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ACCUMULATION OF SOME HEAVY METALS IN LARVAE OF *CONTRACAECUM* SP. AND THEIR HOST TIGRIS CATFISH *SILURUS TRIOSTEGUS* HECKEL, 1843 IN BAGHDAD, IRAQ

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ABSTRACT

This study was achieved to investigate the accumulation of some heavy metals included: Cadmium, Lead and Nickel in the tissues (gill, intestine, liver, muscles and skin) of *Silurus triostegus* Heckel, 1843 (Siluriformes, Siluridae) and its larval stage of the nematode *Contracaecum* sp. (Rhabditida, Anisakidae). As well as to assess the infection patterns of *Contracaecum* among *S. triostegus* specimens which were purchased fresh from the local market in Baghdad. One hundred and nine nematodes specimens in larval stage were recovered from the fish host; the overall prevalence of *Contracaecum* sp. was 38.6%. The sex of the host was not significantly (P > 0.05) associated with the infection of this nematode.

Results showed that the overall mean intensity of *Contracaecum* sp. was 6.41; mean intensity did not differ significantly (P > 0.05) between the fish sexes.The lead (Pb) was the only element detected in all fish tissues investigated as well as in the parasite, while the cadmium and nickel elements were not detected in all specimens. Skin and muscles of the fish, as well the parasite *Contracaecum* sp. contained the lowest lead levels compared to other fish tissues (gill, intestine and liver), although no significant differences were noticed among all investigated tissues and the parasite regarding the concentration of Pb.

Keywords: Accumulation, *Contracaecum*, Heavy metals, Iraq, *Silurus triostegus*.

INTRODUCTION

Extreme action in the industrial and agricultural has undoubtedly boosted heavy element concentrations in natural water streams; as a result, determining the amounts of heavy metals in fish, especially those which are widely consumed by human, has lately got some attentions. It is critical to determine the potential harm of fish consumption to human health (Djedjibegovic *et al*., 2020). Many researches on heavy metal levels in the aquatic ecosystem have been published (Pandey, 2006).

A wide range of creatures have been studied to see if they can be used as biological markers of various types of pollution in the aquatic environment (El-Shafei, 2015). Certain species have been discovered as being very sensitive to aquatic contaminants, either in terms of their functional response or their propensity to accumulate certain poisons in a dose-time dependent manner (Vinodhini and Narayanan, 2008).

Tigris catfish, *Silurus triostegus* (Siluriformes, Siluridae) has been used to identify heavy metal pollution in freshwater environments and can be considered a reliable bioindicator for biomonitoring (Karadede *et al*., 2004; Rasheed, 2012; Jawad *et al*., 2020). As seen by various reviews, there has recently been growing attention to the interaction between parasitism and pollution in the aquatic ecosystem (MacKenzie *et al*., 1995; Aldhamin *et al*., 2021). Heavy metal concentrations in fish parasites have been discovered to be quite high, primarily in adult acanthocephalan worms, but also to a lesser extent in the adult cestodes (Aldhamin *et al*., 2021). Limited investigations were achieved regarding the accumulation of heavy metals in parasites and their fish hosts in Iraq.

The current study was conducted to compare the accumulation of some haevy elements in the different tissues of *S. triostegus* and in its nematode *Contracaecum* sp., as well as to investigate the infection patterns of nematode in Tigris catfish.

MATERIALS AND METHODS

Specimens' collection

Tigris catfish, *Silurus triostegus* (n=44) were purchased fresh from some local markets in Baghdad during the period between July to December 2020. Using a cooling box, the fish specimens were transferred to the laboratory in Iraq Natural History Research Center and Museum within 24 hours. The fish was identified following Coad (2010); Weight and total length were recorded for all obtained fish. The fish body weights were between 0.664 and 1.680 Kg and the total lengths were between 43 to 63 cm. The sex was identified for each fish (female, n=25; male, n=19). All fish carcasses were dissected. Approximately 5 g of the skin, gills, muscles, intestine and liver of the fish were dissected, washed by double deionized distilled water, dried with filter paper, weighed, and kept at – 20 ^{o}C until analysis (El-Shafei, 2015).

Identification of nematodes

The mesenteries and intestine tract of each fish were isolated, then opened and checked for nematodes. The isolated larvae of the nematodes were collected separately from body cavity and intestine of each fish, washed with saline solution (0.9% NaCl), fixed in 70% ethyl alcohol, cleared with lactophenol, and identified microscopically to a genus level according to the morphological description of Anderson (2000). All nematodes were weighted, washed, dried and preserved frozen until subsequent analysis.

Heavy metal analysis

The analyses of the heavy metals (Nickel, Cadmium and Lead) were done according to the procedure described by Lazarus *et al*. (2013). After thawing, 4 to 5 g wet weight of homogenized fish tissues and up to 1.1 g of nematodes were weighed into reaction vessels, followed by 2.5 ml hydrogen peroxide (30% H_2O_2) and 1.3 ml nitric acid (65% HNO_3). Using the microwave digestion method, the vessels were cooked for 90 minutes at around 175°C. After digestion, the clear sample solution was transported to a 10 ml volume with deionized distilled water in a glass container. Subsequently, the concentrations of Nickel, Cadmium and Lead were analyzed using spectrometry (CARBOLITE, England).

Data analysis

Infection prevalence was calculated as a percentage of infected/examined hosts (Bush *et al*., 1997). Using Fisher's exact tests and the chi-square test in quantitative parasitology 3.0 (Rózsa *et al*., 2000), differences in prevalence between male and female hosts were investigated. Bush *et al*. (1997) defined infection intensity as the number of parasites in an infected host divided by the number of infected hosts, with 95 percent CIs of mean intensity calculated using bootstrap testing (Rózsa *et al*., 2000). Using a Mood's median test, mean infection intensities were statistically compared between males and females. The variance/mean ratios (s^2/m) were used to determine parasite aggregation among hosts. QP 3.0 was used to conduct all statistical evaluations of intensity and aggregation.

The variations in heavy metals accumulation among different host tissues and *Contracaecum* sp. were compared using a one-way ANOVA. Statistical Package for the Social Sciences was used to conduct the analysis (SPSS Inc, Chicago IL, USA). P value of less than 0.05 was considered statistically significant in all statistical analyses.

RESULTS AND DISCUSSION

The examination of the body cavity and intestine of the fish revealed that *Contracaecum* Railliet & Henry, 1912 (Rhabditida, Anisakidae) was the only genus identified. Seventeen out of 44 fish specimens (38.6%) were found parasitized with the larval stage of *Contracaecum* sp. (Pl. 1). This nematode taxa has been previously reported from the Tigris catfish, *Silurus triostegus* in different parts of Iraq (Abdulkarim and Abdullah, 2010; Al-Moussawi *et al*., 2018; Mhaisen and Abdul-Ameer, 2021). Mhaisen and Abdul-Ameer (2021) showed that *S. triostegus* was detected as a host of *Contracaecum* species larvae in 22 local studies, as well as, *Contracaecum* species has been recorded from other fish species such as *Planiliza abu* (Heckel, 1843), *Acanthobrama marmid* Heckel, 1843, *Arabibarbus grypus* (Heckel, 1843), *Capoeta trutta* (Heckel, 1843), *Carasobarbus luteus* (Heckel, 1843), and *Chondrostoma regium* (Heckel, 1843) (Mhaisen *et al*., 1988; Abdullah *et al*., 2021). Adults of different species of the genus *Contracaecum* were also reported from some bird species in Iraq such as *Ardea alba* Linnaeus, 1758, *Ardea purpurea* Linnaeus, 1766, *Ardeola ralloides* (Scopoli, 1769), *Botaurus stellaris* (Linnaeus, 1758), *Ceryle rudis* (Linnaeus, 1758), *Egretta garzetta*

(Linnaeus, 1766), and *Phalacrocorax carbo* (Linnaeus, 1758) (Al-Awadi *et al*., 2010; Al-Moussawi and Mohammad, 2011; Alqaisi *et al*., 2020).

Sex of the host was not significantly associated with the occurrence of *Contracaecum* sp. (X^2=2.14; df=1; p=0.143), although the occurrence of *Contracaecum* sp. among the females was higher (48%) than of the males (26.3%) (Tab.1). This result was likely due to slight differences in exposure or resistance between the sexes to some infective stages of parasites, and no differences in the diet and what containing of the possible intermediate hosts of both sexes.

One hundred and nine nematodes larvae were recovered from fish specimens (range= 2-13 nematode larvae/fish). The overall mean intensity of *Contracaecum* sp. was 6.41. For subsets of hosts (Tab. 2, Diag. 1), the mean intensity did not change significantly between sexes (t = 0.4; bootstrap p = 0.711), although the intensity of *Contracaecum* sp. was slightly higher in males than females. Similarities in host susceptibility or host contact with the parasite in the same group are likely to generate such a pattern of intensity (Moravec and Jirků, 2014).Variance /mean for the larval stage of *Contracaecum* sp. showed an aggregated distribution of the parasite across examined fish (Tab. 2, Diag. 2). The aggregation of *Contracaecum* sp. showed to be high (5.64) across the fish. Moreover, the aggregation was higher in males (7.62) than females (4.72). Parasites were aggregated across the host population, with the majority (74/109, 67.88%) of the parasite population concentrated into a minority (7/44, 15.9%) of the host population. The distribution of *Contracaecum* sp. across the fish host in the present study is similar to the distribution of most macro parasites among their hosts (Wilson *et al*., 2002).

The lead was the only element detected in all fish tissues investigated as well as in the parasites, while other elements (Cd and Ni) were not detected in all samples which is likely due to their low concentrations.

This result agreed with Karadede and Unlu (2000), who did not detect the levels of three heavy metals in addition to Cd and Pb in the muscles of six studied cyprinids collected from the Atatürk Dam Lake on Euphrates River in Turkey, *Acanthobrama marmid* Heckel, 1843, *Capoeta trutta* (Heckel, 1843) , *Carasobarbus luteus* (Heckel, 1843), *Chalcalburnus mossulensis* (Heckel, 1843) (synonym of *Alburnus mossulensis* Heckel, 1843, *Chondrostoma regium* (Heckel, 1843) and *Cyprinus carpio* Linnaeus, 1758. Current result was also agreed with Suhendan *et al*. (2010) who showed Cadmium was not detected in *Silurus triostegus* and other six cyprinid fish species: *Acanthobrama marmid*, *Aspius vorax*, *Capoeta trutta*, *Carasobarbus luteus*, *Chalcalburnus mossulens* and *Cyprinus carpio*.

The lead concentrations in Tigris catfish tissue samples and *Contracaecum* larvae specimens are illustrated in Table (3). It is evident that the skin, muscles and the nematode *Contracaecum* sp. biomass contained the lowest lead levels (1.16 ± 1.4) ppm, (1.4 ± 1.3) ppm and (1.4 ± 0.99) ppm respectively, compared to other tissues (intestine, liver and gills), although no significant differences (F-test= 1.32, P value= 0.26) were noticed among all investigated tissues and the parasites regarding the concentration of Pb. Of all the tissues investigated, the mean value of lead accumulation was maximum in the intestine followed by gills and liver. Lower accumulations were seen in *Contracaecum* sp. This result agreed with some other investigators who showed that the parasitic nematode *Anguillicola crassus* has less accumulated lead concentrations than their fish hosts (Sures *et al*., 1994; Zimmermann *et al*., 1999). Tenora *et al*. (1999, 2000) found higher amounts of metals in nematodes than in their fish hosts in their experiments. Additional influences that may affect metal accumulation include the parasite's developmental stage and the length of time it has been present in a particular host (Bergey *et al*., 2002). The properties of the parasite, its stage, the heavy element, the parasite's position in the host, and the host species are likely to cause differences in accumulation levels.

Finally, it is concluded that *Contracecum* sp. is highly aggregated in *Silurus triostegus* as well as this worm has the ability to accumulate Pb in low levels comparing to the tissues of fish.

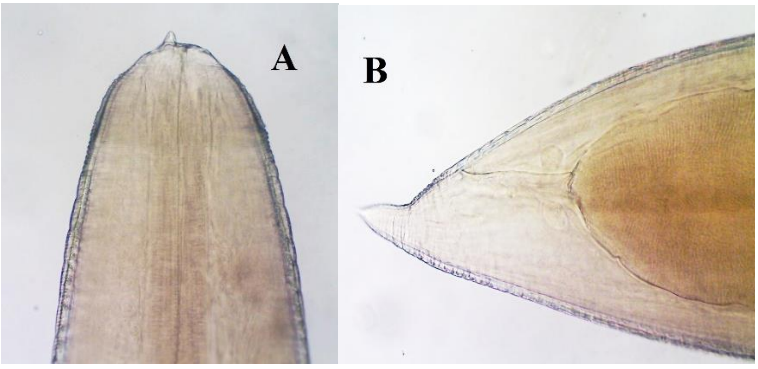

Plate (1): Anterior (A) and Posterior (B) extremity of *Contracaecum* larvae recovered from *S. triostegus* (100X).

Table (1): The infection rate of *Contracaecum* sp. among the males and females of *S. triostegus*.

Population	Number of examined fish	Number of infected fish (%)
All	44	17 (38.6%)
Female	25	12 (48%)
Male	19	5 (26.3%)

X^2=2.14; df =1; p=0.143

Table (2): The mean intensity and Variance/mean ratio of *Contracaecum* sp. among males than females of *S. triostegus*.

Population	Number of fish examined	Mean intensity	Var/mean ratio
All	44	6.41	5.64
Female	25	6.17	4.72
Male	19	7	7.62

t = 0.4; bootstrap p = 0.711

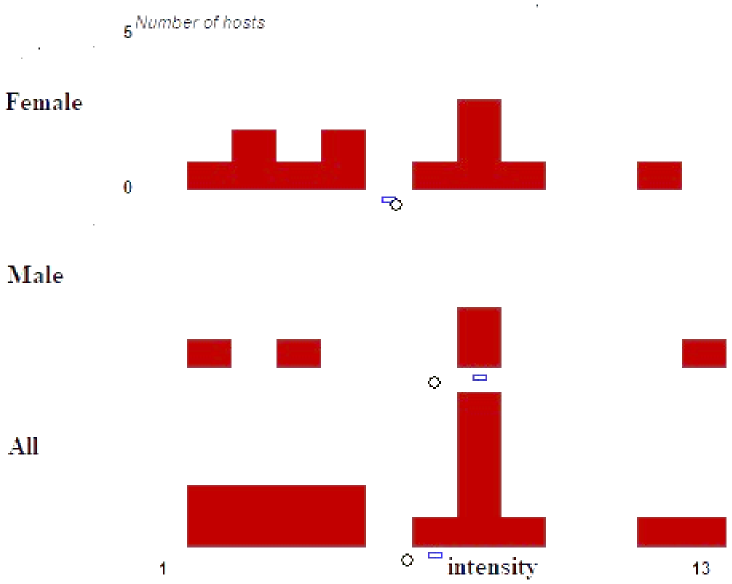

Diagram (1): The intensity of *Contracaecum* sp. (larval stage) collected from *S. triostegus*.

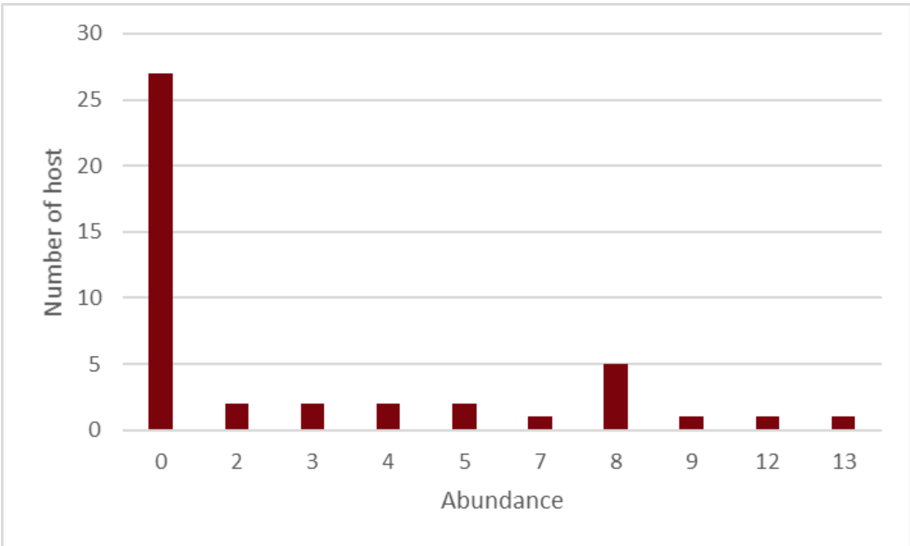

Diagram (2): Distribution of *Contracaecum* sp. abundance (number of parasites per host, including uninfected hosts) among n = 44 *S. triostegus* examined.

Table (3): The mean ± SD of Pb concentrations in different tissues of *S. triostegus* and of *Contracaecum* sp.

Tissue	Pb concentration ± SD (ppm)
Skin	1.16 ± 1.4
Gills	1.9 ± 1.16
Muscles	1.4 ± 1.3
Intestine	2.08 ± 1.2
Liver	1.7 ± 1.2
Nematodes	1.4 ± 0.99

F-test= 1.32, P value= 0.26

CONFLICT OF INTEREST STATMENT

"The authors have no conflicts of interest to declare".

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تراكم بعض العناصر الثقيلة في يرقة الدودة الخيطية *Contracaecum* sp. ومضيفها سمكة جري دجلة *Silurus triostegus* Heckel, 1843 في بغداد، العراق

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الخلاصة

أجريت هذه الدراسة للتعرف على تراكم بعض المعادن الثقيلة (الكادميوم والرصاص والنيكل) في بعض انسجة (الخياشيم والأمعاء والكبد والعضلات والجلد) سمكة جري دجلة *Silurus triostegus* Heckel ,1843 (Siluriformes, Siluridae) والطور اليرقي للدودة الخيطية *Contracaecum* sp. (Rhabditida, Anisakidae) المعزولة من التجويف الجسمي والامعاء لهذه الاسماك. تم تقييم أنماط الاصابة بالدودة الخيطية في (44) عينة من اسماك *S. triostegus* والتي تم شراؤها من السوق المحلي في بغداد و عزل (109) عينة ليرقات الدودة الخيطية *Contracaecum* sp. من هذه الاسماك. بلغت النسبة المئوية للاصابة بيرقات الدودة الخيطية 38.6٪، و لم تُظهر النتائج علاقةً معنويةً بين جنس الاسماك و الاصابة بيرقة الدودة الخيطية (P > 0.05).

كما أوضحت النتائج أن متوسط كثافة الاصابة بيرقة الدودة الخيطية بلغ 6.41. لم يختلف متوسط شدة الاصابة معنوياً (P 0 >.05) بين الجنسين. كان الرصاص هو

العنصر الوحيد الذي تم اكتشافه في جميع أنسجة الأسماك التي خضعت للفحص وكذلك في يرقة الدودة ، بينما لم يتم اكتشاف العناصر الأخرى في جميع العينات. احتوى جلد وعضلات الأسماك ، وكذلك *Contracaecum* sp. على أقل مستويات الرصاص مقارنة بألانسجة الأخرى للاسماك (الخياشيم والأمعاء والكبد) ، على الرغم من عدم وجود فروق معنوية ($P > 0.05$) بين جميع الأنسجة التي تم فحصها واليرقة فيما يتعلق بتركيز الرصاص.