that increase the oven temperature to 103 C^0 until dry the sample, weight 0.0103 gm from the dry sample then added 9 ml HNO3 %69 + 1ml H_2O_2 % 40.5 weights for 30 min, finally put in the microwave digestion after digestion complete to 50 ml ready to reading by ICP-OES instrument.

2.6. Statistical analysis

A two-tailed t-test was used to compare the significant variations in heavy metal concentrations between the four study locations. The statistical analysis was performed using XLSTAT software 2016.

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III. Results

3.1. Morphological identification

From the gills of two fish species that were dissected, many encysted metacercariae were removed. Their cysts were round or oval in form, visible to the eye, and contained just one metacercaria once they had ruptured. The bodies of *Clinostomum* spp. metacercariae extracted from a number of sickened fish were ligula-shaped. The mouth of the metacercariae was situated in the middle of its subterminal oral sucker. The oesophagus was relatively short, the ventral sucker was larger than the oral sucker and located in the front part of the body, and the digestive system was made up of two large, wrinkled, unbranched, blind-ending intestinal caeca. Insufficient development was observed in the reproductive organs, particularly the ovaries and testes (Fig. 3). All parasites found have been identified as *Clinostomum* spp. metacercariae based on the descriptions presented by Abdullah et al. (2023) and Gibson et al. (2002).

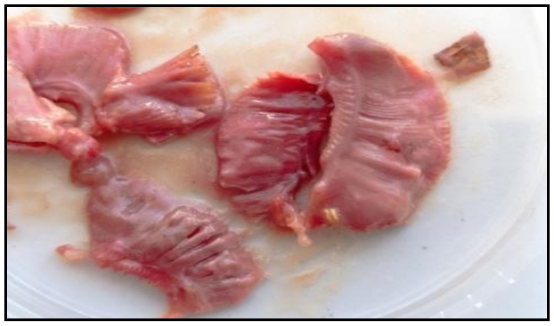
Figure -3: *Clinostomum complanatum*

3.2. Prevalence of infection

A total of 70 fish specimens, *Squalius cephalus* (n = 40) and *Squalius lepidus* (n = 30), were examined for parasitic infection. Out of these, 13 individuals were found to be infected, resulting in an overall prevalence of 18.57%. In *S. cephalus*, 8 out of 40 individuals were infected, corresponding to a prevalence of 20%. A total of 23 parasites were collected from this fish species, mean intensity was 2.87. In *S. lepidus*, 5 of 30 fish were infected, representing a prevalence of 16.66%. A total of 12 parasites were isolated, with a mean intensity of 2.4. These findings indicate that both *S. cephalus* and *S. lepidus* are susceptible to parasitic infection, with a slightly higher prevalence and mean intensity observed in *S. cephalus*.

Table 1. The prevalence values (%) and mean intensity of *Clinostomum complanatum* in *Squalius cephalus* and *Squalius lepidus* fish host.

Fish species	Fish examined	Infected fish	Prevalence %	No. of parasite	Mean intensity
Squalius cephalus	40	8	20	23	2.87
Squalius lepidus	30	5	16.66	12	2.4
Total	70	13		35	



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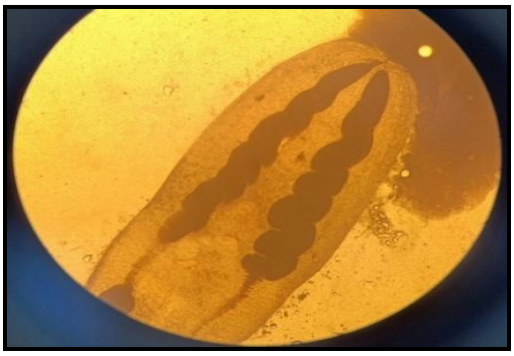

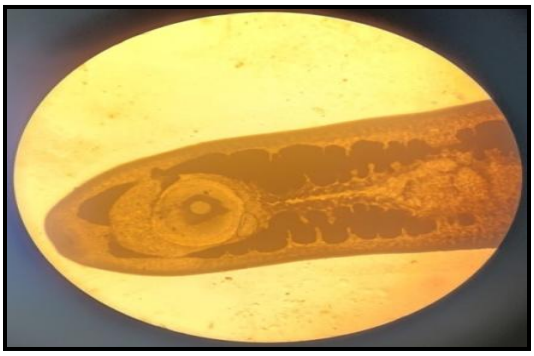

3.3. Levels heavy metals accumulated in water

Five heavy metal concentrations were measured in water samples from four locations in the Sarchnar from Sulaimanyah city, Iraq: iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb) (Table 2). Lead, copper, and cadmium were all below the detectable level at all four sites, according to our testing, which means they were either very low or not detected at all. Zinc levels, however, were detectable everywhere and ranged from 11.52 µg/L at Site 3 to 22.17 µg/L at Site 4, significantly surpassing our 2.06 µg/L threshold. The highest levels of zinc are found at Site 4. Our statistical analysis revealed a significant difference in zinc levels between the locations ($p = 0.049$), indicating only minor variations in zinc pollution. With corresponding measurements of 11.33 µg/L and 16.99 µg/L, iron levels were over the limit at Sites 2 and 4 and below the detection limit at Sites 1 and 3. This would imply that different environmental factors are contributing to elevated iron levels in particular locations. In conclusion, zinc was found everywhere, iron levels differed by location, and lead, copper, and cadmium were not found.

Table 2. Concentration of Cd, Cu, Pb, Zn and Fe from four study sites in Sarchnar water.

Site	Heavy metal µg/L				
	Cd	Cu	Pb	Zn	Fe
1	<3.0	<5.99	<5.7	12.57	<3.37
2	<3.0	<5.99	<5.7	11.90	11.33
3	<3.0	<5.99	<5.7	11.52	<3.37
4	<3.0	<5.99	<5.7	22.17	16.99
LOD (µg/L)	0.94	1.79	1.7	0.61	1.12
LOQ (µg/L)	3.0	5.99	5.7	2.06	3.37
t-test				0.049*	

LOD (µg/L): Limit of detection
LOQ (µg/L): Limit of quantification
p-value (Two-tailed) < p alpha (0.05)

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3.4. Levels heavy metals accumulated in *Clinostomum complanatum*

Cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn) concentrations were measured in the parasite *Clinostomum complanatum*, which was identified in infected freshwater fish from the Sarchnar area in Sulaimanyah city, Iraq (Table 3). The detected level of cadmium was 3174.7 mg/kg dry weight, which is significantly greater than the detection limit of 3.0 µg/kg. Zn in high concentrations (1116.5 mg/kg), which is higher LOQ 2.0 µg/kg. However, the levels of Pb and Cu were both lower than their LOQ (10.0 µg/kg and 5.0 µg/kg for) respectively. However the accumulation pattern in the parasite was Cd > Zn > Pb > Cu.

Table 3. Concentration of Cd, Pb, Cu and Zn in *Clinostomum complanatum*.

Fish parasite	Heavy metal			
	Cd (mg/Kg)	Pb (µg/Kg)	Cu (µg/Kg)	Zn (mg/Kg)
Clinostomum complanatum	3174.7	<10.0	<5.0	1116.5
LOD (µg/Kg)	0.96	3.7	1.7	0.61
LOQ (µg/Kg)	3.0	10.0	5.0	2.0

LOD (µg/Kg): Limit of detection
LOQ (µg/Kg): Limit of quantification

IV. Discussion

This is the first study to use the Clinostomum complantum as bio indicator for water contamination with heavy metals. The parasite has a complex life cycle, with freshwater fish acting as the second intermediate host, snails as the first intermediate host, and piscivorous birds as the last host. This Clinostomum metacercaria was first found in Iraq from B. luteus and A. vorax in Basrah's Mehaijeran Creek (Khamees, 1983). It was then documented from 22 diverse fish hosts, with the exception of *G. kurdistanicus* (Mhaisen, 2016). Abdullah et al. (2018) from *Glyptothorax kurdistanicus*, Abdullah et al. (2023), and Al-Maliki et al. (2018) from *Alburnus mossulensis* (also known as *A. sellal*), *Capoeta damascina, Garra rufa,* and *Squalius cephalus* from Swarian station on the Gheshlagh River in Kurdistan Province, Iran, exhibited a strong resemblance to the metacercariae in this study. Simsek et al. (2018) discovered similar results from *S. cephalus* in the Central Anatolia Region of Turkey.

Morphologically, the metacercariae gathered from the two fish host species used in this study were similar. Other studies have noted the occurrence of this parasite in 27 different fish species in Iraq (Mhaisen, 2023). Thus, *Squalius cephalus* from the current study may represent a newly added host of this parasite in Iraq.

Clinostomum complanatum was shown to be extremely common in *Squalius cephalus* and *Squalius lepidus*, with infection rates of 20% and 16.66%, respectively, in our study. Our findings are in line with those of Abdullah et al. (2023), who discovered *C. complanatum* in many fish species gathered from surface waters in the Sulaimani city of Iraq. *Capoeta trutta*, *Capoeta umbla*, *Carasobarbus kosswigi*, *Cyprinion macrostomum*, *Garra rufa*, *Alburnus sellal*, *Squalius lepidus*, and *Planiliza abu* were among these fish species. A total of 1.8%, 5%, 20%, 1.5%, 1.7%, 4.9%, 4.9%, and 1.3% were reported as infection prevalence rates. According to Gholami et al. (2011), the prevalence of the larvae of the *C. complanatum* metacercariae in *Aphanius dispar*, which were collected from the Mehran River in southern Iran, was 4.12%. Additionally, Malek and Mobedi (2001) found that the metacercaria prevalence in *Capoeta capoeta* gracilis, which was sampled from the Shiroud River in Iran, was 47.3%. This result is similar to Ahammed-Shareef and Abidi (2012) found *C. complanatum* metacercariae in Channa punctatus from India, based on the infection rate record of 24.7%. The spiny eel, *Mastacembelus mastacembelus*, was collected from the Greater Zab River in Erbil Province by Bashě and Abdullah (2010) and found *Clinostomum complanatum* metacercariae in branchial cavity they found that the

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prevalence of these metacercariae was 0.78 %. Al-Maliki et al. (2015) determined the prevalence of *C. complanatum* metacercariae in Qurna, Basrah Province, was 26.1% in *Tilapia zillii* (*Coptodon zillii*).

Heavy metals are among the most dangerous contaminants found in aquatic ecosystems due to their persistence, bioaccumulation, and rare biodegradation (Keke et al., 2020). These contaminants could affect parasite performance and survival (Akinsanya et al., 2020). Results showed that the concentration of zinc (Zn) exceed from the (LOQ), with a value of 2.06. The concentration of Fe in site 2 and 4 above the LOQ (3.37). In four sites the level of Pb, Cu and Cd below their LOQ (3.0, 5.99, and 5.7), respectively. Fe levels in sites 1 and 3 were lower than the LOQ of 3.37. The concentration of Pb, Cu, and Fe lower than Rashid and Mohammed (2015). Mashaly et al. (2023) examined the amount of Cd and Pb in water samples were greater than the WHO (2022) and "Drinking Water Standards" (EWQS, 2007) allowable limits in all sites of study.

Mashaly et al. (2020) reported that the parasitic species could be used as biological markers for environmental pollution. In this study, *C. complanatum* had the highest concentrations of Cd, and Zn. This could be because of the fish's diet, the amount of algae and the sediment content of the water. According to Sures (2003), fish parasites can effectively detect contamination with heavy metals in aquatic habitats. Therefore, parasites have the potential to act as bio indicators of heavy metal contamination in water. Both fish and their parasites are clearly valuable tools for assessing the quality of the ecosystems they inhabit (Omer *et al*., 2021). Digenean parasites obtained higher concentrations of all the heavy metals that were examined than gills, gut, and water samples, according to Mashaly et al. (2021), the exception was chromium, which was shown to be the highest concentration in the gills.

V. Conclusion

Based on this information, the present study is the first to use *C. complanatum* as a bio indictor to assess the amount of heavy metal contamination in the Sarchnar from Sulaimanyah city, Iraq. The ecological findings demonstrated that *S. cephalus* had the greatest prevalence values of *C. complanatum*. The Zn content at site 4 is higher than the LOQ. Furthermore, the results demonstrated that in all of the study sites, parasite tissue had higher concentrations of zinc and cadmium than water, suggesting that *C. complanatum* may be used as a bio indicator of heavy metals. Changes in environmental pollutants have a positive effect on the parasite.

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