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## The Role of Helminths Infecting *Columba livia domestica*, Pigeon in Bioremediation of Heavy Metals Accumulation (Mn, Zn, Fe)

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### ABSTRACT

*Columba livia domestica* pigeons were collected from Ismailia city, Egypt, during the year 2017. They were found to be infected by intestinal helminths, two cestodes, *Raillietina echinobothrida* and *Cotugnia polyacantha* and a nematode, *Ascaridia columbae*. The heavy metals levels (Mn, Zn, and Fe) in pigeon's muscles and intestine, in addition to helminths, were measured. *R. echinobothrida* revealed the highest in heavy metals accumulation compared to *C. polyacantha* and *A. columbae*. Bioaccumulation factor was most obvious and dominant in muscles than intestine of infected pigeons in respect to *R. echinobothrida*. *C. livia* model ranked in concentration from highest to lowest as follows: (Mn>Zn>Fe), followed by *A. columbae*. *C. livia* model. *R. echinobothrida* / *C. livia*, *C. polyacantha* / *C. livia*, and *A. columbae* / *C. livia* are considered effective models for monitoring heavy metals pollution for Mn, Zn, and Fe, especially in areas where pollution levels are still relatively low, and have an essential role in bioremediation of heavy metals accumulation in pigeon's tissues. This study also emphasized how environmental science and parasitology might profit from each other. Further studies should be executed in studying the role of pigeon's helminths in monitoring heavy metals pollution.

### INTRODUCTION

There is a necessity to evaluate the relationship between parasitism and pollution considering parasitism as a crucial factor in evaluating environmental stressors (Hudson *et al.*, 2006; Sures, 2006). Heavy metals, although naturally occurring, are often mobilized into birds and the environment at faster-than-normal rates due to anthropogenic activities (Sharma and Agrawal, 2005).

Parasites respond to anthropogenic pollution in different ways which made it attracting attention as potential indicators of environmental quality (Sures, 2008; Vidal-Martínez, 2010). Heavy metals are considered as critical contaminants in the environment, due to their high potential to enter and accumulate in food chains (Erdogrul and Erbilir, 2007). Unfortunately, less literature is available on the use of pigeon's endoparasites in environmental impact studies. In the past two decades, manuscripts including studies of host-parasite models challenged by heavy metal exposure were increased (Barus *et al.*, 2012; Nachev *et al.*, 2013; Hassan *et al.*, 2016; 2018).

Wild animals are normally exposed to heavy metals in their natural habitats. (Kalas *et al.*, 2000). Birds are among the possible biomonitoring species, that are widely used to assess environmental contamination, and over the last decades, the feral pigeon (*Columba livia*) is assessed as a bio indicator of pollution (Nam *et al.*, 2004; Klein *et al.*, 2008). In this context,

feral pigeons have a significant epidemiological role, since they can be reservoirs and potential vectors of a large number of zoonotic pathogens (Magnino *et al.*, 2009; Karatepe *et al.*, 2011).

Monitoring the interactions between pollution and parasites have been increasing over the last years as some helminths are able to accumulate heavy metals in concentrations higher than their hosts (Sures, 2003; 2004). Directions have been shown to be highly promising and relevant, considering parasites as accumulation indicators for selected pollutants (Sures *et al.*, 2017)

Heavy metals pollution threatens pigeon's tissues. Moreover, health risks accompanied with its consumption. Therefore, there an increasing world attention towards heavy metal contamination in pigeon's tissues (Begum and Sehrin, 2013). One of the most known pigeons is *Columba livia domestica*. Considering the helminths/pigeon systems, the main target of the present study was to assess the accumulation of trace elements (Mn, Zn, Fe) in two cestodes *Raillietina echinobothrida* and *Cotugnia polyacantha*, and a nematode, *Ascaridia columbae* and in *C. livia* tissues in Ismailia city, Egypt, thus evaluating three models *R. echinobothrida* /*C. livia*, *C. polyacantha* /*C. livia*, and *A. columbae* /*C. livia* as promising bioindicator systems.

## MATERIALS AND METHODS

### 1. Sample Collection:

Thirty *C. livia* pigeons were collected from Ismailia city, Egypt during year 2017. They were transferred to the laboratory, and euthanized, then dissected and the separated parts of the alimentary canal of the pigeons were taken in 0.85% normal saline solution to collect helminths parasites. Each section of gastrointestinal tracts of was opened separately. The removed contents were examined under a stereomicroscope for the presence of helminths. Identification of the species was confirmed on the basis of light microscopic examination according to

Yamaguti (1961) and Soulsby (1982). Samples of muscles and intestine of infected and uninfected pigeons, in addition to helminths were collected, stored in glass vials and deep-frozen until later processing for trace element analysis (Danilewicz *et al.*, 2002).

### 2. Heavy Metal Analysis:

The samples were weighed (0.5 gm) in the small beaker; 3.5 ml of concentrated HNO<sub>3</sub> and 10.5 ml of concentrated HCL were added and left for 24 hours. The samples were heated for digestion with more addition of HNO<sub>3</sub> till the entire brown fumes disappeared. The digested samples were filtered and diluted with distilled water to 25 ml (Saleh and El Shenawy, 2001). The heavy metals concentrations in the digested intestine, muscles and helminths were measured by Plasma Optical Emission-Mass Spectrometer. Results were expressed as micrograms per gram (µg/g) wet weight.

### 3. Statistical Analysis:

Differences between element concentrations among the analyzed tissues were detected by ANOVA followed by Tukey's test. Correlation analysis was used to detect relationships between toxic element levels in pigeon's tissues and parasites. Statistical analysis was performed using the SPSS 20 software package. For all tests, a significance level of P<0.05 was applied. The bioaccumulation factors (BFs) were determined as the ratio of the element concentration in the parasites to that in tissues.

## RESULTS

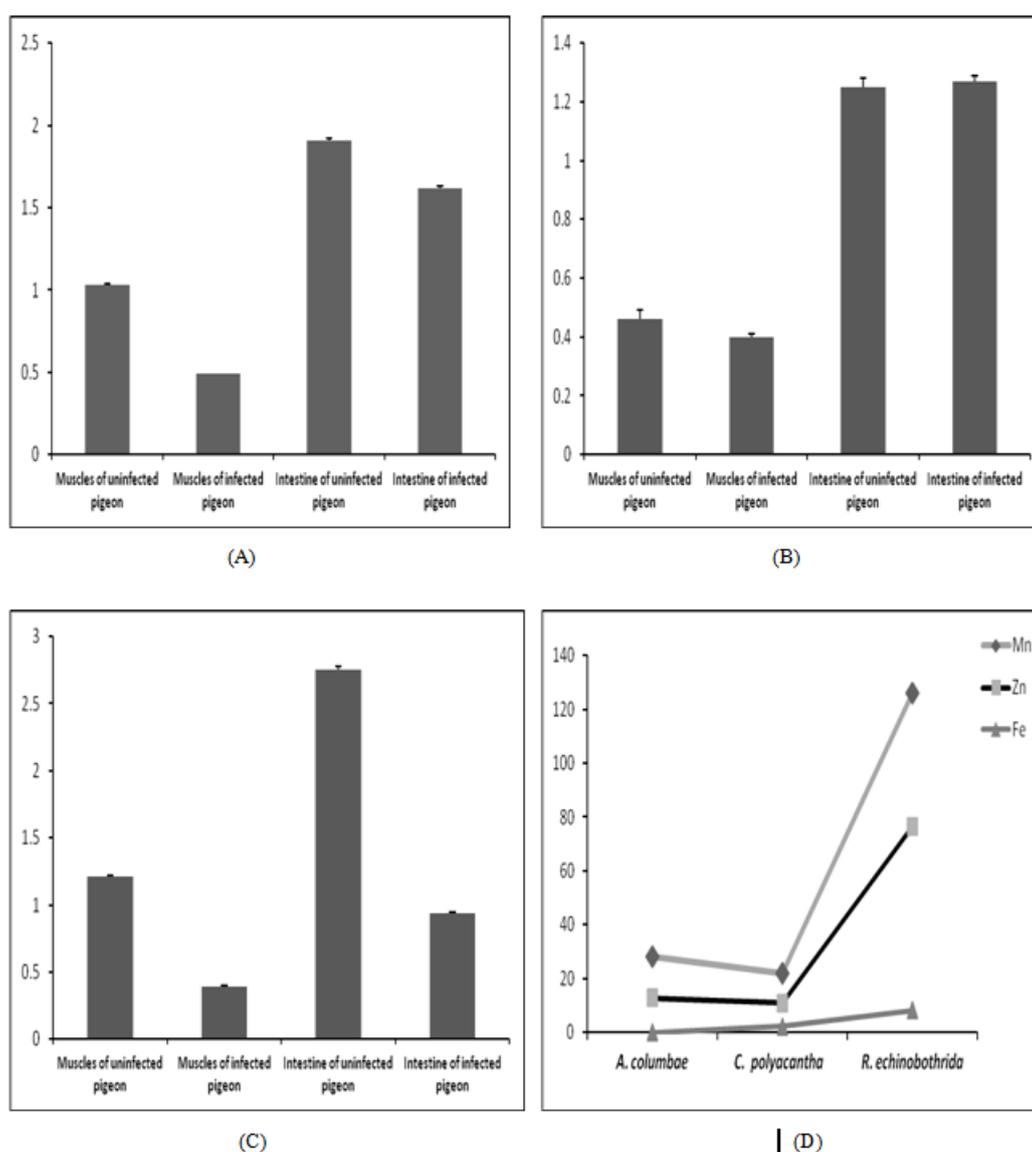
### 1. Heavy Metals Concentrations in Pigeon's Tissues and Helminths:

All element levels were found to vary according to analyzed tissue and parasites (Figure 1). Considering the pigeon's tissues, mean concentration of Mn in muscles and intestine of uninfected pigeons was 1.03±0.01 µg/g; 1.905± 0.009 µg/g and that of the infected ones was 0.49±0.004 µg/g ; 1.617±0.01 µg/g, respectively (Figure 1,A). *R. echinobothrida* revealed a higher levels in concentrating Mn as many times as that in

tissues of pigeons ( $126.15 \pm 1.13 \mu\text{g/g}$ ), followed by *A. columbae* ( $28.25 \pm 0.39 \mu\text{g/g}$ ) ( $P < 0.001$ ) and the lowest was recorded by *C. polyacantha* ( $21.75 \pm 0.14 \mu\text{g/g}$ ) (Figure 1,D).

The concentrations of Zn in uninfected and infected tissues of pigeon did not vary significantly. *R. echinobothrida* is also the highest in accumulation of Zn ( $76.69 \pm 0.26 \mu\text{g/g}$ ) concerning uninfected and infected pigeons tissues and parasites ( $P < 0.001$ ) (Figure 1, B), followed by *A. columbae* ( $12.69 \pm 0.29 \mu\text{g/g}$ ), while *C. polyacantha* was the least ( $10.91 \pm 0.18 \mu\text{g/g}$ ) (Figure 1, D).

The average content of Fe was  $1.21 \pm 0.01 \mu\text{g/g}$  in the uninfected pigeon, while it was  $0.39 \pm 0.01$  in muscles of infected pigeons ( $p < 0.001$ ). Similarly, for intestine, the mean concentration of Fe in the intestine of uninfected pigeons was  $2.75 \pm 0.03 \mu\text{g/g}$ , while in the infected pigeon's intestine; it recorded  $0.94 \pm 0.01 \mu\text{g/g}$  accumulating capacity (Figure 1, C). The role of *R. echinobothrida* is always evident as, it has high tendency to concentrate Fe compared to uninfected pigeons tissues ( $8.26 \pm 0.17 \mu\text{g/g}$ ) ( $P < 0.001$ ).



**Figure 1.** (A) Mean concentration of Mn in muscles and intestine of uninfected and infected pigeons. (B) Mean concentration of Zn in muscles and intestine of uninfected and infected pigeons. (C) Mean concentration of Fe in muscles and intestine of uninfected and infected pigeons. (D) Mean concentration of Mn, Zn, and Fe in *R. echinobothrida*, *C. polyacantha* and *A. columbae*, ( $\mu\text{g/g}$ , wet weight)

## 2. Relations between heavy metals concentrations in pigeon's tissues and helminths:

Positive and negative correlations were observed between the evaluated heavy metals in pigeon's tissues and helminths (Table 1). There was a positive correlation between the levels of Zn in *A. columbae* and Fe in muscles ( $R=0.999$ ,  $P< 0.02$ ). Also, the relation between Mn levels in *R. echinobothrida* and *A. columbae* ( $R=1$ ,  $P<$

$0.02$ ). Furthermore, a positive correlation was detected between levels of Fe in *R. echinobothrida* and Mn levels in *C. polyacantha* ( $R=1$ ,  $P< 0.001$ ). Contrarily, negative correlations were detected concerning the levels Mn in *C. polyacantha*, Fe ( $R= - 0.998$ ,  $P< 0.04$ ) and Zn ( $R= - 0.997$ ,  $P< 0.04$ ) in muscles. In addition to the levels of Fe in *R. echinobothrida* and Zn in muscles ( $R= - 0.997$ ,  $P< 0.04$ ).

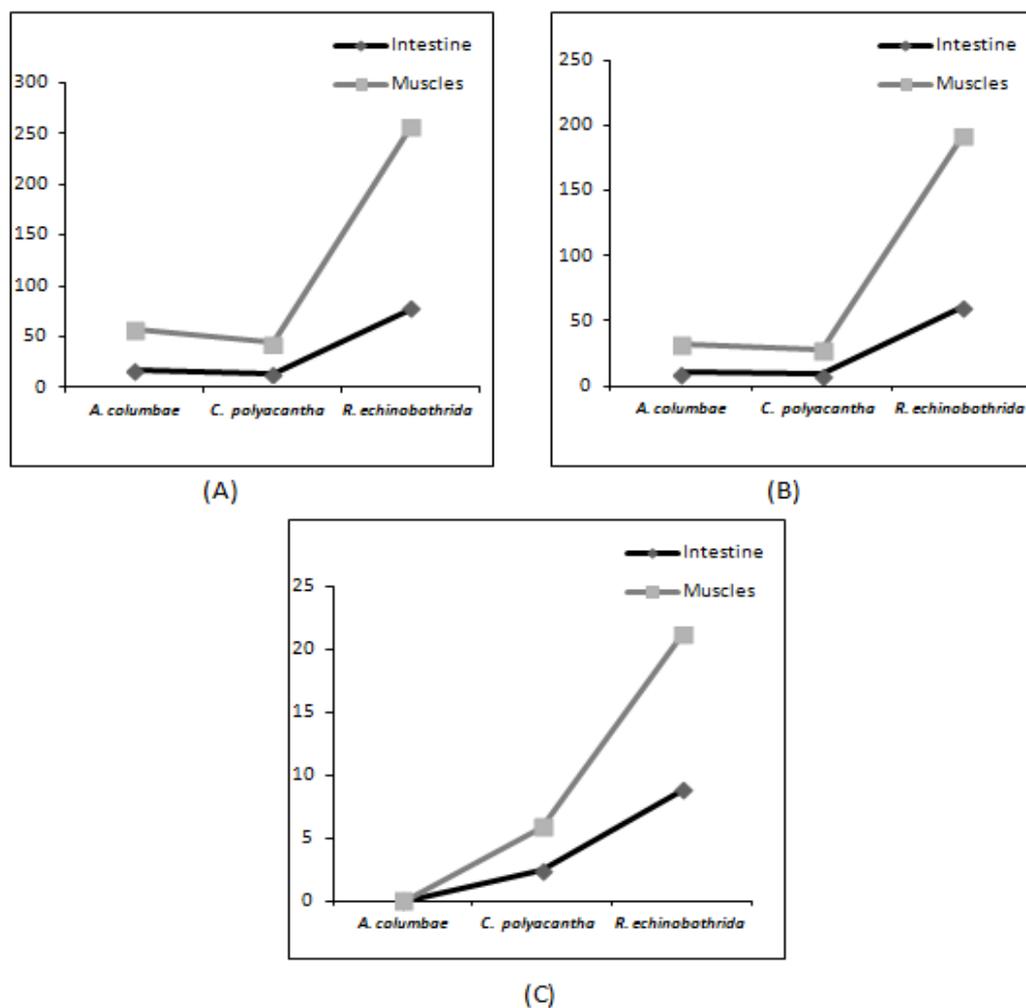
**Table 1. Correlations among element levels, tissues of *C. livia*, and helminths.**

Helminths - pigeons tissues	R	P<
<i>A. columbae</i> Zn * Fe muscles	0.999	$P<0.02$
<i>C. polyacantha</i> Mn * Fe muscles	-0.998	$P<0.04$
<i>C. polyacantha</i> Mn * Zn muscles	-0.997	$P<0.04$
<i>R. echinobothrida</i> Fe. * Zn muscles	-0.997	$P<0.04$
<i>R. echinobothrida</i> Mn * Mn <i>A. columbae</i>	1.000	$P<0.02$
<i>R. echinobothrida</i> Fe * Mn <i>C. polyacantha</i>	1.000	$P<0.001$

## 3. Bioaccumulation factors:

Bioaccumulation factors were most obvious and effective in muscles than intestine of infected pigeons with *R. echinobothrida* \ *C. livia* model being the most conspicuous (Figure 2). The highest BF registered was by *R. echinobothrida* as it accumulates Mn 257.45 times than that in *C. livia* muscles, Zn (191.73) and Fe (21.18). Similarly for the ratios between accumulation of *R. echinobothrida* and the

intestine ranked in concentration from highest to lowest as follows: (Mn>Zn>Fe) (78.01, 60.39, 8.79), respectively. The *A. columbae* \ *C. livia* model revealed also high rates of BFs. The highest BF was for Mn, as *A. columbae* accumulates Mn 57.65 times than muscles followed by Zn 31.73. On the other hand, *C. polyacantha* \ *C. livia* model revealed low BFs for Mn (44.39), Zn (27.28) and Fe (5.85) compared to *R. echinobothrida* and *A. columbae*. The same pattern was verified in the intestine (Mn>Zn>Fe) (13.45, 8.59, 2.43), respectively.



**Fig.2.** Bioaccumulation of Mn (A), Zn (B), and Fe (C) concerning helminths / *C. livia* models

## DISCUSSION

Ignoring parasites in the assessment of pollution on organisms is now recognized as a potential bias in studies, which may then lead to false conclusions (Evans *et al.*, 2001). Recently, evaluating environmental pollution has emerged via the role of parasites ability to concentrate inorganic elements, particularly, heavy metals at much higher levels compared to free-living organisms (Taraschewski, 2000). They have the ability to concentrate heavy metals levels that are up to 2,000% above background levels (Sures, 2003; 2004).

In the present study, we found out that pigeons infected with cestodes and nematodes had lower concentrations of

heavy metals in muscles and intestine than that in the uninfected pigeons. In this context, Sures *et al.* (2002) concluded that some parasites of the terrestrial hosts accumulate toxic elements more efficient than their hosts. Similarly, Barus *et al.* (2003) reported that helminths of terrestrial mammals are not as effective as those from fishes and birds in the accumulation of heavy metals. Whereas, intestinal parasites accumulated more metals compared to parasites inhabiting the body cavity (Nachev *et al.*, 2013). Torres *et al.* (2010) proposed the model *Raillietina micracantha* / *Columba livia* from the city of Santa Cruz de Tenerife (Canary Islands, Spain) as a promising bioindicator in evaluating environmental

pollution with heavy metals particularly Pb and Mn.

There was species specificity in the response of helminths to heavy metal accumulation. *R. echinobothrida* and *C. polyacantha* have an effect different capacity in accumulation of heavy metals from their host tissues. Thielen *et al.* (2004) supported the hypothesis that parasites that haven't digestive tract concentrate metals in rates higher than that detected in host tissues. These results have coincided with that reported by Torres *et al.* (2006) that cestodes accumulate heavy metals effectively. Also, Cestodes have a higher metal accumulation capacity compared to nematodes that aren't suitable as sentinels (Lafferty, 1997). Also, Barus *et al.* (2007) reported that heavy metal accumulation in the host tissues wasn't influenced by nematodes presence.

The preference of parasites for bile absorption from their host gut is the main reason for achieving high levels of heavy metal concentration as most vertebrates minimize the impact of harmful substances by surrounding them with bile, thus parasites absorb the pollutants from their host gut and accumulate them in their bodies (Sures, 2003).

The essential role of helminths as a heavy metal sanitizer for the host has raised a controversial question of whether it might be beneficial to the vertebrate host to be infected by these worms (Sures and Siddall, 1999; Taraschewski, 2000; Malek *et al.*, 2007). On another point of view, Szefer *et al.* (1998) adopted the opinion that the higher bioaccumulation by helminths may reflect the higher ability of the host to clear heavy metals. It's important to point out that the ability of the parasites to accumulate metals is affected by numerous factors including the nature of the metal itself (Sures *et al.*, 1998).

In conclusion, *R. echinobothrida* /*C. livia*, *C. polyacantha* /*C. livia*, and *A. columbae* /*C. livia* are considered effective model in monitoring heavy metals pollution for Mn, Fe, and Zn especially in areas where pollution levels are still relatively low. In addition to the role of *R. echinobothrida*, *C. polyacantha*, and *A. columbae* in

bioremediation of heavy metals accumulation in muscles and intestine of pigeons. The present outcome exhibited disparity between the accumulation capacities of helminths, as, there was species specificity in heavy metal accumulation, despite that *R. echinobothrida* and *C. polyacantha* are cestodes, and their bioaccumulation levels were different. This study also emphasized how environmental science and parasitology might profit from each other. The results obtained emphasize that not only does pigeons helminths appear to be a suitable bioindicator for Mn, Zn, and Fe, but it also indicates that pollution might have been underestimated in the past. Further studies should be executed in this field considering the type of metal as accumulation capacity was different in relation to Mn, Zn, and Fe.

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