Energy Dispersive X-Ray Microanalysis in conjunction with Scanning Electron Micrography to establish nematodes as bioindicators in marine fish environment

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1 **Energy Dispersive X-Ray Microanalysis in conjunction with Scanning Electron**
2 **Micrography to establish nematodes as bioindicators in marine fish environment**

3
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12
13 **Abstract:** Energy Dispersive X-Ray Microanalysis (EDXMA) has been used as the non-invasive
14 technique on Indian helminthes to explore the role of nematode parasites as bioindicators in the marine
15 ecosystem of Central West coast of India for the first time. The investigation incorporates assertions on
16 the possible benefit of such technology to elucidate bioremediating prospects that could be helpful to
17 establish helminth parasites as a tool representing Bioindicators. The accumulation of Sulphur and Iron
18 were analysed from a raphidascaroid roundworm, *Rostellascaris spinicaudatum* (Malhotra and Anas)
19 parasitizing marine catfish, *Arius maculatus* from the Central west coast of India at Goa. Quantitatively,
20 the cuticle on oral armature comprised as much as ten times more Sulphur than iron content in the
21 roundworm under study. However, only Carbon and Oxygen were detected over caudal papillae, where
22 no metals or other elements were recorded.

23
24 Key words: *Rostellascaris spinicaudatum* (Malhotra and Anas, 2001), bioindicators, metal, iron, sulphur,
25 bioremediation, raphidascaroid, environmental impact assessment, Central West coast of India.

INTRODUCTION:

Helminth parasites as Accumulator Effectors have been gaining ground in the role of Bioindicators in India. Bioremediation using live organisms is a challenging task. However, utilizing naturally available tools as predictors is even a greater sensitive challenge. Several earlier reports have illustrated that the findings of toxicological investigations could be falsified if parasitic organisms are ignored (Turčeková *et al.*, 2002). The accumulation bio-indication studies were involved in such studies conducted on cestodes and acanthocephalans. Some of the commonly occurring pollutants that have been acting in a natural environment directly or synergistically are inorganic metals *viz.* Fe, Zn, Pb, Cd, Cu and Mn (Baby *et al.*, 2010). Since past three decades considerable interest has been generated in parasitic bioindicators to monitor aquatic biota to thrash out intricacies in the host-parasite interactions. The contribution to the discovery of parasites as water quality indicators was rightly acknowledged, but until recently the potential of bio-adaptability of endoparasites to accumulate biologically available metals in their bodies, when parasitized in fish and other aquatic biotas, was little understood. Certain contributions (Galli *et al.* 1998) appreciated the utility of parasites to assert the possibility of their role as a specialist bio-indicator that were competent to evade detection by the ordinarily available methods of hydrological monitoring. The role of cysteine as both catalyst and precursor in prebiotic peptide synthesis has been illustrated by Islam *et al.* (2020). The noticeable binding affinity of cysteine with iron to generate complexes with the potential to regulate protein function was elaborated. The amino acid, Cysteine interacted with environmentally abundant iron. These conclusions were also supported by the assertion by Baby *et al.*, (2010).

METHODOLOGY:

The roundworms were extracted from the marine catfish, *Arius maculatus* at the Central West coast of India at Goa were washed with Hank's medium and then fixed in 4% (w/v) Gluteraldehyde buffered with 0.1M sodium cacodylate (pH 7.4) containing 3% (w/v) sucrose for 4.5 h at 4° C. to conduct X-Ray Scanning Electron Microanalysis. The fishes were collected from the fresh landings at Jetty at Panjim, Goa. The tissues used for these investigations were head of *R. spinicaudatum* and the region of

pre-caudal papillae. SEM photomicrographs were prepared with the specimens attached to the stubs, for elaborate analysis of the anterior (head) and the posterior (pre-caudal papilla) regions of the worm. Each body organ of three worms of the same stage of development were subjected for analysis, and these were impinged twice to extract data on elements as well as metals. EDXMA (Energy Dispersive X-Ray Microanalysis) was conducted using JSM6510LV JEOL (Japan) equipped with Oxford Instrument INCAx-act energy-dispersive x-ray analyzer, in conjunction with SEM analysis at USIF (University Sophisticated Instrumentations Facility), Aligarh Muslim University, Aligarh, U.P., India, and the results presented as Figs. 1, 2, 3, 4 along with quantitative data on elements analysed from the tissues.

RESULTS:

The histograms evidently illustrated micro-quantities of C, O, S and Fe detected from Figs. 1,2 elaborating comparative assessment of contents of C, O, S and Fe. Though in microquantities iron content was higher than sulphur in different parts of the body, as analysed, but no metal deposition on papilla was detected but for the universal presence of C and O in both the regions studied. The values of iron element in trace quantities obtained from the present analysis as well as reported from other studies have been summarized in Table1. The differentiating pattern of elemental distribution was notably specific with regard to body organs of the worm; iron and sulphur being encountered over the cuticle of oral armature(Figs. 1, 2, 3; Table 2), while no sulphur occurred over caudal papilla that was scrutinized for element analysis (Figs. 4, 5, 6; Table 3). Quantitatively, the content of sulphur analysed was at least ten times higher than iron content in the cuticle of the roundworm under study, that were expressed as weight percent.

DISCUSSION

Non-invasive detection of metals

The differential element's accumulator response from the varied nematode body region's measurements of *R. spinicaudatum* was in evidence. The evidence of occurrence of sulphur and iron was

82 available in anterior part of body in the region of the head of the worm (Figs. 1-3). But no deposition of
83 any trace metal or other elements were recorded (Figs. 4-6) from the surface of the caudal papilla in the
84 hinder region of the body. These observations support the role of these worms as Accumulation Effector
85 as a component of the environmental impact assessment (EIA).

86 Heckmann *et al.* (2007) enumerated advantages to avoid multiplicity of elemental run for
87 detection and diagnosis of chemical elements by application of Energy Dispersive X-Ray Microanalysis
88 (EDXMA). The sulphur ion contributors, disulphide bonds in the twin amino acids, Cysteine and Cystine
89 that were the part of protein infrastructure of mammalian hair and horns as well as their probable
90 association with acanthocephalan egg shells and hooks were emphasized by Heckmann *et al.*, (2007).
91 These amino acids also contributed to the strength of microstructure within the outer cuticle that also
92 facilitated movement of fluids across to further smoothen the physiological functioning of worm's various
93 systems. These macro and micro elements are important structural and functional factors, as they are
94 involved in the architecture of many enzymes and other complex molecules (Sures, 2004). The
95 predominant accumulation of essential metals like Cu, Fe, Zn and Se occurred with inhalation of food by
96 the adult roundworms (Bird and Bird, 1991; Szefer *et al.*, 1998). The parallels in the morphological
97 infrastructure and biology of the nematodes could be drawn vis-a-vis the requirements of essential
98 elements enumerated above. Their larvae equip themselves well before encapsulation to feed on blood
99 and connective tissues available during migration through tissues of their fish hosts. The revelations by
100 Bird and Bird (1991) and Szefer *et al.* (1998) on the microstructure that assigned specific properties to the
101 cuticle of nematodes made it obvious that the infrastructure around wall of larvae was much more simpler
102 than the complex cuticularized skin of their adult counterparts. This indeed facilitated much more
103 smoother movement of required fluids across physiological systems of larvae.

104 The bioremediating effect of helminth parasites of fish in polluted water bodies has been a serious
105 subject of study in recent years (Khalaj *et al.*, 2007). It has been claimed that the nematode
106 infracommunities were remediating (pollutant is in priority because of its persistence) by triggering
107 inherent accumulating instincts to absorb chemical elements inside soft body tissues of the fish. Indeed

the survival of the fish in aquatic bodies laden with metal polluted loads were adequately supported. Physiologically, the developmental phase of parasitized helminthes and secondly the time period spent while passing through the tissues of internal organs of its fish host virtually equipped the worm with an enriched potential to accumulate metal. Other supplemental factors could be the metal characterized by specific chemical elements, site of worm infesting within the host as well as the habitat of fish.

The inorganic elements inherent in the complex long-chain fatty acids are not available to roundworms through their *de novo* synthesis, therefore, their compulsive dependence on hosts to acquire these for completion of their metabolic activities is obvious. The essential inorganic elements required for development and growth of roundworms have to be obtained from their host's body organs (Wang *et al.*, 2020). The elements of primary significance from digested and absorbed, dietary nutrients are important precursors for the synthesis and metabolism of cells, most of which need be extracted from those hosts of nematodes parasitizing them (Wang *et al.*, 2020; Khalaj *et al.*, 2007).

The efficiency of the technique facilitating attainment of peak height to detect even trace amount of chemical elements in the tissues of fish after a 50-second run for each specimen has been reliable (Heckmann, 1996). The emphasis given on technology oriented surveillance of metals assisted in the detection of iron as well as sulphur only in the region of head and in no other part of body of the roundworm in the present study. Thus the strength of cephalic armature is apparently specifically identical in the same way as the hardness functioned in a manner as to give strength to the hooks and proboscis in the anterior part of body of acanthocephalan (Heckmann *et al.*, 2007).

In earlier literature the outcome of the surveillance strategy adopted for organisms of the aquatic ecosystems by the application of atomic absorption spectrometer was reviewed by Sures (2003). Stedman (2001) emphasized that the disulfide bonds coupled with sulphur ions that are the essential constituents of the chemical bond infrastructure of the amino acids, viz. cystine and cysteine, are the common structural constituents of keratin too in the horns of mammalian hosts which are placed higher in the ladder of evolution contrary to the parasitized host fishes which were placed at the lowest order in the series of evolution.

On similar lines the strength assigned to the head of *R. spinicaudatum* due to sulphur and iron detected in the region could be similar to the hardness of the mammalian tissue constituents described above, to assist the former in attachment and penetration through the tissues during worm migration, as similar in function of proboscis hooks and egg shells of acanthocephalan, earlier concluded by Heckmann *et al.*, (2007). In further studies, Azmat *et al.*, (2008) emphasized that nematode parasites like *Echinocephalus* sp. and *Ascaris* sp. are sensitive indicator of heavy metals in aquatic ecosystems.

Heckmann *et al.* (2007) enumerated advantages to avoid multiplicity of elemental run for detection and diagnosis of chemical elements by application of Energy Dispersive X-Ray Microanalysis (EDXMA). The sulphur ion contributors, disulphide bonds in the twin amino acids, cysteine and cystine that comprised protein infrastructure of mammalian hair and horns as well as their probable association with acanthocephalan egg shells and hooks were emphasized by Heckmann *et al.*, (2007).

The hardness, strength and rigidity provided by Carbon and Sulphur to the chitinized components around armature equipped head of a variety of Anisakid and Raphidascaridoid worms draws parallel to the conclusions of similarity drawn with constituents of keratin i.e. the infrastructure components of hairs, horns, hooves, beaks, shells, nails, claws of higher vertebrates by Radwan *et al.*, (1912). It was significant that the study by Antoniou and Tselentis (1993) held the constitution of cuticle with specified elements like S, Ca and P accountable for the strength in acuarid roundworms in addition to the specialized cordons. The examples of the marine nematodes, *Tripyloides marinus*, *Ascaris suum* and *Xiphinema vuittenezi* were also cited. The site specific distribution of Sulphur at the tips, mid and basal parts of spines was also encountered by the application of Energy Dispersive X-Ray Microanalysis (EDXMA) method by Heckmann *et al.* (2017).

The critical role of sulphur in several life processes including the protective role of sulphur-coupled ligands that occurred in natural environment has been on record (Colovic *et al.*, 2018). The adverse toxic effect and accumulation of metal ions is prevented on the body of higher mammals due to the active intervention by naturally occurring sulphur in the biological systems. The chelating sites generated by the sulphur-containing amino acids provide a protective platform for eliminating toxic

metals. In view of this, it is significant that such amino acids represent an effective constituent of cell antioxidant system. On similar lines Nozaki *et al.* (1998) also illustrated the critical role played in the antioxidative defense of enteric parasites by the cysteine biosynthetic pathway.

The highly recognized macro-bioelement of the heavy metals has been iron, as emphasized by Nies (1999). In his opinion, it acted as a heavy metal filter to facilitate improved health conditions of the host. They may also have a beneficial effect on the health of their hosts by acting as heavy metal filters. Resultantly iron was considered to be the biologically most important cation. Oscar *et al.* (2003) concluded that the accumulator organisms reflect the element contamination level of their environment. The enhanced affinity of nematodes parasitizing alligators to Fe, Pb and Cd within the aquatic environment was concluded by Tellez and Merchant (2015) in their investigations. Conclusively, a lot variety of investigations conducted worldwide on fish parasitic organisms have so far focused on cestodes (Turčėková *et al.*, 2002), acanthocephalan (Galli *et al.*, 1998; Thielen *et al.*, 2004; Heckmann *et al.*, 2007) and other minor organisms (Galli *et al.*, 1998). The deposition of metals, including iron on the body of another marine nematode of Anisakidae viz., *Contracaecum* sp. (third stage larvae) was reported by Leite *et al.* (2017), while members of Camallanidae namely, *Procamallanus* reportedly accumulated inorganic metal, Fe on its body (Akinsanya and Kuton, 2016b).

The reason of high concentration of iron analysed by Sures and Siddal (1999) in the experiments conducted on *Hysterothylacium reliquens* was explained by Sures *et al.*, (1999a). He assigned this to the enhanced bioavailability of metals within the physiological system of the experimental organism, i.e. fish due to the presence of bile acid inside the alimentary canal at the site of infection. Calculated bioavailability of the iron amino acid chelate was 75.0% compared to 27.8% for FeSO₄. As suggested by Fairweather- Tait, *et al.* (1992), this study also demonstrated that the greater bioavailability of the iron amino acid chelate allows for the rapid incorporation of iron into hemoglobin first, followed by a quicker repletion of depleted iron pools (i.e. serum ferritin) than is possible with FeSO₄ (Pineda and Ashmead, 2001). This study may well open up a new area to intricately explore the possible amending effects of parasites on the biomarker responses particularly because most of the time moderate levels of iron in

water or nutrients provide a supportive strength for the survival, growth and development of nematodes parasitic in fish (Sures *et al.*, 2017). This conclusion emanated from the fact that the mixing of nitrates and iron in the upwelling zones of riverine stretch, the mixing of dietary feed with the available iron in the euphotic zone habitation of *A. maculatus*, could easily provide enhanced iron rich dietary constituents to this fish host through which the cuticle of nematodes, *R. spinicaudatum* availed opportunity to absorb extra iron available.

CONCLUSIONS:

An elaborate narrative has been developed by Parasitologists to assert (Sures and Siddall, 1999) that the environmental value of certain endoparasites should credibly be required a more emphatic attention. The approaches, methodology and academic prowess of ecological parasitologists and aquatic ecologists must address to common goals at large to develop skills to substantiate parasitic bioindicators. The earlier studies have faltered to ignore the significance of helminth parasites as contributors to sinks for heavy metals (Sures and Siddall, 1999) within a fish host. It is thus quite likely that a host fish could have much more potential to tolerate heavy metals in the aquatic ecosystems than that have been actually reported (Hofer and Lackner, 1995; Gunkel, 1994; Köck, 1996) till recent times notwithstanding the heavier amount of contaminants that helminthes parasitizing such fish could have been known (Sures, 2001). Therefore, this is the first validated attempt in the Gangetic freshwater ecosystems to confirm the role of nematodes as sensitive bioindicators that could prove to be vital as a viable tool for environmental monitoring. In a way, like the response of organisms to abiotic factors, such organisms/animals/plants that were susceptible to a wide range of parasitic organisms or their developmental stages could be termed as Euryparasitic, and when such fauna had a narrow range of susceptibility to parasitic organisms or their developmental stages, these are termed as stenoparasitic. Morsy *et al.* (2011) elaborated sustainability potential of anisakid worms, a closer taxonomic ally of rhabdascaridoid roundworms that are the subject of present study, as potential instruments to monitor environmental pollution which had the capacity to help in the survival of their fish hosts by removing heavy metals from within the aquatic ecosystems.

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Conflict of interest: The authors declare that there is no conflict of interest.

Ethical approval: No approval by an ethical committee was required to achieve the goals of the present study.

REFERENCES:

- Akinsanya B, Kuton MP (2016a) Bioaccumulation of heavy metals and parasitic fauna in *Synodontis clarias* (Linnaeus, 1758) and *Chrysichthys nigrodigitatus* (Lacepede, 1803) from Lekki Lagoon, Lagos, Nigeria. Asian Pac J Trop Dis 6:615–621 doi: 10.1016/S2222-1808(16)61096-4
- Akinsanya B, Kuton MP (2016b) Parasitic diseases and heavy metal analysis in *Parachanna obscura* (Gunther 1861) and *Clarias gariepinus* (Burchell 1901) from Epe Lagoon, Lagos, Nigeria. Asian Pac J Trop Dis 6:685–690 doi: 10.1016/S2222-1808(16)61110-6
- Antoniou M, Tselentis Y (1993) Studies on *Echinococcus granulossus* using the scanning electron microscope II. The hooks. Parasitol Res 79:543-546 Springer-Verlag.
- Azmat R., Fayyaz, S, Kazi, N, Mahmood, SJ, Uddin F (2008) Natural Bioremediation of Heavy Metal through Nematode Parasite of Fish. Biotechnology 7 (1): 139-143.

238 Baby J, Raj JS, Biby ET, Shankarganesh P, Jeevitha M.V, Ajisha, S.U., Rajan SS (2010) Toxic effect of
 239 heavy metals on aquatic environment. Intern. J Biol Chem Sci 4(4): 939-952. ISSN 1991-8631.

240 Bird AF, Bird J (1991) The Structure of Nematodes. New York: Academic 2.

241

242 Colovic MB, Vasic, VM, Djuric DM, Krstic, DZ (2018) Sulphur-containing amino acids: Protective role
 243 against free radicals and heavy metals. Curr Med Chem 25(3):324-335. doi:
 244 10.2174/0929867324666170609075434.

245

246 Dural M., Genc E., Sangun M.K., Güner Ö. 2011. Accumulation of some heavy metals in
 247 *Hysterothylacium aduncum* (Nematoda) and its host sea bream, *Sparus aurata* (Sparidae) from North-
 248 Eastern Mediterranean Sea (Iskenderun Bay). *Environ Monit Assess* 174(1-4): 147-155.
 249 DOI:10.1007/s10661-010-1445-0

250

251 Fairweather- Tait SJ, Fox TE, Wharf SG, Ghani NA (1992) A preliminary study of the bioavailability of
 252 iron- and zinc- glycine chelates. Food Add Contam 9: 97-101.

253

254 Foden, C.S., Islam, S., Fernandez-Garcia, C., Maugeri, L.M., Sheppard, T.D. and Powner, M.W. (2020)
 255 Probiotic synthesis of cysteine peptides that catalyze peptide ligation in neutral water. PLOS One, 370
 256 (6518):865-869 doi: 10.1126/science.abd5680.

257

258 Galli P, Crosa G, Occhipinti-Ambrogi A (1998) Heavy metals concentrations in acanthocephalan
 259 parasites compared to their fish host. Chemosphere 37: 2983–2988.

260

261 Geetanjali, Malhotra, Sandeep K, Malhotra A, Ansari Z, Chatterjee A (2002) The role of nematodes as
 262 bioindicators in marine and freshwater habitats. Current Sci, 82: 505-507.

263

Genc E, Sangun MK, Dural M, Can MF, Altunhan C (2008) Element concentrations in the swimbladder parasite *Anguillicola crassus* (Nematoda) and its host the European eel, *Anguilla anguilla* from Asi River (Hatay-Turkey). *Environ Monit Assess*, 141:59–65

Gunkel G (1994) Bioindikation in aquatischen Ökosystemen. Fischer Verlag, Jena, Stuttgart.

Heckmann R A (1996) Energy dispersive x-ray microanalysis in conjunction with electron optics, a tool for analyzing aquatic animal diseases and deaths. *Micro Anal* 17:27–29.

Heckmann RA, Amin OM, Standing MD (2007) Chemical Analysis of Metals in Acanthocephalans Using Energy Dispersive X-Ray Analysis (EDXA) in Conjunction with a Scanning Electron Microscope (SEM). *Comp Parasitol* 74(2): 388–391.

Hofer R and Lackner R (1995) *Fischtoxikologie - Theorie und Praxis*. Fischer Verlag, Jena.

Khalaj V, Eslami H, Azizi M, Nuria R-G, Bromley M (2007) Efficient Downregulation of *alb1* Gene Using an AMA1-Based Episomal Expression of RNAi Construct in *Aspergillus fumigates*. *Microbiol Let* 270(2): 250-254.

Köck G (1996) *Die toxische Wirkung von Schwermetallen auf Fische*. In: Steinberg C, Bernhardt H and Klapper H (eds.) *Handb Angew Limnol* pp 1–167. Ecomed Verlagsgesellschaft, Landsberg am Lech.

Le ITY, Zimmermann, S., Sures, B. 2016. How does the metallothionein in induction in bivalves meet the criteria for biomarkers of metal exposure. *Environ. Pollut.*, 212, 257-268.

288 Leite L.A., Pedro N.H., de Azevedo R.K., Kinoshita A., Gennari R.F., Watanabe S., Abdallah V.D. 2017.
 289 *Contracaecum* sp. parasitizing *Acestrorhynchus lacustris* as a bioindicator for metal pollution in the
 290 Batalha River, southeast Brazil. *Sci. Total Environ.* 575:836–840. doi: 10.1016/j.scitotenv.2016.09.132.
 291
 292 Malhotra Sandeep K, Anas M (2001) A unique piscine ascaridid *Rostellascaris spinicaudatum* gen et sp n
 293 with key to genera of subfamily Ascaridinae. *In*: Professor V.N. Capoor Commem Vol, GBP Univ Agric
 294 Technol, Pantnagar 20-25, 1-3 Nov.
 295
 296 Morsy K, Bashtar A-R, Abdel-Ghaffar F, Mehlhorn H, Al Quraishy S, El-Mahdi M, Al-Ghamdi A,
 297 Mostafa N (2012) First record of anisakid juveniles (Nematoda) in the European seabass *Dicentrarchus*
 298 *labrax* (family: Moronidae), and their role as bio-indicators of heavy metal pollution. *Parasitol Res* 110:
 299 1131–1138 doi: 10.1007/s00436-011-2600-4
 300
 301 Nachev M, Schertzinger G, Sures B. (2013) Comparison of the metal accumulation capacity between the
 302 acanthocephalan *Pomphorhynchus laevis* and larval nematodes of the genus *Eustrongylides* sp. infecting
 303 barbel (*Barbus barbus*). *Parasit Vect* 6, 21. <https://doi.org/10.1186/1756-3305-6-21>.
 304
 305 Nies DH (1999) Microbial heavy metal resistance. *Appl Microbiol Biotechnol* 51: 730-750.
 306
 307 Nozaki T., Asai T., Kobayashi S., Ikegami F., Noji M., Saito K.,and Takeuchi T. (1998) Molecular
 308 cloning and characterization of the genes encoding two isoforms of cysteine synthase in the enteric
 309 protozoan parasite *Entamoeba histolytica*. *Mol Biochem Parasitol* 97 (1-2): 33-44.
 310
 311 Oscar R, Roberto C, Maria BG, Marta D, Paolo L (2003) Trace element concentrations in freshwater
 312 mussels and macrophytes as related to those in their environment. *J Limnol* 62(1): 61–67.
 313 doi:10.4081/jlimnol.2003.61

Pineda D, Ashamead H DeWa (2001) *The absorption and metabolism of iron amino acid chelate*. Archivos Latinoamericanos de Nutricion ver. Imp. ISSN0004-0622. ALANv.51.1supl.1 Caracasmr.:19pp.

Radwan NA, Abou Shafeey HE, Khalil AI (2012) Chemical characterization of tegumental spines of four digenean species using energy dispersive X-ray microanalysis (EDXA). Internat Jour Parasitol Res ISSN: 0975-3702 & E-ISSN: 0975- 9182, 4(2), 100-105.

Stedman J K (2001) *Stedmans Medical Dictionary for the Health Professions*. 4th ed. Lippincott, Williams and Wilkins. Philadelphia. 1597 pp.

Sures, B (2003) Accumulation of heavy metals by intestinal helminths in fish: an overview and perspective. Parasitol. 126 (Suppl.): S53–S60.

Sures, B. (2004). Environmental parasitology: relevancy of parasites in monitoring environmental pollution. Tr Parasitol 20, 170–177.

Sures B, Siddall R (1999) *Pomphorhynchus laevis*: the intestinal acanthocephalan as a lead sink for its fish host, chub (*Leuciscus cephalus*). Exp Parasitol 93: 66–72.

Sures B, Siddall R, Taraschewski H (1999) Parasites as accumulation indicators of heavy metal pollution. Parasitol Today 15: 16-21.

Sures B, Nachev M, Selbach C, Marcogliese DJ (2017) Parasite responses to pollution: what we know and where we go in Environmental Parasitology. Parasit Vect, 10: 57pp.

Szefer P, Rokicki J, Frelek K, Skora K, Malinga M. (1998) Bioaccumulation of selected trace elements in lung nematodes, *Pseudalius inflexus*, of harbor porpoise (*Phocoena phocoena*) in a Polish zone of Baltic Sea. Sci Tot Environ 220: 19-24 doi:10.1016/S0048-9697(98)00221-6.

Tellez M, Merchant M (2015) Biomonitoring heavy metal pollution using an aquatic apex predator, the American alligator, and its parasites. PLoS ONE 10:e0142522. doi: 10.1371/journal.pone. 0142522.

Thielen F, Zimmermann S, Baska F, Taraschewski H, Sures B (2004) The intestinal parasite, Budapest, Hungary. Environ Poll 129, 421–429.

Turčeková L, Hanzelová V, Špakulová M (2002) Concentration of heavy metals in perch and its endoparasites in the polluted water reservoir in Eastern Slovakia. Helminthologia 39(1):23–28

Wang, X., Zhang, L., Zhang, L., Wang, W., Wei, S., Wang, J., Che, H. and Zhang, Y. (2020) Effects of excess sugars and lipids on the growth and development of *Caenorhabditis elegans*. Gene and Nutrition. 15, 1. <https://doi.org/10.1186/s12263-020-0659-1>

Explanation of Figures

Fig.1. SEM of head of *Rostellascaris spinicaudatum* (Ventral view)(Purple square represents measured area). Typical chart recording elemental composition using energy –dispersive X-ray Microanalysis (EDXMA) in conjunction with a Scanning Electron Microscope.

Fig.2. Head of *Rostellascaris spinicaudatum* for observed chemical elements after a 50-Sec run include specific peaks of different elements.

Fig.3. Bar diagrams to show quantitative illustration of peaks as depicted in Fig. 2.

Fig.4. SEM of pre-cloacal papillae (Dorsal view)(Purple square represents measured area). Typical chart recording elemental composition using energy –dispersive X-ray Microanalysis (EDXMA) in conjunction with a Scanning Electron Microscope.

366 **Fig.5.** Pre-cloacal papilla (Dorsal view) of *Rostellascaris spinicaudatum* for observed chemical elements
367 after a 50-Sec run include specific peaks of different elements.

368 **Fig.6.** Bar diagrams to show quantitative illustration of peaks as depicted in Fig. 5.

369

Figures

Figure 1

SEM of head of *Rostellascaris spinicaudatum* (Ventral view)(Purple square represents measured area). Typical chart recording elemental composition using energy –dispersive X-ray Microanalysis (EDXMA) in conjunction with a Scanning Electron Microscope.

Figure 2

Head of *Rostellascaris spinicaudatum* for observed chemical elements after a 50-Sec run include specific peaks of different elements.

Figure 3

Bar diagrams to show quantitative illustration of peaks as depicted in Fig. 2.

Figure 4

SEM of pre-cloacal papillae (Dorsal view)(Purple square represents measured area). Typical chart recording elemental composition using energy –dispersive X-ray Microanalysis (EDXMA) in conjunction with a Scanning Electron Microscope.

Figure 5

Pre-cloacal papilla (Dorsal view) of *Rostellascaris spinicaudatum* for observed chemical elements after a 50-Sec run include specific peaks of different elements.

Figure 6

Bar diagrams to show quantitative illustration of peaks as depicted in Fig. 5.

Supplementary Files

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