Cestodes are Bioremediation Tools by Absorbing the Heavy Metals from Their Hosts

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Abstract

The helminthes may act as indicators of pollution of the environment where their hosts live. The present study was conducted to evaluate the role of cestodes in the elimination of some toxic minerals such as cadimum (Cd) and chrome (Cr) in wild rats (*Rattus norvegicus*) which has been used as a model. The results showed that there are high concentrations of Cd and Cr in kidneys and livers compared to intestines and muscles of captured rats from industrial and agricultural areas in Diyala Province. The concentrations of both Cd and Cr were significantly higher ($P< 0.05$) in the cestodes than in the rat tissues. The bioaccumulation factor for Cd increased 3.2 folds and 2.1 folds in the worms than in the intestines and muscles of the rats, respectively in agricultural areas and 2.8 folds and 1.4-fold in worms than in the intestines and muscles of rats captured the in industrial areas. For Cr, the bioaccumulation factor increased 3.1 folds and 2.8 folds in worms than in the intestines and muscles of rats from the agricultural areas while it increased 2.7 folds and 1.4-fold in worms than in the intestines and muscles in the industrial areas. In conclusion, and in comparison, between infected and non-infected rats and the bioaccumulation factors in the present study, it is possible to propose the ability of the cestode (*Hymenolepis diminuta*) to absorb some toxic elements such as Cd and Cr from their hosts**.**

Keywords: bioremediation, cestodes, Diyala province, wild rats.

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Cestodes are Bioremediation Tools by Absorbing the Heavy Metals from Their Hosts

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الديدان الشريطية اداة للمعالجة الحيوية بواسطة امتصاص المعادن الثقيلة من مضائفها

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الخلاصة

قد تمثل الديدان مؤشرا لتلوث البيئة التي يعيش فيها مضيفها.لقد اجريت الدراسة الحالية لتقييم دور الديدان الشريطية في ازالة بعض العناصر السامة مثل الكادميوم والكروم في الجرذان البرية (*Rattus norvegicus*) كموديل. اظهرت النتائج ان هناك تراكيز عالية من عنصري الكادميوم والكروم في الكلية والكبد مقارنة بالامعاء و العضلات للجرذان المصطادة من بعض المناطق الصناعية والزراعية في محافظة ديالى. وكانت تراكيز كل من الكادميوم والكروم اعلى معنويا ($P<0.05$) في الديدان الشريطية مقارنة بانسجة الجرذان. وكان عامل التراكم الحيوي للكادميوم يزيد بمقدار 3.2. و 2.1 اضعاف في الديدان الشريطية عن ما هو عليه في امعاء وعضلات الجرذان، على التوالي، في المناطق الزراعية وازداد بمقدار2.8 و 1.4 اضعاف في الديدان عن ما هو في الامعاء والعضلات على التوالي، في المناطق الصناعية. وبالنسبة للكروم ازداد معامل التراكم الحيوي بمقدار 3.1 و 2.8 اضعاف في الديدان الشريطية عما هو عليه في امعاء وعضلات الجرذان في المناطق الزراعية بينما ازداد بمقدار 2.7 و 1.4 في الديدان عن ما عو عليه في امعاء وعضلات الجرذان في المناطق الصناعية. وكاستنتاج، وبالمقارنة بين الجرذان المصابة وغير المصابة وعامل التراكم الحيوي في الدراسة الحالية، من الممكن اقتراح ان للديدان الشريطية (*Hymenolepis diminuta*) القدرة على تخليص مضائفها من بعض العناصر السامة مثل الكادميوم والكروم وقد تمثل مؤشرا مهما على تلوث البيئة.

الكلمات المفتاحية: المعالجة الحيوية، الديدان الشريطية، الجرذان البرية، محافظة ديالى.

Introduction

It has been suggested that the helminthes of varying animals can act as indicators of pollution in their environment [1]. Numerous industries and anthropogenic activities are the main reasons for aquatic ecosystem pollution [2]. Since heavy metals are considered to one of the most common form of aquatic pollution, helminthic parasites are of interest as a factor for sequestration and accumulation of these pollutants [3]. Many studies showed that fish acanthocephalans, cestodes and nematodes have the capacity to absorb Cu, Fe, Zn, Mn, Pb and Cd [4, 5, 6, 7]. Among all these helminthes, acanthocephalans have the highest capacity to sequestrate these pollutants (heavy metals) and considered more appropriate for this process when compared with other helminthes [1, 8].

In addition to aquatic environment, contamination of soil by heavy metals occurs due to numerous industrial and anthropogenic activities such as industrial (solid, liquid and gaseous) and domestic effluents, application of fertilizers, mine tailings, pesticides, batteries, alloys, chemical laboratories and medical industries and their wastes, electroplated metal parts, spill of hydrocarbons on the ground and the byproducts of fuel combustion in the electrical power station and in the automobile emissions [9]. The heavy metals can be transported to humans through the food chain, where they harm biological reactions, long lasting harm to vital

Few studies have been conducted on the bioaccumulation of heavy metal in the terrestrial mammals [10, 11, 3]. In Iraq, the accumulation of heavy metals in helminthes have been studied in aquatic ecosystems and birds in Ninvah and AL-Najaf provinces and both studies revealed the capacity of helminthes to absorb the heavy metals [12, 13, 14].

There are several health risks associated with heavy metals and can they lead to various disorders in humans and they may affect several organs such as liver, kidney, lung, bones, placenta, brain and the central nervous system, negatively and lead to damage and change their structures and functions [15]. Moreover, other damages that have been observed include reproductive, and development toxicity, hepatic, haematological and immunological effects and can also result in excessive damage due to oxidative stress induced by free radical formation [16].

Although it seems unusual, some studies have indicated the possibility of using some parasites such as *Trichuris suis*, hookworm and *Taenia solium* in the treatment of some autoimmune disorders such as Celiac diseas, Crohn’s disease and Irritable Bowal Syndrome [17- 21]. This may open a new area of research (parasitotherapy) to use the parasites as tool for the elimination of toxic agents from humans and the companion animals. The objective of the present study was to evaluate the role of cestodes in the elimination of some toxic minerals such Cadmium (Cd) and Chrome (Cr) in wild rats (*Rattus norvegicus*) as a model.

Materials and methods

Eighty adult rats (*Rattus norvegicus*) were captured using traps placed in the selected areas. Thirty-eight rats from industrial areas and forty-two from agricultural areas (in the center of Baquba city) were collected between October 2015 and October 2016. The captured rats were transported to the laboratory, euthanized and dissected immediately.

Adult worms were removed, identified and counted. The removed worms were identified as *Hymenolipes diminuta*. The organs and tissues (intestines, kidneys, livers and muscles) were placed in petri dishes and washed with distilled water and kept at -20^{0}C for future processing. Tissue and worm samples were thawing and weighted (30 mg to 150 mg of cestode and tissue samples for each animal). Each weighted sample was digested in the flask containing 5 ml of Perchloric acid and 7 ml of 0.1 N concentrated HNO3 and then heated at 50C° for 90 min. Five ml of 20% HCl (0.1 N) was added to the mixture. The samples were filtered using Whatman filter NO42 paper and placed in a 100 ml volumetric flask and was made up to the mark with a distilled water.

Metal analysis was done using Flame Atomic Absorption Spectroscopy (FAAS). **Standard** solutions of cadmium and chrome were used for preparing the calibration curve to measure the concentration of these elements in mg/g wet weight.

The bioaccumulation factor of heavy metals in heminthes were calculate according to the formula proposed by Sures et al. [22].

Statistical analysis

The data were analyzed by t–test analysis to compare between infected and non-infected groups and also between the collection areas (SPSS. 8).

Results

The results of the current study showed that 45% of the captured rats were infected with *Hymenolepis diminuta*. In the industrial areas, the percentage of infection with this cestode was 42.1% while in the agricultural areas it was 47.6% (Table 1) and the statistical analysis showed no significant differences between the two areas regarding the percentage of infections.

Table1: The percentage of infection among captured rats from the industrial and agricultural areas, Diyala Province.

Location	No. Examined	No. Infected	Percentage infection (%)	P-value
Industrial areas	38	16	42.10	P= 0.6208
Agricultural areas	42	20	47.61	
Total	80	36	45.00	

When the comparison between the concentrations of cadmium and chrome in agricultural and industrial areas in different tissues/organs was done, the results showed that there were no significant differences. The highest concentration of chrome was in the liver of rats captured in the industrial area (59.7 ± 3.3 mg/g wet weight) while the highest concentration of chrome was in kidneys (55.6 ± 2.3 mg/g) of the rats captured in the agricultural areas. For cadmium, the highest concentration (29.0 ± 1.8 mg/g) was in the muscles of the rats captured at the industrial areas while the highest concentration of cadmium (32.5 ± 2.9 mg/g) was found in in the kidney of rats captured at the agricultural areas (Table 2).

Table 2. Comparison between the concentrations of Cadmium and Cr in agricultural and industrial areas in different tissues/organs and parasites.

Tissue	Cadmium (mean ± SE) mg/g		P-value	Chrome (mean ± SE) mg/g		P-value
	Agricultural	Industrial		Agricultural	Industrial	
Intestine	14.4 ± 1.2	14.9 ± 1.2	NS	24.1 ± 2.1	30.1 ± 2.5	NS
Kidney	32.5 ± 2.9	28.4 ± 1.3	NS	55.6 ± 2.3	52.6 ± 2.5	NS
Liver	29.4 ± 1.9	29.0 ± 1.8	NS	53.9 ± 3.1	59.7 ± 3.3	NS
Muscles	28.9 ± 1.2	26.4 ± 1.1	NS	26.9 ± 2.5	34.9 ± 3.4	NS
Parasites	34.9 ± 1.9	40.5 ± 2.9	NS	61.4 ± 6.4	76.3 ± 6.1	NS

The concentrations of cadmium and chrome in the host and parasites are displayed in Figures (1, 2, 3, 4). In non-infected rats, both cadmium and chrome in the intestines and kidneys were significantly higher than in the infected rats (the mean of Cadmium and Chrome concentrations in the intestines and kidneys in non- infected rats were 33.3, 66.2; 62.4, 63.9mg/g, respectively, compared with infected rats 14.4, 24.1; 32.5, 55.6 mg/g, respectively). Figure 1 showed that the concentration for both elements was higher in the kidney than in the intestines and Chrome was higher than Cadmium in both organs.

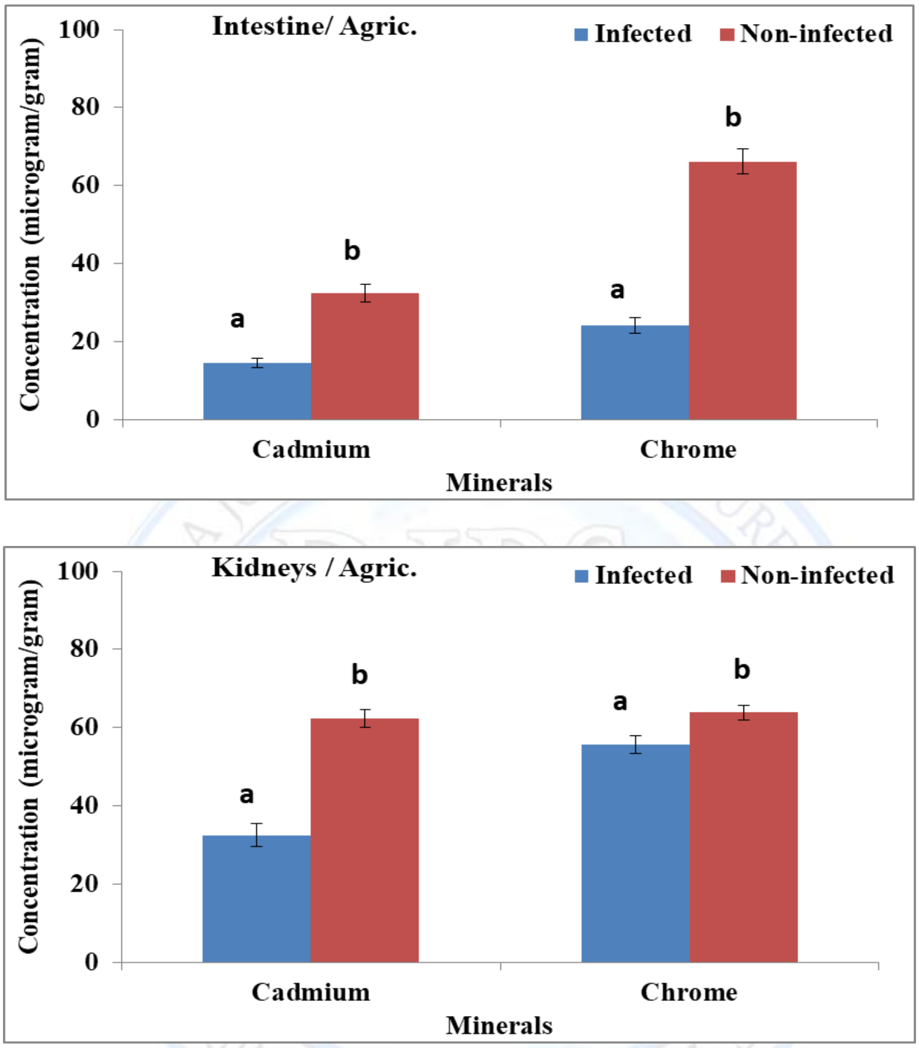

Figure 1. Concentrations (microgram / gram) of cadmium and chrome in the intestines (upper panel) and kidneys (lower panel) of wild rats (*Rattus norvegicus*) collected from agricultural areas around Baquba City, Diyala Province

In the livers and muscles, the concentrations of both cadmium and chrome decreased significantly ($P< 0.05$) in infected rats (29.4 and 53.9 for Cd; 28.9 and 26.9 for Cr, respectively) compared with those of non-infected (64.8mg/g and 68.7mg/g; 48.01mg/g and 40.96 mg/g, respectively) rats and the same results obtained in muscles for both elements as showed in Figure 2. Both elements were higher in liver than in muscles in infected and non-infected rats (Figure 2).

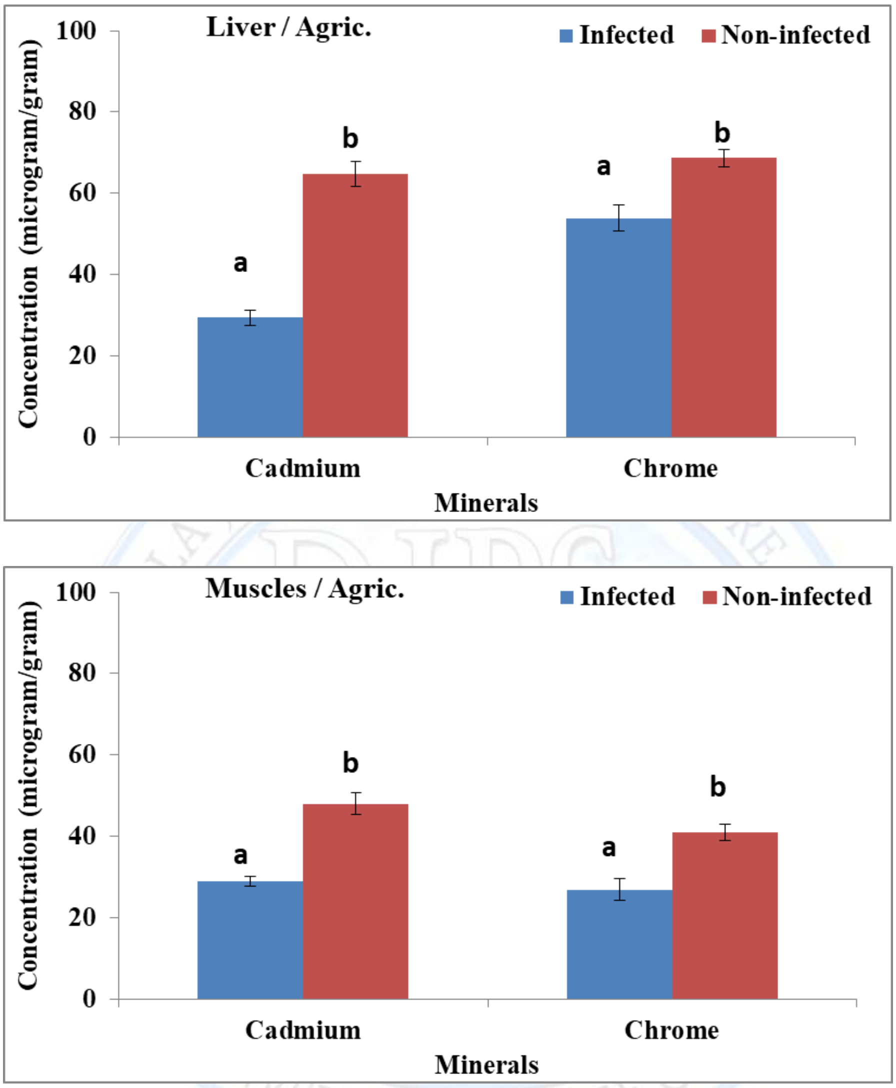

Figure 2. Concentrations (microgram / gram) of cadmium and chrome in the livers (upper panel) and muscles (lower panel) of wild rats (*Rattus norvegicus*) captured at the agricultural areas around Baquba City, Diyala Province.

In the industrial areas, the mean concentrations of cadmium and chrome were significantly higher (P< 0.01) in the intestines and kidneys of non-infected rats than those in infected rats and the chrome in both organs was higher than cadmium in both infected and non-infected rats. The mean concentrations of both elements were higher in the kidney than in the intestines (26.4 mg/g and 52.6mg/g in kidney verses 14.99mg/g and 30.04mg/g) (Figure 3).

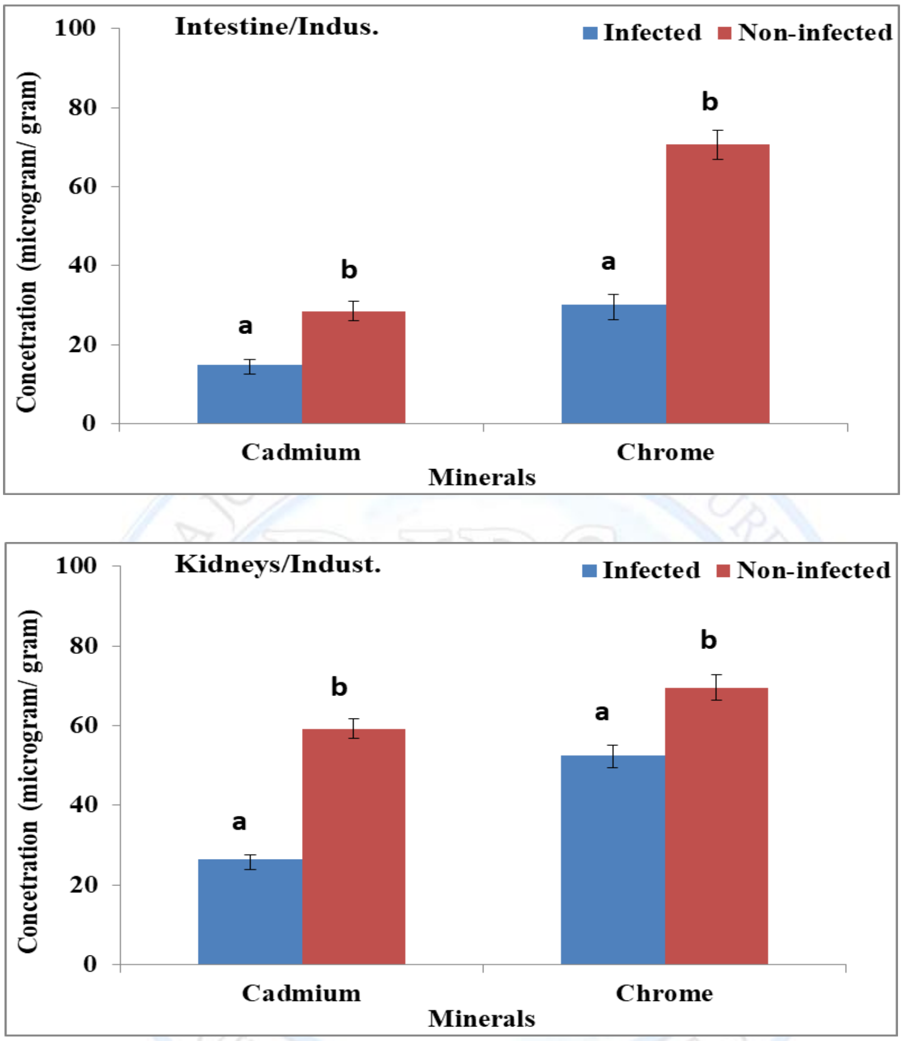

Figure 3: Concentrations (microgram / gram) of Cadmium and Chrome in the intestines (upper panel) and kidneys (lower panel) of wild rats (*Rattus norvegicus*) collected from industrial areas around Baquba City Centre, Diyala Province.

Both cadmium and chrome decreased in the livers and muscles of infected rats compared with non-infected rats and the mean concentrations of chrome was significantly higher in the livers of infected rats than in the kidneys while the cadmium had comparable levels in in the livers and kidneys of infected rats (Figure 4).

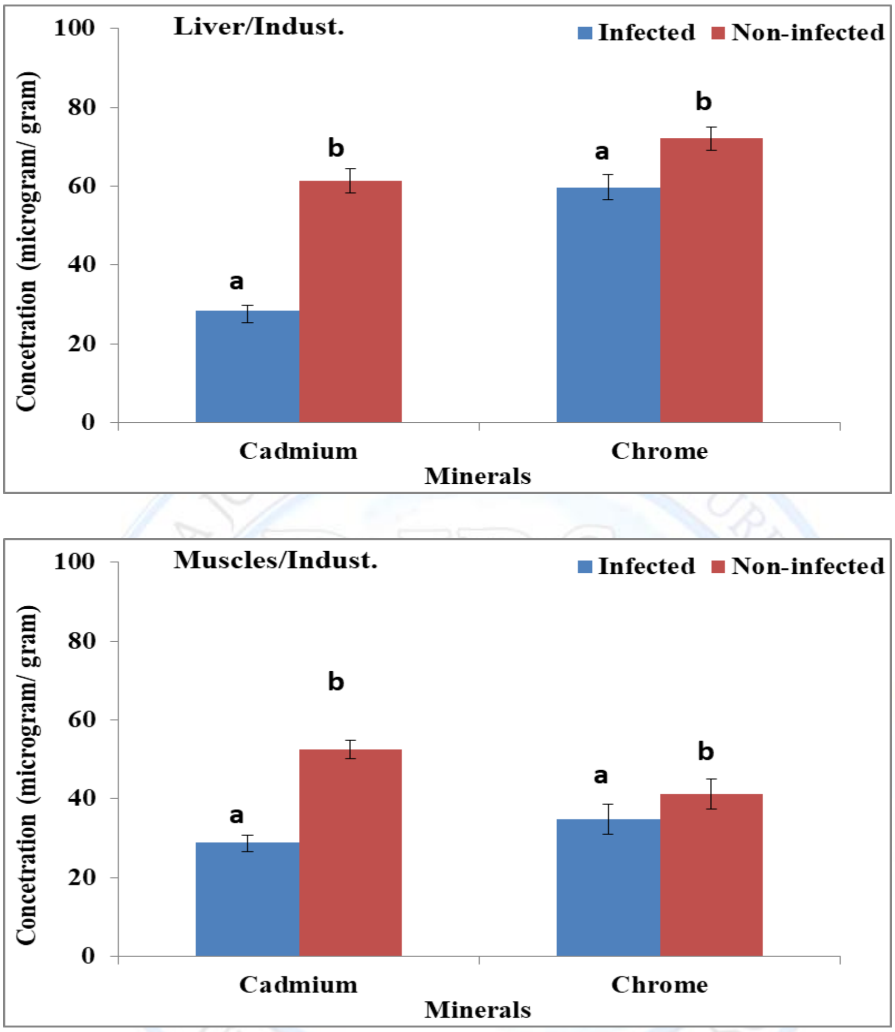

Figure 4. Concentrations (microgram / gram) of cadmium and chrome in the livers (upper panel) and muscles (lower panel) of wild rats (*Rattus norvagicus*) captured at the industrial areas around Baquba City Centre, Diyala Province.

The concentrations of both cadmium and chrome were significantly higher ($P< 0.05$) in the cestodes than in the host tissues. The mean concentration of cadmium in cestodes of infected rats was 40.5 mg/g while the mean concentration of chrome was 76.3 mg/g in cestodes of infected rats in the agricultural area. The concentration of chrome was higher than cadmium in cestodes in both industrial and agricultural areas (Figure 5).

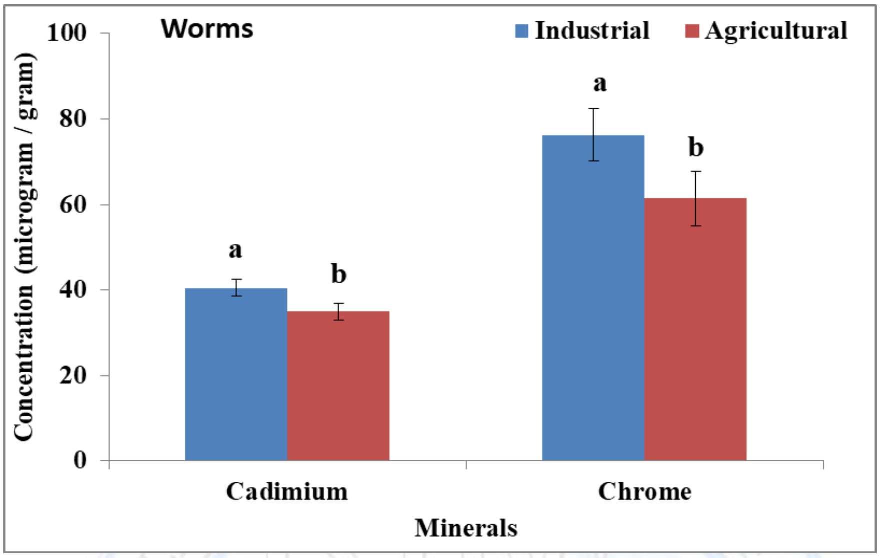

Figure 5. Concentrations (microgram / gram) of cadmium and chrome in the cestodes (*Hymenolipes diminuta*) obtained from infected rats captured at industrial and agricultural areas around Baquba City Centre, Diyala Province.

The bioaccumulation factor for cadmium increased 3.2-fold and 2.1-fold in helminthes than in the intestine and muscle of the rats, respectively in agricultural areas and 2.8-fold and 1.4-fold in helminthes than in the intestine and muscle in industrial areas. For chrome, the bioaccumulation factor increased 3.1-fold and 2.8-fold in helminthes than in the intestines and muscles in agricultural areas while it increased 2.7-fold and 1.4-fold in helminthes than in the intestines and muscles in industrial areas as showed in Table 3.

Table 3. Bioaccumulation factors calculated for the analyzed metals (Cadmium and Chrome) in parasites and rat tissues and organs.

Tissue	Cadmium		Chrome	
	Agricultural	Industrial	Agricultural	Industrial
Intestine	2.6	2.5	2.4	2.7
Kidney	1.1	1.5	1.1	1.5
Liver	1.1	1.3	1.2	1.4
Muscles	2.3	2.2	1.2	1.4

Discussion

Insufficient attention is paid to study the pollution of areas that may be subjected to several types of direct or indirect anthropogenic activities and the role of animals and their parasites to be a factor to decrease or increase this problem. However, some studies proposed that parasites can be a tool for bioremediation of some toxins, including heavy metals from their hosts [5, 7, 11]. Some studies have indicated the possibility of using some parasites in the treatment of some diseases [17,18, 20, 21]. Thus, future studies should focus on using the parasites as a tool for the elimination of toxic agents from humans and the companion animals (parasitotherapy).

Although the results of the present study revealed that there were no significant differences in the percentages of infections of wild rats with *H. diminuta* in industrial and agricultural areas, slightly higher prevalence of infection was recorded in agricultural areas than industrial areas and this agrees with the findings of some previous studies [23, 24]. This may indicate that the rats are exposed to infection in equal chances and as Chaisiri *et al*. [24] mentioned that the diversity and prevalence of parasites were affected by habitat type, with domestic habitats being a high-risk area for helminthes transmission.

By comparing the concentrations of heavy metals in the rat tissues (intestine, kidney, liver and muscles), different concentrations of cadmium and chrome in different tissues (Table 2) in infected and non-infected rats (Figures 1-4) were revealed. The concentrations of both elements were higher in kidneys and livers than in the intestine and muscles in both infected and non-infected rats in industrial and agricultural areas in the present study. The present results may be supported by the findings reported by other authors who revealed that the cadmium and chrome are accumulated in the liver and kidneys more than in other host tissues [14,25, 26, 27]. This may due to the fact that the rats pick food and water from the ground, which may be contaminated with different pollutants including heavy metals [27] and these pollutants absorbed by the intestine and reached to the liver and kidneys by the blood stream. Both livers and kidneys play an important role in the processes of metabolism and detoxification [12].

It has been reported that after clearance from the blood stream, cadmium accumulates in many tissues including the liver and kidneys which concentrate higher levels of this metal, mainly due to their ability to synthesize large quantities of metallothionein, which in turn plays an important role in the homeostatic of essential metals, such as zinc and copper, and the detoxification of non-essential metals, such as cadmium and lead [28].

The results of the present study revealed that the concentrations of cadmium and chrome decreased in non-infected rats in comparison with the infected ones and the concentrations of both elements in *H. diminuta* were higher than the concentrations in all tissues of rats. These findings may indicate the possibility of using *H. diminuta* as indicator for environmental pollution with some heavy metals. This result agreed with those of many previous studies [3, 25, 26, 29] that referred to the importance of helminthes as a systemic control using suitable species or helminthes biomonitors and bioremediation of toxic environmental pollutants.

Few published studies are available on the usage of the mammalian endoparasites in environmental-impact studies. It is known that some parasites (particularly, acantocephalans and cestodes) of the terrestrial hosts accumulate toxic elements exceed the values detected in the tissues of their hosts. Although the mechanism whereby cestode-infected animals accumulate less heavy metals than parasite-free ones is unknown, it may be partially due to their lower metabolic activities [29] or may be due to the fact that the helminthes are lacking digestive tracts (Cestoda, Acanthocephala) and using their teguments for absorbing substance from the intestines of their hosts [30].

The higher bioaccumulation factors in cadmium and chrome- exposed tapeworms observed in the present study are consistent with the results of the previous field studies [31, 32, 33] which showed elevated (2–3-fold) tapeworm bioaccumulation factors in rodents living in polluted areas. This may agree with the hypothesis that cestodes with a relatively large absorbance surface should reach high bioaccumulation factors. It is known that the intestinal cestodes are taking up bile salts produced in the liver through the hepatic intestinal cycle and using them for their egg formation. Sures *et al*. [34] reported that the mechanism which enables cestodes to absorb metals from the intestinal lumen of the host is the presence of bile acids, which form organo-metallic complexes that are easily absorbed by the worms due to their lipophilicity [34, 29]. By comparing between infected and non-infected rats and the bioaccumulation factors in the present study, it is possible to propose the relative ability of cestode *(H. diminuta*) to remediate the cadmium and chrome from their host this and this assumption may be supported by the findings of Hassan *et al.* [7]. In conclusion, this study supports the hypothesis that helminthes can be used as a bioremediation tool via absorbing the heavy metals from their host and also be used as indicators of pollution in the environment where their hosts live.

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