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Investigation of Acanthocephalan parasites in some marine fishes as a bio-indicator for heavy metals pollution

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ABSTRACT

A total of 120 marine fish as (60 *Siganus revulatus* and 60 *Mulloides flavolineatus*) were collected from Suez Canal area at Suez Governorate to investigate the presence of acanthocephalan parasites and its relation with some heavy metals. Residues of lead (Pb), zinc (Zn), copper (Cu), cadmium, (Cd) and iron (Fe) were detected in water, acanthocephalan parasites and in the organs of infested and non-infested fishes (livers, gills, and musculature) of the examined fishes using atomic absorption spectrophotometer. The clinical picture of naturally infested fishes showed no pathognomonic signs except abdominal distension or emaciation in some fishes. Postmortem lesions were in pale liver and internal organs with presence of acanthocephalan in the opened viscera or perforated intestine. Some cases showed congestion in the liver with petechial hemorrhage. The total prevalence of infested fishes with acanthocephalans was 30%. The isolated Acanthocephala were identified as *Neohydinorhynchus macrospinosus* from *S. revulatus* 36.66 % and *Echinorhynchus sp., Serrasentis sagittifer from M. flavolineatus* 23.33%. The morphological descriptions of each isolated parasites were described.

The present study indicated that the average of heavy metal residues in organs of the infested fishes were lower than its residues in organs of the non-infested fishes. Also, the acanthocephalan parasites are able to accumulate heavy metals in their tissues higher than fish tissues and could be considered as a bio-indicator for heavy metals pollution besides being a competitor to fish organs for heavy metals accumulation.

The obtained results of heavy metals in the edible parts of the fish demonstrated at the safe levels for human consumption and its concentrations in the musculature are generally accepted by the worldwide enactment limits.

Key words: Marine fish - *Siganus revulatus* – *Mulloides flavolineatus* - acanthocephalan - bio-indicator - heavy metals - lead – cadmium- iron- pollution

Introduction

Acanthocephalans are a group of endoparasitic helminths found commonly in both marine and freshwater fishes. They are characterized by complex life cycle including arthropods as intermediate hosts and vertebrates as definitive or paratenic hosts. They cause pathological conditions in many fishes (**Nickol, 2006**). Attachment by the armed proboscis may cause mechanical damage which affects the architecture of the intestinal tissues and the digestive and absorptive efficiency of the fish. Heavy infections may cause obstruction of the gut and invasion or migration of the parasites to uncommon locations **(Sanil *et al.*, 2010 and Sakthivel *et al.*, 2014).**

Adult acanthocephalan infects fish (definitive hosts) belongs to four classes which are Palaeacanthocephala, Archiacanthocephala, Polyacanthocephala and Eoacanthocephala **(Weber *et al.*, 2013).** Heavy metals bioaccumulation in fish and subsequent distribution in organs is inter-specific. Moreover, many factors can affect metal uptake like age, sex, reproductive cycle, size, feeding behavior, swimming patterns and living environment (**Zhao *et al.*, 2012).**

Aquatic pollution is considered a big problem in many freshwater and marine environments as it negatively affects the health of the respective organisms. (**Mouillot *et al.*, 2005** and **Farombi *et al.*, 2007; Kaoud and El-Dahshan, 2010** and **Noor El Deen *et al.*, 2011**). The relationship between parasitism and pollution is not simple. It involves a double edged phenomenon in which infection by parasites may increase host susceptibility to pollutants or pollutants may result in an increase or decrease in parasite prevalence in fish, by impairing the immune response of the host. Heavy metals such as copper, lead, cadmium and more specifically mercury are harmful to most organisms even in very low concentrations. Pollutants may affect the intermediate or alternate hosts in the parasite life cycle and on free-living life cycle stages of parasite invasion.

Accumulation of heavy metals in fish by direct absorption from water through their gills and skin or by consumption of contaminated food then enter the fish`s blood stream and then accumulated into their tissues, mainly liver and kidney where they are excreted and bio-transformed or taken up by the food chain to consumers (**Ju *et al.*, 2012; Obaidat *et al.*, 2015** and **Mziray and Kimirei, 2016).** So, fish consumption can be a major route for human exposure to heavy metals (**Chen *et al.*, 2010).** Cadmium low levels may cause kidney damage; while, severe exposure causes carcinogenicity, nephrotoxicity, coronary atherosclerosis, reproductive disorder, and neurological disorders (**Nawrot *et al.*, 2010).**

Therefore, the aim of the current investigation is to determine the role of the isolated acanthocephalans as bioindicator of heavy metals pollution.

Material and Methods

Fish: A total number of 120 a live marine fishes [60 *Siganus revulatus* (segana) and 60_*Mulloides flavolineatus* (barbony)] were collected randomly from different places in Suez Canal area at Suez Governorate. They were transferred to the laboratory of Fish Diseases and Management Department, Faculty of Vet. Medicine, Suez Canal Univ. in plastic bags partially filled with their natural water in a short period of time according to **Langdon and Jones (2002).**

Clinical picture: Clinical and Post-mortem examinations of fishes were carried out to detect any abnormalities by naked eyes. Internal organs were thoroughly examined with the help of a stereoscopic dissecting microscope according to **Noga (2010).**

Parasitological examination: The freshly recovered acanthocephalan parasites were refrigerated overnight to extend the proboscis. Then they were fixed in alcohol-formalin-acetic acid (AFA). The specimens stained with Semichon's aceto-carmine, dehydrated through an ethanol series, cleared in clove oil, then got rid of oil by xylene and mounted in Canada balsam **(Pritchard and Kruse, 1982).** Identification of the isolated acanthocephalan was performed according to **Youssef and**

Derwa (2005), El-Lamie (2007), Bayoumy *et al.* (2008), Abdel-Mawla & Abo-Esa (2011), Al-Zubaidy and Mhaisen (2012), Abdel-Ghaffar *et al.* (2014) and Sakthivel *et al.* (2014).

Water samples preparation for heavy metals measurements: Four water samples were collected from the same locality of fish capture, kept refrigerated and transferred cold to the laboratory for analysis. 5 ml of concentrated Nitric acid was added to 250 ml of water sample at the time of analysis and evaporated to 25 ml. The concentrate was transferred to a 50 ml flask and diluted to the mark with distilled de-ionized water **(Parker, 1972).**

Fish samples preparation for heavy metals measurements: Fishes were dissected to get gills, liver and muscle, kept frozen until heavy metals analysis. To the dried sample, add 2 ml. HNO3 and swirl. Evaporate carefully just to dryness on hot plate, transfer to cooled furnace, slowly raise temp. to 450ºC-500ºC and hold at this temp. for 1 hour. Add 10 ml of 1N HCl and dissolve ash by heating cautiously on a hot plate. Transfer to 25 ml volumetric flask and add HCl as necessary. Cool and dilute with deionized water to volume then stored at 4ºC until analysis **(Official Methods of Analysis, 1980).**

Parasites preparation for heavy metals determination: Samples were weighted (around 100–150 mg wet weight) and digested in Teflon vessels with 2 ml HNO3 and 1 ml H2O2 at 90 °C in an oven and left overnight, according to the method mentioned by **Brázová et al. (2012).** After the complete digestion, samples were diluted with 30 m of Milli-Q water and then analyzed for trace elements by the mentioned instrument.

Different investigated heavy metals in acanthocephala parasite and in the different fish organs were measured with Atomic Absorption Spectrophotometer (Model Thermo Electron Corporation, S. Series AA Spectrometer, UK,) at central lab of Faculty of Agriculture, Suez Canal University

Results and Discussion

Clinical and Postmortem findings: The naturally infested fishes were apparently healthy and no pathognomonic signs except abdominal distension or emaciation in some fishes **(Plate 1: A&B).** The postmortem findings showed paleness in liver and the internal organs with presence of acanthocephalan in the opened viscera or perforated intestine. Some cases showed congestion in the liver with petechial hemorrhage **(Plate 1: C&D).** These postmortem findings are similar to that recorded by **El-Lamie (2007), Abdel-Mawla and Abo-Esa (2011) and Salah Eldeen *et al*. (2014).**

<u>Parasitological finding</u>:

Phylum: Acanthocephala

Family: Neohydinorhynchidae

Genus: Neohydinorhynchus

Species: *Neohydinorhynchus macrospinosus* Amin and Nahhas (1994)

It isolated from the intestine of infested *Siganus revulatus* revealed cylindrical body with widest part near the middle and gradually tapering anteriorly and posteriorly. Proboscis is barrel shaped with 9-11 rows of 5 hooks on each row. 4 cement glands arranged in pairs one after the other. Proboscis receptacle was double walled while Leminisci were enclosed in membranous sac. Male reproductive system occupied up to two-thirds of trunk. Testes were spherical to ovoid in shape and tandem in position. Gravid uterus was filled with eggs **(Plate 2: A, B, C, D &E).** This description was coincided with that recorded by **Youssef and Derwa (2005), El-Lamie (2007) and Abdel-Mawla & Abo-Esa (2011).**

Family: Echinorhynchidae

Genus: Echinorhynchus

Species: Echinorhynchus sp.

It isolated from the intestine of infested *Mulloides flavolineatus.* All worms had a milk-white color, the sex of adult Echinorhynchus sp. clearly distinguishable. The trunk elongate, Proboscis cylindrical, stout, claviform, densely armed with numerous rows of strongly recurved hooks, which vary in size according to their position. Worms' possess 18-19 longitudinal rows, with 4 hooks per row in male, two elliptical testes. Female usually larger, possess 14- 18 longitudinal rows, with 6-10 hooks per row. The body cavity of the female filled with fusiform shape eggs, **(Plate 2: F, G, H, I and J).** This description agrees with that described by **Bayoumy *et al.* (2008), Sakthivel *et al.* (2014).**

Family: Rhadinorhynchidae

Subfamily: Serrasentinae

Species: *Serrasentis sagittifer*

It isolated from the intestine of infested *Mulloides flavolineatus.* Body was cylindrical, elongated and creamy white in color, proboscis club-shaped, broad at its anterior part, narrowed towards its posterior, and covered with numerous uniform spines arranged longitudinally. Spines were triangular, arrow shaped, the proboscis is followed by a short spineless neck region followed by the body proper (trunk) which is supported by multiple combs of spines (16–24 incomplete rows) on its ventral surface. The proboscis of the male was long and at the widest point. Trunk was spinose at its anterior part. Testes were ovoid, tandem in position, Body of the female worm was larger than that of the male. Vagina was surrounded by two pairs of vaginal muscles with the uterus having a conical shape **(Plate 2: K & L).** This description agrees with that described by **Al-Zubaidy and Mhaisen (2012) and Abdel-Ghaffar *et al.* (2014).**

<u>Prevalence of the isolated parasites:</u>

The total prevalence of acanthocephala in the examined marine fishes was 30%. This result was higher than that reported by **Al-Zubaidy and Mhaisen (2012)** who recorded that the total prevalence was 13.1% in some marine fishes.

Table (1) showing that the prevalence of *Neohydinorhynchus macrospinosus* isolated from *Siganus revulatus* was 36.66%. This was nearly similar to the results obtained by **Abdou and Mahfouz (2006)** which was 33% *Sclerocollum rubrimaris* acanthocephala from *Siganus luridus* and **Salah Eldeen *et al*. (2014)** 38% Sclerocollum sp. from *Siganus revulatus.* It was higher than that obtained by **Youssef and Derwa (2005), El-Lamie (2007), Abdel-Mawla and Abo-Esa (2011)** who recorded the prevalence in siganid fish was 5.6%, 15% and 12.6% respectively**.** Meanwhile, it was less than that obtained by **Abdou and Mahfouz (2006)** who recorded the same prevalence of Sclerocollum sp infested *Siganus rivulatus* and *Centropristis filamentosus* from the Red Sea was 59 %.

The prevalence of Acanthocephala (Echinorhynchus sp and *Serrasentis sagttifer***)** from *Mulloides flavolineatus* was 23.3% which nearly the same results recorded by **El-Ashram and Shager (2008)** 25% *Serrasentis sagttifer* from *Scombermorus maculates* and **Al-**

Zubaidy and Mhaisen (2012) 24% from *Pomadasys argenteus* fish. Meanwhile, higher than that obtained by **Bayoumy *et al.* (2008)** 16.2% *Echinorhynchus gadi from Mullus surmuletus,* **Abdel-Mawla and El-Ekiaby (2012)** 7% from Seabass fish and **Debenedetti *et al.* (2013)** 3% *Echynorhynchus gadi* from *Mullus barbatus* , **Öztürk and Yeşil (2017)** 1.21% *Acanthocephaloides irregularis* from Red Mullet and lower than that obtained by **Abo-Esa (2007)** 35% *Serrasentis sagttifer* from *Mullus barbatus* and **Abdel-Ghaffar *et al* (2014)** 57.14 % *Serrasentis sagttifer* from gilthead Sea bream. This variation in prevalence may be attributed to the unequal samples, difference of fish species, species of parasites and different sites from which samples collected as well.

Table (2) showing heavy metals concentration (mg/l) in water. Cadmium concentration was 0.0002 and 0.0003 mg/l in two samples and not detected in the others. Lead concentration in one sample was 0.0049 and not detected in the rest. The average of Pb, Zn, Cu, Cd and Fe concentrations were 0.0012, 0.0148, 0.0049, 0.0001and 0.0227 mg/l, respectively. All investigated metals were lower than the permissible limits mentioned by **WHO (2011).**

Table (3) showing that there were no Pb residues detected in all investigated organs except in infected gills of *Siganus revulatus*. The absence of lead from different investigated fish organs may be due to the much decreased pb concentrations in water. Similar result obtained by **Victor *et al.* (2012)** who reported that pb concentrations in muscles, gills and livers increased as its concentration in the surrounding medium increase. **EOS (1993)** recommended 2 mg/Kg in fish muscle and **WHO (2011)** recommended 25 µg/kg body weight/day for an adult daily intake; consequently, there were no health risk on the consumption of the edible muscles of *S. revulatus* and *M. flavolineatu* but there main risk in bioaccumulation of heavy metals in edible muscles.

Zn concentrations in livers, gills and musculature of the infested fishes were lower than its concentrations in the non-infested organs. Zn residues in different investigated organs in both infested and non-infested organs had the order: livers > muscles > gills **(El-Moselhy *et al.*, 2014)**. The obtained results showed that Zinc values in examined fishes were lower than the acceptable concentration (50 mg/kg) as mentioned by **FAO (1983), (40 mg/kg) EOS (1993)** and according to

WHO (2011) which recommended that daily intake for an adult is 1 mg Zn/kg of body weight/day, so a normal consumption of these fish species considered quite safe for human.

Copper concentrations in the infested fish among different organs were lower than its concentrations in the non-infested ones. Among different organs, Cu in both infested and non-infested organs followed the order: liver > gills > muscles. Similar results were observed by **El-Moselhy *et al.* (2014), Omayma *et al.* (2015). All C**opper concentrations that detected in the edible muscles during the present work was below the permissible concentration (20 mg/kg) mentioned by **FAO (1983**), and **(20 mg/kg) EOS (1993)** so they quite safe for human consumption.

There was no detected cadmium in infested fish. On the other hand, liver and musculature of non-infested fishes had some cadmium residues (**Table, 3).** There were no health risk on the consumption of the edible muscles of the fish, where the permissible cadmium concentration mentioned by **FAO (1983) and EOS (1993)** are 0.5 mg/kg.

Concerning Fe values in the non-infested organs were higher than its values in the infested one. Average iron in both infested and non-infested organs followed the order: liver > gills > muscles. The concentration were below the permissible limits (30 mg/kg) mentioned by **EOS (1993)** and **WHO (2011)** who reported that the recommended daily intake for an adult is: up to 50 mg Fe/day. So, a normal daily diet including this fish species poses no health risk to consumer.

The obtained results noticed that the bioaccumulation of different metals in different organs was higher in non-infested fishes in comparison to infested fish with acanthocephalan. Similar results were obtained by **Shahat *et al*. (2011)** with acanthocephalan and nematodes**, Baruš *et al.* (2012)** with cestodes and **Hassan *et al.* (2017*)*** who reported that the heavy metals exhibit a significant decrease in fish tissues infested with helminthes. The liver was the target organ for Cu, Zn and Fe accumulation (**Zhao *et al.*, 2012 and El-Moselhy *et al.*, 2014)**. The lowest residues of all investigated metals were in musculature of fish may be attributed to muscle is generally not an active tissue and considered to have a weak accumulating potential (**Saeed and Mohammed, 2012; Baruš *et al.*,**

2012; El-Moselhy *et al.*, 2014; Abdel-Mawla and Shalaby, 2014 and Omayma *et al.*, 2015).

Heavy metal residues were higher in the isolated acanthocephalan than organs of the same infested fishes. Similar results detected by **Tekin-Özan and Kir (2007), Baruš *et al.* (2012)** and **Hassan *et al.* (2017)** who revealed that parasite could be used as an indicator of the metal pollution and at the same time could also minimize the bioaccumulation of heavy metals in fish tissues. Even the parasites minimize the bioaccumulation of heavy metals in fish tissues but presence of the parasites itself are repulsive to the consumers.

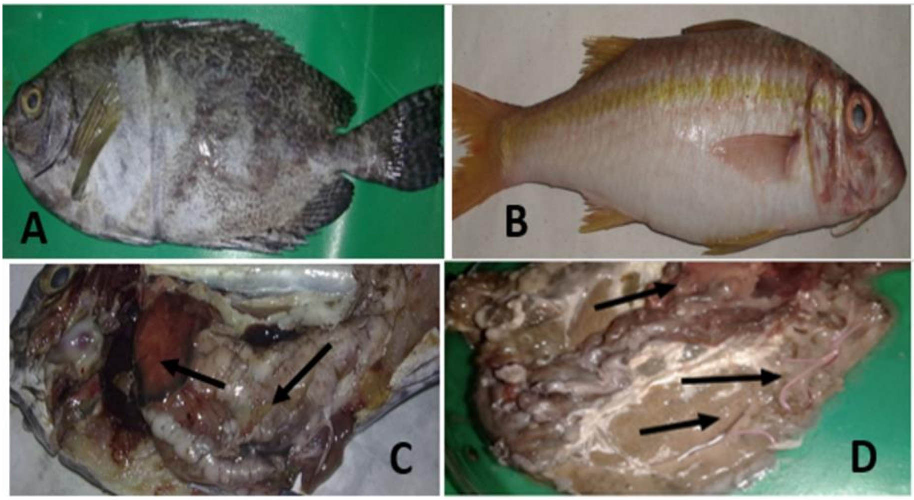

Plate (1): A: Macroscopic appearance of *Siganus revulatus* showing abdominal distention, **B:** slight abdominal distention in *M. flavolineatus*. **C:** congested liver with petechial hemorrhage and pale viscera of *S. revulatus*. **D:** paleness in liver and intestine with presence of acanthocephalan in the opened viscera of *S. revulatus.*

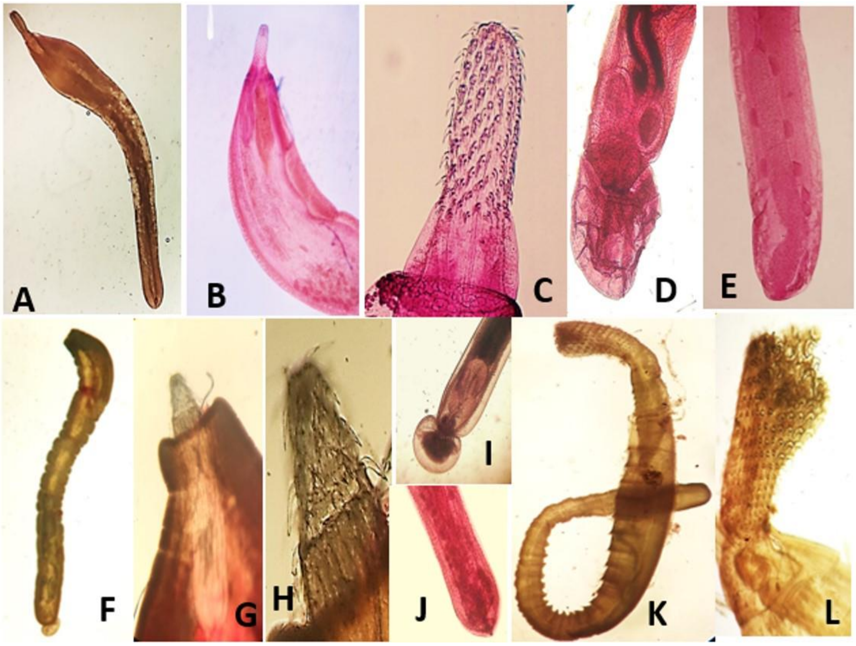

Plate (2): A: Whole female Acanthocephala (*Neohydinorhynchus macrospinosus*) by Dissecting microscope **B:** anterior part showed the two Leminisci. X100 **C:** high magnification of thorny proboscis with hooks rows. X400 **D:** male posterior end. X400 **E:** female posterior end. X400 **F:** whole male Acanthocephala Echinorhynchus sp. by Dissecting microscope **G:** anterior portion showed the two Leminisci. X100 **H:** high magnification of thorny proboscis with hooks. X400 **I:** male posterior end. X400 **J:** female posterior end. X400 **K:** whole male *Serrasentis sagittifer* by Dissecting microscope **L:** anterior portion showed thorny proboscis with hooks. X100.

Table (1): The prevalence of the isolated acanthocephala from different marine fishes

Fish species	Total No of Exam	Total No of infest.	% of infest.	Acanthocephala species	Intensity of acan. /fish
Siganus revulatus (Segana)	60	22	36.66	- *Neohydinorhynchus macrospinosus*	2-8
Mulloides flavolineatus (barbony)	60	14	23.33	- *Echinorhynchus sp* -*Serrasentis sagittifer*	1-3
Total	120	36	30		

Table (2):Heavy metal concentrations (mg/l) in the collected water samples from Red Sea

Reading	Pb	Zn	Cu	Cd	Fe
1	ND	0.024	0.0026	0.0002	0.0126
2	0.0049	0.0116	0.0063	ND	0.0234
3	ND	0.0123	0.0067	ND	0.0301
4	ND	0.0115	0.0041	0.0003	0.0246
average	0.0012	0.0148	0.0049	0.0001	0.0227
PL	0.01	No health-based guidline value has been recorded	2	0.003	No health-based guidline value has been recorded

PL: permissible limits (mg/l) according to **WHO (2011)**

ND: Not Detected.

Table (3): Residues of some heavy metals (ppm) in different investigated organs and musculatures of *Siganus revulatus* , *Mulloides flavolineatus* and *Acanthocephalan* parasite.

Fish sp.	Organs	Fish	Pb (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Cd (mg/kg)	Fe (mg/kg)
Siganus revulatus	Livers	Infested	0	0.6892	0.4287	0	16.9505
		Not infested	0	4.4110	4.7371	0.0075	26.5018
	Gills	Infested	0.0052	0.1179	0.0371	0	1.9247
		Not infested	0	0.2289	0.2459	0	4.0294
	Muscles	Infested	0	0.2855	0.0585	0	0.3819
		Not infested	0	0.3885	0.1115	0.0012	2.7347
Neohydinorhynchus macrospinosus		**Average**	0.006	5.0328	5.6314	0.0085	27.345
Mulloides sp.	Livers	Infested	0	0.3034	0.3245	0	15.7435
		Not infested	0	3.487	2.9108	0.005	24.2833
	Gills	Infested	0	0.0902	0.0371	0	1.9247
		Not infested	0	0.1528	0.2647	0	3.7534
	Muscles	Infested	0	0.1357	0.0085	0	0.3819
		Not infested	0	0.3885	0.2151	0.0007	2.3107
Echinorhynchus sp		**Average**	0	4.0215	3.0135	0.007	25.0215
Maximum acceptable* concentrations			**0.025**	**1**	**0.025**	**0.5**	**50**

Maximum acceptable* concentrations: mg/kg for Cd **(FAO, 1983), EOS (1993)** and as recommended daily intake (mg/kg of body weight) as **WHO (2011)** for the other elements.

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استبيان الاصابة بديدان الاكانثوسيفيلا فى بعض الأسماك البحرية كمؤشرحيوي للتلوث بالمعادن الثقيلة

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الملخص العربى

أجريت هذه الدراسة علي ١٢٠ سمكه بواقع ٦٠ سمكة من أسماك السيجان و٦٠ سمكة من البربوني) , و التى تم تجميعها عشوائياً من منطقه قناه السويس فى محافظة السويس لإستبيان وجود الديدان الرأس شوكية وعلاقتها ببعض المعادن الثقيلة. كما تم قياس تركيزات كلا من الرصاص(Pb) والزنك (Zn) والنحاس(Cu) والكادميوم (Cd) والحديد (Fe) في عينات المياه وطفيليات الأكانثوسيفيلا و أعضاء الأسماك المصابة والغير مصابة (الكبد والخياشيم، والعضلات) باستخدام طيف الامتصاص الذري.

أسفر الفحص الاكلينكي للأسماك المصابة انها سليمة ظاهريا وعدم وجود علامات مرضيه مميزة بإستثناء انتفاخ طفيف فى البطن أو هزال فى بعض الأسماك. الصفة التشريحية للأسماك كانت عباره عن شحوب فى الكبد والأعضاء الداخلية مع وجود الديدان الرأس شوكية في الأحشاء أو الأمعاء. بعض الأسماك يظهر عليها احتقان فى الكبد ونقط نزفية .

كان معدل الإصابة الكلية بالديدان الرأس شوكية ٣٠٪. وكانت الأكانثوسيفيلا المعزولة هى نيورهايدينورينكس ماكروسبينوسس من أسماك السيجان بنسبه إصابه ٣٦,٦٦% و إكينورينكس سبيسز و سيراسينتس ساجيتيفير من أسماك البربوني بنسبه ٢٣,٣٣%. كما نوقشت الصفات المورفولوجية لكل الطفيليات المعزولة.

أشارت الدراسة الحالية إلى أن متوسط متبقيات المعادن الثقيلة في أعضاء الأسماك المصابة كان أقل من بقاياها في أعضاء الأسماك غير مصابة . طفيليات الأكانثوسيفالا قادرة على ان تراكم المعادن الثقيلة في أنسجتها بنسبه أعلى من أنسجة الأسماك و يمكن اعتبار هذا مؤشرا بيولوجيا لتلوث المعادن الثقيلة إلى جانب كونه منافسا لأعضاء الأسماك لتراكم المعادن الثقيلة .

أشارت النتائج التي تم الحصول عليها من المعادن الثقيلة في الأجزاء الصالحة للأكل من الأسماك إلى مستويات آمنة للاستهلاك الادمى وتركيزاتها في العضلات عامه مقبولة من قبل حدود التشريعات الدولية مع الاهتمام بحاله الاصابه بالطفيليات فى الاسماك من عدمه طبقا لبروتوكول صحه وسلامه الغذاء المصري التى لاتسمح بتداول او عرض او استهلاك أسماك مصابة بالطفيليات بلحومها.