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Research Article

Bioremediation of heavy metals in animal Manure by earthworms

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Abstract

The study aims to treat cow and buffalo dung from the heavy elements cadmium and chromium. The results showed the absence a significant effect of Lumbricus terrestris and Eisenia fetida by worms. Significant differences between the two types of worms in the average accumulation of cadmium in dung, as well as no significant differences in the average accumulation of cadmium in the tissues of the two types of earthworms. As for the average concentration of chromium in dung, there were significant differences between the two types of worms, as the average concentration of chromium was It reached 14.491 ppm for L.terrestris and 16.316 ppm, while for E.fetida The average accumulation of chromium in the tissues of worms was significant effect, as the highest average was in L. terrestris, which reached 17,050 ppm compared to E. fetida, which reached 11,558 ppm.

Keywords: Earthworms, heavy metals, Buffalo manure, cow manure

Introduction

The earth is the first recipient of waste and chemicals that are currently used, and when they are present in the soil, they become part of a cycle that affects all forms of life, such as Manure containing impurities such as cadmium, chromium, and lead, as well as solid waste such as sewage waste, urban garbage, factory waste, and the use of sewage waste. For irrigation. Soil pollution may lead to the loss of many green areas and their transformation into desert areas (Abdul-Jabbar et al., 2017). Pollution is one of the most important problems faced by humans at present due to its relationship to human health and other organisms, as human attempts to reduce pollution are insufficient, and even attempts to make improvements to it are also insufficient (Namik, 2021).

The great interest in heavy metals compared to other toxic substances is due to their easy absorption by living organisms and their ions being highly toxic (Hadid, 2017). In addition to its non-biodegradable nature, it remains bound for very long periods and then easily accumulates in soil organisms and seeps into groundwater and becomes a threat to biodiversity (Ekperusi and Aigbodion, 2015).

Earthworms reduce the toxic effects of heavy metals through the physiological metabolic processes that occur inside them (Suthar et al., 2014). It reduces the rate of its presence during the vermicompost formation process through two methods of cellular adaptation:

The first method: the heavy metal binds to the nucleus of the protein found in the worms, forming nuclear bodies.

As for the second method: it is the cytoplasmic method, which is linked to the manufacture of a special metal to bind to proteins, which is metallothionein, which is a thiol-rich substance that binds heavy metals to proteins and thus has a major role in removing toxins. There are other methods, such as antioxidants and the enzyme cytochrome p450 (Hussain et al., 2021).
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Martial and methods

The experiment began on 4/15/2022 and ended on 7/15/2022. Plastic boxes were used and covered with burlap bags in preparation for spreading them with decomposed Manure. 2 kilograms of cow manure were placed inside them in three replicates, and 2 kilograms of buffalo manure in three replicates were taken from each. Repeat samples to measure heavy metals and the carbon to nitrogen ratio before added the worms.

After that, 50 grams of E. fetida worms were placed in 6 boxes covered with decomposed cow manure, and 50 grams of L. terrestris worms were placed in 6 boxes covered with decomposed buffalo manure. The temperature, humidity, and pH were measured during the experiment period. Other measurements were taken at the end of the experiment.

Figure 1. worms in animal manure

Figure 2. Worm breeding boxes

1- Estimate of heavy metals in the manure before and after feeding

Manure samples were analyzed before the start of the experiment and after the end of the experiment, with 3 samples from each experimental unit. The samples were dried in an oven at a temperature of 48 °C for a whole day, and then they were ground by an electric mill and passed through a sieve with a capacity of 2 mm. The samples were weighed with a sensitive electric balance and collected in envelops Until the process of digestion was carried out. Heavy metals were estimated according to the method of Everson by taking 1 gm of manure samples in 10 ml of hydrochloric acid (Everson ,1975). Then, the samples were placed at low temperatures until boiling for 10 minutes almost dry. Then the solution was filtered through filter papers in Beakers with a capacity of 100 ml, the volume was completed with distilled water free of ions, and the heavy metals chromium and cadmium were measured using an atomic absorption spectrophotometer.

Digestion and analysis of heavy metal in earthworms before and after feeding in an experiment manure

The worms were digested according to the method of Moopam (Moopam ,1982). Samples were taken from the worms, washed with distilled water, dried in an oven, and then ground with an electric mill. Weighed 1 gm of the ground worms in a sensitive electric balance, then digested in 3 ml of a mixture of perchloric acid $HClO_4$ and nitric acid HNO_3 concentrated in a proportion. 1:1 in glass tubes, then the tubes were shaken well and left for 12-16 hours to complete the digestion process. and the tubes were placed in a water bath at a temperature of 70°C for 30 minutes and transferred to a heating plate to complete the digestion process. After that, the filtrate was taken and the volume was completed with distilled water free of ions to 25 ml, then the samples were stored in tightly sealed plastic bottles until measurements were made for cadmium and chromium, using a Flame Atomic Absorption Spectrophotometer.

Results and discussion

The concentration of cadmium in manure

It is noted from table 1 showed no significant differences between the worms and no interaction between them, but there are apparent differences between E.fetida and L.terrestris in the percentage of cadmium accumulation in manure, as the highest average concentration of cadmium in the vermicompost of L.terrestris was 0.605 ppm and less average concentration of cadmium was 0.507 ppm. The removal efficiency of a metal depends on its type and valence in general (Mostafaii et al., 2016) As for the interaction, the results show that there are no significant differences between the accumulation of cadmium and the types of manure and worms, but there are apparent differences, as the highest percentage of accumulation reached 0.790 ppm in cow manure before feeding for the two types of worms, and the lowest percentage of accumulation reached 0.346 ppm in cow manure for the E.fetida worm after feeding.

As for manure, the results showed significant differences, as the highest average concentration of cadmium in cow manure before feeding was 0.790 ppm, and the lowest value for buffalo manure after feeding was 0.433 ppm. (Xio et al., 2021) stated that a lack of cadmium in the soil means a decrease in its toxicity. The decrease in cadmium concentration was higher in cow dung from 0.790 ppm before feeding to 0.536 ppm after feeding compared to buffalo manure. The reason may be that cow dung is ideal for worms in terms of the mixture of protein and carbohydrates (Ghimire, 2008).

Tabel 1. Cadmium concentration in manure ppm

Mean	Average manure after feeding		Average manure before feeding		Manure / Worms
	Buffalo	Cow	Buffalo	cow	
0.507	0.426	0.346	0.466	0.790	*E.fetida*
0.605	0.440	0.726	0.466	0.790	*L.terrestris*
	[b] 0.433	[b] 0.536	[b] 0.466	[a]0.790	Mean

Interference: n.a Worms: n.a

*Different letters indicate a significant difference at p≤ 0.05 according to Duncan's multiple range tests.

Measuring the concentration of cadmium in worms

The results of Table 2 showed that there are no significant differences between E.fetida and L.terrestris, but there are apparent differences, as the highest average concentration of cadmium in L.terrestris reached 1.750 ppm compared to the average concentration of cadmium in E.fetida of 1.443 ppm , When comparing the averages of manure before feeding and after feeding, the manure of cows and buffaloes after feeding recorded the highest concentration, reaching 1,840 and 1,716 ppm, respectively. These results are consistent with (Zaltauskaite and Sodiene, 2010), who found a clear relationship between the ability of worms to absorb cadmium. In the environment in which they are located, and since the worms feed on manure, we notice the absorption of cadmium by these worms. Takacs et al., 2016 found that cadmium accumulated in the tissues of worms and changed the physical and chemical state of the soil and the movement of heavy metals during composting. When L. terrestris absorbs cadmium, it stimulates metallothionein, which protects from heavy metal toxicity and oxidative stress (Calisi et al., 2014). Detoxification of heavy metals may be due to the binding of the heavy metal to proteins (Akbar et al., 2011). The results are also consistent with Laszezyca et al., 2004, who found higher cadmium accumulation in L. terrestris compared to *E. fetida*. Referring to the previous Table 1, we notice that the average concentration of cadmium in the manure after feeding has decreased compared to the average concentration of cadmium in the manure before feeding, as the highest average concentration of cadmium in the worms reached 1,840 ppm, compared to the lowest concentration of the element, which reached 1,390 ppm before nutrition. All treatments increased the accumulation of cadmium in the tissues of the worms, as cadmium is one of the elements that accumulates most in the tissues of earthworms, and at the same time its toxicity is removed in a very effective way, as it is deposited in the chlorogenic cells of earthworms, resulting in high concentrations in the tissues It is not fatal (Richardson et al., 2020).

Table 2. Cadmium concentrations in worms. ppm

mean	Average manure after feeding		Average manure before feeding		Manure / worms
	Buffalo	cow	Buffalo	cow	
1.443	1.966	1.480	1.080	1.246	E.fetida
1.750	1.466	2.200	1.700	1.633	L.terrestris
	1.716	1.840	1.390	1.440	mean

Interference: n.a Worms: n.a Manure: n.a

*Different letters indicate a significant difference at p≤ 0.05 according to Duncan's multiple range tests.

Measuring chromium concentrations in manure

The results of Table 3 showed that the highest average concentration of chromium was recorded in manure that E.fetida grown on it, 16.316 ppm, compared to manure that L.terrestris grown on it, which reached 14.49 ppm. We note from Table 4 that the accumulation of the element chromium in the tissues of L. terrestris reached an average concentration of 17,050 ppm. In contrast, the element decreased in the manure environment, as in Table 3, where the average concentration of chromium reached 14,491 ppm, *L. terrestris* can accumulate heavy metals in its tissues, and this accumulation depends on the concentration of metals in the soil, and thus heavy metals are reduced in the resulting vermicompost (Broma et al., 2009).

As for manure, the results showed significant differences, as the highest average concentration in cow manure before feeding reached 20.666 ppm, and the lowest percentage of chromium accumulation in buffalo manure before feeding reached an average concentration of 9.0333 ppm. We note that the average concentration of the chromium element decreased after feeding compared to before feeding, as it reached 20.666 ppm before feeding and reached 18.433 ppm after feeding. The results are consistent with (Bhartiya and Singh, 2020), as the chromium element decreased significantly in the final vermicompost of cow manure compared to At the beginning of the experiment.

As for the interaction, the table shows that there are significant differences in the accumulation of chromium in E.fetid and L.terrestris manure, as the highest percentage of accumulation of chromium in cow manure before feeding for both types of worms at a rate of 20.66 ppm, and the lowest percentage of accumulation was for buffalo manure. The amount grown in *L. terrestris* reached 8.766 ppm after feeding.

Table 3. Concentrations of chromium in manure, ppm

Mean	Average manure after feeding		Average manure before feeding		Manure / Worms
	Buffalo	cows	Buffalo	cow	
a 16.316	bc 18.200	c 17.266	b 9.1333	a 20.666	E.fetida
b 14.491	d 8.766	ab 19.600	b 8.933	a 20.666	L.terrestris
	c 13.483	b 18.433	b 9.0333	a 20.666	Mean

Different letters indicate a significant difference at p≤ 0.05 according to Duncan's multiple range tests.

Measuring chromium concentrations in worms

The results of Table 4 recorded the highest average concentration of chromium in L. terrestris which reached 17,050 ppm, compared to the average concentration of chromium in *E. fetida*, which reached 11,558 ppm. The accumulation of chromium in worms is higher than in their polluted environment, which may be due to the content of organic matter. Microorganisms in the worm's environment play a vital role in the bioavailability of chromium and are of great importance in the case of earthworms (Tozser et al., 2022). As shown in Table 3, the average chromium element in the environment of L. terrestris reached 14,491 ppm, compared to the accumulation of the element in its tissues, which reached 17,050 ppm. *E. fetida* is characterized by low concentrations of chromium within its tissues, and multiple environmental contamination and metal interactions may be potential limiting factors for metal accumulation in earthworm tissues (Dicarlo et al., 2020).

As for manure, the results show significant differences, as the highest average absorption of chromium in buffalo manure after feeding was 19.633 ppm, and the lowest average recorded in buffalo manure was 9.466 ppm before feeding. The results are consistent with Asman et al (2023) as they found an increase in the accumulation of chromium in the tissues of *E. fetida* compared to the beginning of the experiment. In contrast, the concentration of the element decreased in the worms' environment. The vermicomposting process works to reduce the content of heavy elements as a result of their accumulation by earthworms (Gong et al., 2018). We also notice in Table 3 that the concentration of chromium in manure was lower before feeding compared to after feeding, as the average concentration of chromium before feeding was 20.666 and 9.0333 ppm for cow and buffalo manure, respectively, and after feeding it was 18.433 and 13.483 ppm for cow and buffalo manures, respectively. The digestive system of worms can separate heavy metal ions from complex aggregates between them and humic substances, with the help of enzymes that lead to the assimilation of those ions in the worms' tissues, thus remaining in their bodies instead of returning them to the compost again (Zigmonitene and Liberyte, 2014). This result shows the ability of worms to accumulate chromium in their tissues, which indicates that vermicomposting is an effective way to reduce metal pollution in the soil.

As for the interaction, the results show that the highest average concentration of chromium was in buffalo manure after feeding for L. terrestris, amounting to 23.266 ppm, and the lowest concentration in buffalo manure before feeding for L. terrestris amounted to 8.933 ppm. We note that there is a difference between the average concentration of chromium before feeding and after feeding in the tissues of *E. fetida*, as the average concentration of chromium in cow manure before feeding reached 9,100 ppm compared to the average concentration of chromium after feeding cow manure, which reached 11,133 ppm. The average chromium element in buffalo manure before feeding was 10,000 ppm, compared to the average chromium element after feeding, which reached 16,000 ppm. The results obtained are consistent with the results of (Bhartiya and Singh, 2022), as the accumulation rate of chromium increased for *E. fetida* worms in Cow manure is 9.59% and buffalo manure is 9.08%. It is consistent with the current study in terms of the principle of chromium accumulation in the tissues of the red worm.

Table 4. Concentrations of chromium in worms, ppm

mean	Average manure after feeding		Average manure before feeding		Manure / worms
	Buffalo	**cow**	**Buffalo**	**cow**	
b11.558	b 16.000	c 11.133	c 10.000	C 9.100	E.fetida
a 17.050	a 23.266	a 22.666	c 8.933	bc 13.333	L.terrestris
	a19.633	a 16.900	b 9.466	b 11.216	Mean

*Different letters indicate a significant difference at p≤ 0.05 according to Duncan's multiple range tests .

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