

Parasitic Helminths (Trematoda, Nematoda, Cestoda) as Bioindicators of Heavy Metal Pollution in Marine Ecosystems: A Comparative Study on *Saurida undosquamis*

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Research Article

Keywords: Bioindicators, Parasitic helminths, Heavy metals, Saurida undosquamis, Environmental monitoring, Libyan coast

Posted Date: July 31st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10404413/v1>

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Additional Declarations: No competing interests reported.

Abstract

Parasitic helminths have emerged as promising bioindicators for monitoring heavy metal pollution in aquatic ecosystems. This study investigated the bioaccumulation of four heavy metals (Cu, Zn, Pb, and Cd) in three groups of parasitic helminths (Trematoda, Nematoda, and Cestoda) isolated from the commercially important Brushtooth lizardfish (*Saurida undosquamis*) along the Misurata coast, Libya. A total of 88 fish samples were collected during the hot and cold seasons of 2022, revealing a 100% infection prevalence across all seasons. Heavy metal concentrations in the isolated helminths were analyzed using Atomic Absorption Spectrophotometry (AAS). The results indicated that Copper (Cu) was the predominant metal across all helminth groups, while Cadmium (Cd) and Lead (Pb) exhibited group-specific accumulation patterns. Trematodes and Cestodes exhibited a significantly higher capacity to accumulate toxic metals (Pb and Cd) compared to Nematodes, which showed more stable accumulation patterns and a preference for essential metals. Seasonal variations influenced metal distribution, with generally higher concentrations observed during the cold season, particularly in Cestodes. The distinct accumulation capacities, especially the high bioconcentration of toxic metals in Trematoda and Cestoda, highlight their potential as sensitive and reliable bioindicators for marine heavy metal pollution. This study provides the first baseline data on the use of helminth parasites from *S. undosquamis* for environmental biomonitoring in the Libyan Mediterranean coast.

1. Introduction

Marine environments are continuously exposed to anthropogenic pollutants, with heavy metals representing a persistent and critical threat to aquatic ecosystems. Traditional biomonitoring programs rely heavily on analyzing water, sediments, or fish tissues. However, these matrices have limitations; water and sediment concentrations fluctuate rapidly, while fish possess complex physiological mechanisms that tightly regulate and excrete certain metals, potentially masking the true bioavailable fraction of pollutants in the environment. Consequently, there is a growing need for highly sensitive and reliable bioindicators that can accurately reflect environmental contamination over time.

In recent years, environmental parasitology has highlighted parasitic helminths as exceptional bioindicators of heavy metal pollution. Unlike their hosts, many parasitic worms lack complex excretory and detoxification systems, allowing them to accumulate heavy metals directly from the host's tissues or intestinal contents at concentrations significantly higher than those found in the surrounding environment or the host's organs. This accumulation is facilitated by their large surface-area-to-volume ratio, direct contact with the host's internal environment, and the presence of specific metal-binding proteins (metallothioneins) in their tegument.

The brushtooth lizardfish, *Saurida undosquamis*, is a demersal marine fish of significant ecological and commercial value in the Mediterranean, including the Libyan coast. Previous parasitological surveys have indicated a remarkably high prevalence of helminth infections in this species. Despite this, there is a critical lack of integrated studies utilizing these parasites for environmental biomonitoring along the

Libyan coast. Therefore, the present study aims to: (1) determine the concentrations of Cu, Zn, Pb, and Cd in three groups of parasitic helminths (Trematoda, Nematoda, and Cestoda) isolated from *S. undosquamis*. (2) compare the accumulation capacities and metal distribution patterns among the three helminth groups. (3) evaluate the influence of seasonal variations (hot vs. cold seasons) on heavy metal bioaccumulation in these parasites, thereby assessing their potential as bioindicators for marine pollution in the region.

2. Materials and Methods

2.1. Study Area and Sample Collection

The study was conducted along the coast of Misurata, Libya (Latitude 32.22° N, Longitude 15.06° E). 88 fresh specimens of *Saurida undosquamis* were collected from local fishing boats and the local fish market between February and October 2022, covering both the cold and hot seasons.

2.2. Parasitological Examination and Helminth Isolation

Upon transport to the laboratory, fish were dissected, and the internal organs (gills, stomach, intestines, and muscles) were carefully examined for parasitic helminths using a stereomicroscope. The isolated helminths were categorized into three major groups: Trematoda, Nematoda, and Cestoda. The parasites were repeatedly washed with distilled water to remove any host tissue residues, mucus, or intestinal content, and then blotted dry. Clean helminth samples were pooled according to their taxonomic group and season, weighed, and stored in clean glass vials until analysis.

2.3. Sample Digestion and Heavy Metal Analysis

Helminth samples were digested using a wet digestion method. Approximately 0.1 g (wet weight) of each pooled helminth group was placed in acid-washed glass digestion tubes. Then, 1 mL of high-purity nitric acid (HNO₃, 65%) was added. The tubes were heated in a water bath at 70°C for 24 hours, followed by cooling at room temperature for 72 hours to ensure complete oxidation. The digested samples were filtered and diluted with distilled water to a final volume of 10 mL. Heavy metal concentrations (Cu, Zn, Pb, Cd) were determined using an Atomic Absorption Spectrophotometer (AAS, Hitachi 180 – 30). Results were expressed as micrograms per gram of wet weight (µg/g w.w.).

2.4. Statistical Analysis

Data were analyzed using SPSS version 25. Two-way Analysis of Variance (ANOVA) was employed to determine significant differences in metal concentrations among the three helminth groups and between the two seasons. Statistical significance was set at $p < 0.05$. Results are presented as mean $\pm$ standard error (SE).

3. Results

3.1. Parasitic Infection Prevalence

Parasitological examination revealed a remarkably high infection prevalence; 100% of the examined *S. undosquamis* specimens were infected with parasitic helminths. No significant seasonal fluctuation in the overall infection rates was observed between the hot and cold seasons. The isolated parasites belonged to three major groups: Trematoda, Nematoda, Cestoda.

3.2. Heavy Metal Accumulation in Helminth Groups

The analysis revealed distinct accumulation patterns among the three-helminth groups (Table 1). Copper (Cu) was consistently the predominant metal across all groups in both seasons. However, the accumulation of toxic, non-essential metals (Pb and Cd) varied significantly among the groups. Trematodes and Cestodes exhibited notably higher concentrations of Cd and Pb compared to Nematodes. Conversely, Nematodes showed the highest relative retention of essential metals (Cu and Zn) and the lowest levels of toxic metals. Two-way ANOVA confirmed that the differences in metal concentrations among the three-helminth groups were highly significant ($p < 0.05$).

Table 1
Mean concentrations ($\mu\text{g/g}$ wet weight $\pm$ SE) of heavy metals in three groups of parasitic helminths isolated from *Saurida undosquamis* during hot and cold seasons.

Helminth Group	Season	Cu	Zn	Pb	Cd
Trematoda	Hot	0.149 $\pm$ 0.001	0.135 $\pm$ 0.001	0.068 $\pm$ 0.001	0.083 $\pm$ 0.002
	Cold	0.155 $\pm$ 0.002	0.139 $\pm$ 0.003	0.068 $\pm$ 0.004	0.085 $\pm$ 0.002
Nematoda	Hot	0.153 $\pm$ 0.001	0.137 $\pm$ 0.000	0.056 $\pm$ 0.001	0.062 $\pm$ 0.003
	Cold	0.153 $\pm$ 0.002	0.134 $\pm$ 0.002	0.055 $\pm$ 0.001	0.063 $\pm$ 0.002
Cestoda	Hot	0.143 $\pm$ 0.001	0.133 $\pm$ 0.001	0.055 $\pm$ 0.001	0.076 $\pm$ 0.001
	Cold	0.157 $\pm$ 0.000	0.136 $\pm$ 0.001	0.066 $\pm$ 0.001	0.083 $\pm$ 0.001

3.3. Percentage Distribution and Group-Specific Accumulation

The percentage distribution of heavy metals within the helminth tissues further highlighted the group-specific accumulation capacities (Table 2). In Trematoda and Cestoda, Cadmium (Cd) constituted a remarkably high proportion of the total metal burden (ranging from 18.7% to 19.2%), indicating a strong affinity for this toxic metal. In contrast, in Nematoda, the proportion of Cd was significantly lower (15.2% to 15.4%), while the proportions of essential metals like Cu and Zn were comparatively higher (exceeding 70% combined).

Table 2
Percentage distribution (%) of heavy metals in three groups of parasitic helminths across hot and cold seasons.

Helminth Group	Season	Cu (%)	Zn (%)	Pb (%)	Cd (%)
Trematoda	Hot	34.3	30.9	15.6	19.2
	Cold	34.7	31.2	15.2	19.0
Nematoda	Hot	37.5	33.6	13.7	15.2
	Cold	37.8	33.1	13.7	15.4
Cestoda	Hot	35.1	32.7	13.5	18.7
	Cold	35.5	30.8	15.0	18.7

3.4. Seasonal Variations in Helminth Accumulation

Seasonal fluctuations influenced the accumulation dynamics within the helminths. During the cold season, a general increase in metal concentrations was observed across most groups, most notably in Cestodes, which showed significant elevations in Cu (from 0.143 to 0.157 µg/g), Pb (from 0.055 to 0.066 µg/g), and Cd (from 0.076 to 0.083 µg/g). Trematodes also exhibited slight increases in Cu, Zn, and Cd during the cold season. Nematodes, however, maintained highly stable concentrations across both seasons, showing minimal seasonal variation.

4. Discussion

4.1. Differential Accumulation among Helminth Groups

The present study demonstrated that parasitic helminths possess distinct, group-specific capacities for heavy metal accumulation. The most striking finding was the significantly higher accumulation of toxic, non-essential metals (Pb and Cd) in Trematoda and Cestoda compared to Nematoda (Table 1 & Table 2). This differential accumulation can be attributed to the morphological and physiological differences among the helminth groups.

Cestodes and Trematodes possess a highly specialized outer covering called the tegument, which is in direct contact with the host's intestinal contents or tissues. The tegument is highly metabolically active and lacks a protective cuticle, facilitating the direct, passive, and active absorption of dissolved heavy metals from the host's microenvironment. Furthermore, these groups often lack complex excretory systems, leading to the continuous bioconcentration of metals. Conversely, Nematodes possess a more complex, multi-layered cuticle and a functional excretory system, which may restrict the uptake of toxic metals and allow for better internal regulation, explaining their lower accumulation of Cd and Pb and higher retention of essential metals like Cu and Zn.

4.2. Essential vs. Non-Essential Metals

Across all helminth groups, Copper (Cu) was the predominant metal, reflecting its essential biological role in various enzymatic and metabolic pathways within the parasites. However, the remarkably high percentage of Cadmium (Cd) in Trematoda and Cestoda (accounting for nearly 19% of their total metal burden) is of significant ecotoxicological interest. Cadmium is a highly toxic, non-essential metal that organisms typically attempt to excrete. The ability of these helminths to accumulate Cd at such high proportions suggests the presence of specific metal-binding proteins, such as metallothioneins, within their tissues, which sequester the toxic metal and prevent it from interfering with the parasite's vital physiological processes. This selective sequestration makes them excellent indicators of the bioavailable toxic fraction in the host's environment.

4.3. Influence of Seasonal Variation

Seasonal variations significantly affected metal accumulation in the helminths, with generally higher concentrations observed during the cold season, particularly in Cestodes. This trend may seem counterintuitive, as higher water temperatures in the hot season typically increase metabolic rates. However, the increased accumulation during the cold season could be linked to several factors: (1) reduced metabolic turnover and excretion rates in parasites during colder periods, leading to prolonged retention of metals. (2) Seasonal changes in the host's feeding behavior and diet, which may alter the availability of metals in the host's intestine. (3) Increased bioavailability of certain metals in the aquatic environment during the cold, wet winter season due to terrestrial runoff. Nematodes, on the other hand, showed remarkable stability across seasons, reinforcing the idea that their physiological cuticle provides a robust barrier against environmental fluctuations.

4.4. Helminths as Reliable Bioindicators in Marine Monitoring

The findings of this study strongly support the use of parasitic helminths, particularly Trematoda and Cestoda, as sensitive bioindicators for marine heavy metal pollution. Unlike fish tissues, which tightly regulate essential metals and fluctuate with environmental changes, helminths provide a time-integrated measure of the bioavailable metal burden within the host. The high prevalence of infection (100%) in *S. undosquamis* ensures that sufficient sample sizes can be easily obtained for monitoring programs. By analyzing the parasites, environmental managers can assess the trophic transfer of metals and the internal contamination of the ecosystem without the need for sacrificing large numbers of fish or analyzing complex host organs. Unlike traditional biomonitoring that requires sacrificing large numbers of fish to analyze multiple organs, the high prevalence (100%) and selective bioaccumulation capacity of these helminths offer a non-lethal, cost-effective, and highly sensitive alternative for long-term marine pollution monitoring programs.

5. Conclusion

This study provides the first comprehensive baseline data on the bioaccumulation of heavy metals in parasitic helminths isolated from *Saurida undosquamis* along the Libyan coast. The results highlight

distinct, group-specific accumulation patterns, with Trematoda and Cestoda exhibiting a significantly higher capacity to accumulate toxic metals (Cd and Pb) compared to Nematoda. Seasonal variations moderately influenced accumulation, with higher levels generally detected during the cold season. The remarkable ability of these helminths to bioconcentrate heavy metals, combined with the 100% infection prevalence in the host fish, confirms their immense potential as sensitive, reliable, and easily accessible bioindicators for monitoring heavy metal pollution in marine ecosystems. Continuous biomonitoring using host-parasite systems is highly recommended for the environmental assessment of the Mediterranean coast.

Declarations

Author Contribution

H.M.A. and L.O.E. conceptualized the study and designed the methodology. H.M.A. performed the sample collection, parasitological examination, helminth isolation, laboratory analysis, statistical analysis, and wrote the main manuscript text. K.A.E. assisted in the parasitological examination, helminth identification, and sample sorting. K.S.A., F.M.A., S.E., H.M.S., R.S.A., M.M.Ab., H.A.H., M.A.A., H.I.A., H.E.A., F.F.E., A.I.S., A.M.A., and M.M.Al. assisted in field sample collection, fish dissection, and laboratory preparation. L.O.E. supervised the project, provided critical revision, and edited the manuscript. All authors reviewed and approved the final manuscript.

Acknowledgement

Acknowledgements The authors would like to thank the Department of Zoology, Faculty of Science, Misurata University, for providing the laboratory facilities and technical support necessary to conduct this research. We also extend our sincere gratitude to the local fishermen in Misurata who kindly assisted in the collection of the fish samples.

Data Availability

Data Availability Statement: "The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request."

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