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Removal of Heavy Metals from Industrial Wastewater Treatment Sludge Using Earthworms (*Eisenia Fetida*)

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ABSTRACT

Industrial wastewater sludge is a solid or semi-solid material which contains different toxic substances depending on the type of industry. Different industries need to use of different treatment and disposal methods for their waste and wastewater. Industrial wastewater sludge may be in a high amount and volume, and processing and removal of these sludge are quite complex and difficult problems. In solution of this problem, earthworms belonging to soil macrofauna group greatly contribute to recycling of organic matter. Also, one of the soil treatment or improvement methods is the vermicomposting method using earthworms in the soil polluted with contaminants based on heavy metal.

In this study, different combinations of iron-steel plant wastewater treatment sludge and cow dung were prepared and left for vermicomposting with the earthworm, *EiseniaFetida* (Savigny). Then, the amount of heavy metal received by earthworms in their body structures, the changes of taken metal depending on the time and the amount of iron- steel waste water treatment sludge and the effect of the sludge on the earthworms were evaluated. Experiments were established as 3 replications and after 30th, 90th and 120th days of vermicomposting process, metal analyses were carried out by weighing the live weights of earthworms. As a result of the analysis, it was determined that there was an increase in the receiving of some heavy metals (Cu, Pb, Ni, Zn and Cr) in the body of earthworms depending on the time and the amount of wastewater treatment sludge.

Keywords: Iron-steel plants; Wastewater treatment sludge; *Eisenia Fetida;* Vermicompost; Heavy metal

Introduction

Industrial activities have increased to meet the need for rapid and irregular urbanization and increasing population in parallel with the rapid population growth in the world. Iron and Steel Industry is one of the important industries in Turkey and it is developing continuously. The reason of this is the rapidly growing market for iron and steel products within Turkey and in addition to the domestic market, many of the neighbouring countries that have no iron and steel production can be considered as a potential future market. The wastes are also increasing in parallel to these industrial activities.

Emissions arising from urban and industrial sources and solid and liquid wastes cause an environmental pollution by releasing directly to nature without pre-treatment. Particularly, the industrial wastewater treatment sludge, whose composition changes according to the production activities, poses a significant problem in quantity and volume. According to the characteristics of wastewater, wastewater sludge which contains toxic, pathological, eco-toxic and carcinogenic harmful substances emerges. This restricts the reuse of wastewater treatment sludge and when it is not addressed with an environmental management, it has negative effects on human health and environmental by participating in natural substance cycles. Toxic heavy metal pollution of water and soil is a major environmental problem and most conventional remediation methods do not provide acceptable solutions [1].

It takes a long time to remediate polluted areas and requires high costs for this rehabilitation. Thus, the contaminative effects of the wastewater treatment sludge should be addressed before the transfer to the receiving environments and the waste should be managed. As a result of the processes of the glass, mine, cement, metal coating, metal production, petrol processing, petro-chemistry and hydrocarbon production sectors and facilities producing vehicles and making some recycling (biodiesel) applications, the treatment sludge, which has rich heavy metal content, is formed as a result of refinement of the wastewater.

Many physical, chemical, and biological techniques are applied for the rehabilitation of areas with soil pollution based on heavy metal [2]. Dermont et al., (2008) also reported that technologies available today for the refining of soils contaminated with heavy metal can be examined in four groups (physical, chemical, biological, and thermal methods) [3]. The uses of these methods also enable to reduce the damage of the treatment sludge with heavy metal content. One of the soil treatment or improvement methods is the refining of the soil contaminated with heavy metal-based contaminants with vermicomposting method by using soil earthworms [4,5].

Earthworms are an important indicator for ecosystem health and numerous studies have been conducted on the response of earthworms to metals [6]. Earthworms and a number of microorganisms work together throughout the vermicompost process to stabilize organic material and does not include the thermophilic stage [7,8] Earthworms which are fed in environments containing heavy metal, can be

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Retransferred to the soil with the way of defecation; whereas, some of the heavy metals are stored in the earthworm tissue [9]. Some studies have revealed that earthworms store heavy metal in the soil in which it interacts with and this reduces total metal concentration in the soil [10-13]. Thus, earthworms can reduce the rate of metals participating in the food chain with the effect of their abilities for heavy metal accumulation during the nutrition event [10,11].

Metal content in vermicomposts can be affected by factors such as physico-chemical features of wastewater, concentration of metals in wastewater, types of earthworms used for vermicomposting, and environmental conditions etc., [14]. Vermicomposting process is affected from two processes. The first process is a decrease in the mass and volume of wastewater due to the resolution and mineralization of organic materials, and the second process is the accumulation of heavy metals in the body of earthworms during the vermicompost process.

In Turkey, industrial wastewater treatment sludge consists of 779 tons of 39 640 tons waste from total hazardous wastes sent to incineration facilities in 2010 in line with the municipal waste statistics created by Turk stat. According to the Turkish Statistical Institute's 2018 data, 22.9 million tons of waste was generated in manufacturing industry establishments, 3.7 million of which were hazardous. It was determined that 71.2%, 80.4% and 83.2% of the discharged wastewater constitute 83.2% of the sectors producing basic metal and chemical products [15].

Recently there is a considerable interest in developing cost effective and environmentally friendly technologies for the remediation of soil and wastewater polluted with toxic trace elements [16].

The aim of this study was to investigate the removal of heavy metals in the wastewater treatment sludge of integrated iron-steel factories by using earthworms, *Eisenia Fetida* and cow dung, sawdusft, and waste of tea leaf. This is the first study conducted to examine the removal of metal from the integrated iron and steel plant sludge using earthworms in Turkey.

Materials and Method

Materials

In this study, iron and steel industry wastewater treatment sludge rich in metal content was obtained from an iron and steel plant located in Karabuk, Turkey. The samples were collected from a decanter unit outlet of a central wastewater treatment plant and brought to the laboratory in a metal box. Earthworms (*Eisenia Fetida*) were obftafin ffrom a company offerfing vermficomposftfing servfices fin a of Samsun, Turkey. The earthworms were brought in a plastic box together with a culture medium and earthworm food. The box was stored in a dark environment at 20ºC-25ºC in the laboratory. Cow dung came from a livestock farm near Karabuk. Sawdust was supplied by a wooden furniture enterprise. Tea pulp was obtained from a cafeteria. Sawdust and waste tea pulp were used as auxiliary materials.

Method

The experiments were conducted by modifying the methods applied by Azizi et al., (2013) and Suthar et al., (2014) and by using the iron and steel industry wastewater treatment sludge (IWS), *Eisenia Fetida* sawdust (SD), cow dung (CD) and waste tea pulp (WTP) as heavy metal removal materials [17,18].

Sterilization was carried out by drying the materials out at 100ºC for 6 hours in order to bring minimum amount of sawdust, waste tea pulp, and cow dung molding and pathogenic elements into the laboratory. Then, a 2-mm long piece of cow dung was ground. Vermicomposting took place in 5-litre rectangular culture containers (11.5 cm x 16.5 cm x 25 cm). The test boxes' covers were drilled in order to ensure air flow. Earthworm cultivation medium was prepared by mixing experimental materials at different rates. The bedding mixtures across all scenarios were moistened with distilled water to maintain the moisture content. The experiment was prepared in 3 replications. Each test box was numbered. The mixing rates of the materials were provided below (by weight):

T1. IWS (100%)

T2. IWS: CD(1:3)

T3. IWS: CD(1:2)

T4. IWS: CD(1:1)

T5. IWS: (SD+WTP) (3:1)

T6. IWS: CD(3:1)

T7. IWS: CD(2:1)

T8. Earthworm Control

The bedding mixtures in all scenarios were moistened with distilled water to maintain the moisture content within their original range. A plastic mesh blanket placed atop each container to prevent sunlight from entering and to prevent the earthworms from escaping [19]. The same series of each scenario, without earthworms, was achieved using the same procedure and designated as the control.

200 g of waste tea post and 50 g of sawdust were added to the test boxes. The materials in the box were homogenized by adding 200 ml of purified water, and then kept under laboratory conditions for 24 hours. Then, 35 earthworms were placed into each test box. The total weight of the earthworms was between 5.53 and 7.624 g. Before they were placed into the boxes, they were taken out of the culture box and cleaned of soil with a soft tip brush, and then sandwiched between damp clothes for 24 hours. The containers were then incubated in a dark environment for 30, 90, and 120 days at 20°C-25°C. The containers were re-humidified by adding distilled water. After incubation, the samples were placed in sealed plastic bags and stored at 4ºC for further analysis. Heavy metal analysis in the vermicompost was carried out on Days 1 and 30. The heavy metal in earthworms was analyzed on Days 0, 30, 90, and 120. All experiments were carried out at laboratories at Karabuk University.

Harvesting earthworms and determining wet and dry weight

At the end of Days 30, 90, and 120, the test boxes were emptied and the earthworms were separated by hand harvesting. The earthworms were then removed from the surface with a soft tip brush and weighed on a precision scale. The material in the experimental box was spread and mixed, whilst the earthworms were sent for analysis. Approximately 50 g of material was taken from the bulk material using the "9 Circle Technique" and placed in a transparent storage container. The earthworms were left on the surface of the storage container to disperse themselves into the container in accord with their own instincts. The numbered and perforated storage containers were packed and sent to the relevant accredited analysis laboratory.

The soil residues on the outer surface of the 35 live earthworms (extracted from the culture medium) were cleaned off and their feces and digestive systems were evacuated in a humid environment at 28°C. They then were weighed on a precision scale to determine how much weight they lost under heat and processed in a drying oven at 105°C for 24 hours.

Statistical analysis

The statistical analysis was carried out on SPSS 16.0 (StandardVersion). One-way analysis of variance (ANOVA) was performed to analyze significant differences between the treatments during vermicomposting at the significance level of 0.05.

Result and Discussion

This study was conducted to remove heavy metals in iron and steel industry wastewater treatment sludge using earthworms. It also examined the earthworms' growth and live weight, how their offspring and cocoons formed, and what impact they had on the compost materials.

Tables 1 and 2 show how much heavy metal the earthworms ingested after Days 30, 90, and 120, in the mixture that were prepared at the ratios of 1: 1 (T4) and 3:1(T6).

According to the analysis data, the amounts of Cadmium (Cd) contained in the earthworm bodies were <0.24 mg/kg in control group. No change was observed at the end of Days 30, 90, and 120.

Liu et al., (2005) found that after incubating sewer sludge for 60 days, the amount of Cd in the earthworm tissues increased up to 10 mg/kg [20]. The metal analysis of the wastewater treatment sludge in the present study revealed that the amount of Cd was 0.0014 mg/ kg. Thus, the Cd concentration in the box of treatment sludge mixed with different supportive materials at different ratios decreased. This decreased the amount of Cd consumed. This was likely caused by the fact that amount of Cd in the earthworm had risen to 10 mg/kg. No Cd increase was found in the earthworm. This was likely associated with the amount of Cd in the material (sludge) (0.0014 mg/kg) in the present study.

Table 1 reveals that the Cu amounts increased by 2.50, 2.34 and 2.90 times, respectively compared to control group at the end of Days 30, 90, and 120. Pb amounts increased by 5.25, 6.19, and 6.32 times, respectively, compared to control group at the end of Days 30, 90, and 120. Ni amounts increased by 57.53, 63.89, and 61.27 times respectively, compared to control group at the end of Days 30, 90, and 120. Zn amounts increased by 44.82, 45.68, and 61.72 times, respectively, compared to control group at the end of Days 30, 90 and 120. On the other hand, Hg amounts did not change compared to the control group at the end of Day 30. It however increased by 24 and 14 times, respectively, at the end of Days 90 and 120. Cr amounts increased by 10.64, 13.32 and 14.41 times, respectively, compared to control group at the end of Days 30, 90, and 120.

Table 1: Heavy metal amounts (mg/kg) in the experiment whereby Iron-Steel Plant treatment sludge and fertilizer were mixed at the ratio of 1:1 (T4).

Parameters	**Control**	**T4**		
		Day 30	**Day 90**	**Day 120**
	Results (mg/kg)			
Cadmium (Cd)	<0.24	<0.24	<0.24	<0.24
Cupper (Cu)	36.4	90.8	85.2	105.4
Lead (Pb)	6.1	32	37.7	38.5
Nickel (Ni)	4.2	241.6	268.3	257.3
Zinc (Zn)	266.2	11 930	12 158	16 430
Mercury (Hg)	<0.1	<0.1	2.4	1.4
Chromium (Cr)	6.1	64.9	81.2	87.9

For the heavy metals (except for cadmium), an increase in the amount of heavy metal that the earthworms absorbed, at the end of Days 30, 90 and 120 was observed when compared with the control group. The metal that the earthworms absorbed most was determined to be zinc, which increased in quantity over time. Investigated removing lead, nickel, and aluminum from industrial treatment sludge using earthworm-based composting method [21]. Upon mixing sewage sludge with sheep manure at different ratios, they determined lead, nickel, and aluminum were removed by 97%, 86%, and 72%, respectively over the 56-day study period.

Aleagha, et al. Conducted a study on bioaccumulation of heavy metals by means of vermicomposting [22]. They added *Eisenia Fetida* into a mixture containing different concentrations of metal compounds. Their results revealed that *E Fetida* accumuflafted Cd, Zn, Ni, Pb, and Cr elements within a 14-day period and they identified a strong correlation between the bioaccumulations of Cr, Pb, and Ni, Cd. Zhu et al., (2014) stated that metal content in vermicomposts, the species of earthworms used for physico-chemical properties of untreated waste, the concentration of metals in untreated waste, and vermicomposting all can be affected by factors such as environmental conditions [14]. Two processes affect the treatment during vermicomposting process. The first process is to decrease the mass and volume of untreated waste due to the resolution and mineralization of organic materials. The second process is to accumulate heavy metals in the earthworms during the vermicomposting process.

According to Table 2 for (T6), amounts of Cu increased by 3.40, 2.79, and 3.41 times, respectively, when compared with control group at the end of Days 30, 90, and 120. Pb amounts increased by 8.07, 6.64, and 7.33 times, respectively, when compared with control group at the end of Days 30, 90 and 120. Ni amounts increased by 81.60, 77.70 and 86 times, respectively, when compared with control group at the end of Days 30, 90 and 120. Zn amounts increased by 79.34, 59.53, and 71.89

times, respectively, when compared with control group at the end of Days 30, 90 and 120. Mercury (Hg) amounts did not change after Day 30 when compared with the control group. However, they increased 23 and 19 times, respectively, when compared with control group at the end of Days 90 and 120. Chromium (Cr) amounts increased by 15.37, 17.19, and 18.19 times respectively, when compared with control group at the end of Days 30, 90 and 120.

Table 2: Heavy metal amounts (mg / kg) obtained from the *E Fetida* analysis in the experiment whereby sludge and manure of Iron-Steel Plants were mixed together at the ratio of 3:1(T6).

Parameters	**Control**	**T6**		
		Day 30	**Day 90**	**Day 120**
	Results (mg/kg)			
Cadmium (Cd)	<0.24	<0.24	<0.24	<0.24
Cupper (Cu)	36.4	123.7	101.4	123.9
Lead (Pb)	6.1	49.2	40.5	44.7
Nickel (Ni)	4.2	342.7	326.3	361.2
Zinc (Zn)	266.2	21 115	15 845	19 135
Mercury (Hg)	<0.1	<0.1	2.3	1.9
Chromium (Cr)	6.1	93.7	104.8	110.9

In general, upon comparing Days 30 and 90 for treatments T4 and T6, it was observed that the amount of zinc in the earthworms' bodies decreased after Day 90 compared to the 30th day. This situation seems to be due to the low organic content depending on sector for which the wastewater treatment sludge is provided, as well as due to the low organic content, the earthworm activity and the amount of zinc taken into the body reduced in parallel with the organic material consumed. Also, zinc, as an essential element, might have been used for metabolic activity of earthworms due to the limited amount of nutrition. According to the analysis done on Day 120—following the addition of nutrients on Day 90—the amount of zinc in earthworms' body increased. Hepşen Türkay (2010) Found in their 90-day study that the zinc in the treatment sludge increased by retaining the tissues of the earthworm used in the experiment and the amount of zinc in the earthworm compost decreased [23]. In the study, Suthar (2008) indicated that metal levels in earthworm tissues were the highest in the combination of the beverage industry treatment sludge (100%)+cow dung (0%) and the lowest in the combination of the beverage industry treatment sludge (20%)+cow dung (80%) [24]. The present study revealed that that metal concentrations in earthworms in T6 (3: 1) dose was more than T4 (1: 1) dose. In their study, Lukkari et al., (2006) determined that by binding metals to organic material (more tightly bound fractions), one could partially reduce how usable the metals were for earthworms [25]. They also noted that the guts of earthworm can affect the mobility of metals due to pH changes and can support their assimilation.

Some studies on removal of metals from treatment sludge using *E Fetida* support the results of the present study. In their study, Liu et al. (2012), who looked at the effects of earthworm (*E Fetida*) activity on the removal of heavy metals from sludge, found that after 120 days of vermicomposting, 5 heavy metals (Cu, Ni, Cd, Pb and Zn) accumulated in the earthworms' bodies, and thereby reduced heavy metal quantities in the vermicompost [26]. They indicated that vermicomposting was an effective means of removing heavy metals. Similarly, Ruiz et al., (2009) conducted a pot experiment on metal (Pb, Zn, Cd and Cu) intake from soil obtained from a mine quarry and that was contaminated by heavy metals [27]. Using maize and barley plants, they found that earthworms had a significant effect on metal content in the soil. They found that metal accumulation rates were significantly higher in corn and barley plants that were accompanied by *E Fetida* -ftype earftThworms.

An examination of the amount of heavy metals accumulated in the earthworms' bodies in boxes T4 and T6 on Days 30, 90, and 120 after vermicomposting revealed that the amounts of metal in the earthworms in T6 box were high. When the amount of metals bound to organic components in the contaminated soil increased, the accumulation of the metal in the body of the interacting lumbricus also increased [28,29]. In their study, Davies et al., (2003) analysed the how toxic lead was on lumbricus [30]. They observed that lead intake to the living body increased linearly over time. The most sensitive parameter showing lead toxicity in the mineral is cocoon production. While the earthworms were found to excrete some of the heavy metals into the environment, they were taken through nutrition, to the receiving environment via defecation and some of them were accumulated in the body tissues [9,10]. Earthworm tissues can contain heavy metals in high concentrations and less metal in their fertilizers [28]. This can depend on factors such as heavy metal concentration in the interacted soil, the physical structure of the soil, and organic content [10]. Paul et al., (2019) also stated that bioaccumulation in the tissues and intestines of the epige lumbricus provided heavy metal immobilization [31].

The present study found that zinc was the most intake component into the earthworm bodies among the heavy metals and there was an increase in the amount of zinc compared to the control depending on the time and feeding. One of the factors that affect heavy metal accumulation in earthworms is the ecological categories [32,6,8] Endogeic earthworms accumulates cadmium in their bodies at higher rates than epigeic and anecic earthworm species. Anecic earthworms, likewise, can accumulate zinc at higher rates than two earthworm species from other ecological categories. The lumbricus of the L. rubellus, for instance, has a higher tolerance to copper-brazed pollution compared to the A. caliginosa. *E. fetida* earthworms show the least toxicity in lead [33].

Amounts of heavy metal in the soil 30 days after vermicomposting

Table 3 shows the results of the analysis made on Day 30 for vermicompost that was prepared by mixing Iron-Steel industry's wastewater treatment sludge with fertilizer at different ratios. Based on Table 3, the pH levels of the soil samples varied between 7.64 and 8.45. There was no significant change in the amounts of cadmium and chrome.

Table 3: Heavy metal amounts in soil analysis 30 days after vermicomposting.

Parameter			T1.Control (Wastewater Treatment Sludge)	T2.Sludge: Cow Dung (1:3)	T3.Sludge: Cow Dung (1:2)	T4.Sludge: Cow Dung (1:1)	T6. Sludge : Cow Dung (3:1)	T7. Sludge : Cow Dung (2:1)
	Unit	Uncertainty of Measurement	Analysis Results					
pH	-	% ± 0,07	7.64	8.45	8.4	8.02	7.82	7.89
Iron(Fe)	mg/lt	% ± 12.9	2.514	25.4	67.6	4.63	0.07	1.05
Cadmium (Cd)	mg/lt	% ± 12.7	0.0014	0.001	0.002	<0.0005	<0.0005	<0.0005
chrome (Cr)	mg/lt	% ± 13.6	<0.001	0.021	0.049	0.003	<0.001	<0.001
Cupper (Cu)	mg/lt	% ± 12.8	0.005	0.059	0.076	0.013	0.005	0.005
Lead (Pb)	mg/lt	% ± 12.6	<0.0005	0.085	0.242	0.017	<0.0005	0.003
Zinc (Zn)	mg/lt	% ± 12.6	0.09	4.83	10.7	1.54	0.207	0.595
TKN	%	% ± 13.4	15.9	16	12.4	12.5	10.2	9.76

Compared to the control group, the values varied between 0.001 ± 12.7-<0.0005 ± 12.7 and <0.001 ± 13.6–0.049 ± 13.6, respectively. For the other metals, some decreased, while others increased depending on the composting mixture ratios.

Iron amounts varied between 2.51 ± 12.9 – 67.6 ± 12.9 and the highest value was 67.6 mg/l in test group T3. Cu amount varied between 0.005 ± 12.8 and 0.076 ± 12.8 and the highest value was 0.076 ± 12.8 mg/l in T3 test. Similarly, lead and zinc amounts were also the highest in T3 test and 0.242 ± 12.6 and 10.7 ± 12.6 mg/lt, respectively. TKN amounts varied between 19.9 ± 13.4 and 9.76 ± 13.4.

The present study's findings are similar to numerous studies conducted with other types of industrial wastewater treatment sludge [34]. Found that Fe content in vermicompost was 27.3%-31% which was higher than the initial mixing, and the change rate of the iron was relatively higher in the first stage of vermicomposting. They also found that the content of Cu in their vermicomposts had decreased by 1.7%-40.8%. Cr content was higher at the rate of 28-80%. A maximum increase was detected in Cr content in vermicompost made using 100% cow dung. During vermicomposting, Zn content showed a variable increase; whereas, Zn content increased between 7.5%-32.8% in the vermicomposts compared to their initial values. Wu et al., (2018) investigated the removal and phytotoxicity of heavy metals during the vermicomposting of treatment sludge by adding different plant wastes [35]. They found that heavy metals in compost material were removed at significant rates through vermicomposting. According to Morgan and Morgan (1992), soil pH is one of the parameters affecting heavy metal accumulation in earthworms [32]. There is a close correlation between the solubility of heavy metals and soil pH. As metal accumulation in earthworm's increases, their pH level decrease [36]. Paul et al., (2019) concluded that amounts of Cu, Zn, Mn, Fe, Pb, Ni, Cd and Cr increased after vermicomposting and their concentration increased as organic material decreased because weight and volume of the mixture reduced [31]. Gupta and Garg (2008) reported similar results by noting that bioaccumulation in the tissues and intestines of the epige lumbricus yielded heavy metal immobilization [37].

The effect of vermicomposting on the growth and live weight of *E. fetida*

The earthworms including their offspring in the containers were counted and weighed at the end of the experiment period. Figure 1 shows total live weight of earthworms on Days 30, 90, and 120 of the experiment period. The 30-day growth of earthworms was examined through vermicompost applications. In the control application, total weights of the earthworms in boxes T8, T2, T3, T4, T6, and T7 were 20.44, 19.12, 18.28, 17.93, 17.76, and 17.63 g respectively. Although the sludge rates differed across each application, the number of earthworms in each box remained constant. However, the earthworms in the combinations of boxes T1 and T5 were unable to survive. Figure 1 shows that the live weight of earthworms was T2>T3>T4>T6>T7 based on the other doses of the control application.

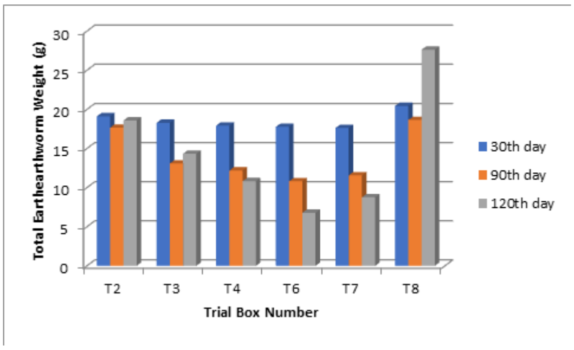

Figure 1: Live weight of the earthworms (*E. fetida*) in trial boxes at the end of days 30, 90 and 120.

The application with closest physical properties to the earthworms in the control application was the box T2 and sex development of the earthworms is close to the control in the tests with low treatment sludge dose (boxes T2 and T3). It was found that earthworms in these doses responded to physical reactions rapidly and they tended to displace themselves. Boxes T6 and T7, which contained high treatment sludge doses, as well as earthworms in half-treatment sludge-fertilizer (T4) darkened and no sex development was observed. The earthworms responded to physical reactions and tended to displace themselves at these doses.

The inspection of the 90-day growth of earthworms with vermicompost applications revealed that their total live weights of the earthworms in boxes T8, T2, T3, T4, T6, and T7 were 18.65, 17.66, 13.08, 12.19, 10.09, and 11.57 g/earthworm, respectively.

The total number of earthworms was 93 in control application box T8, 52 in box T2, 49 in box T3, and 35 in boxes T4, T6, and T7. The results revealed that their live weight was T2>T3>T4>T7>T6 in control application when comparing with other dose applications. The dose that had the closest physical properties to the earthworms in the control application was the box T2. The clitellum structures were observed in the earthworms in boxes T8, T2 and T3. The earthworms in boxes T4, T6, and T7 did not form clitellum structures or were weak. At the end of the application, the earthworms in boxes T8, T2 and T3 responded slowly to physical reactions and showed little tendency to displace themselves. The earthworms in boxes T4, T6 and T7 doses exhibited almost no response to physical reactions and they had difficulties in displacing themselves. The color of the earthworms in these boxes was still dark. In the light of this, 250 g of waste tea pulp was added into each trial box at the end of Day 90 due to the low amount of organic matter in main material (IWS).

Malińska et al., (2014) Indicated that heavy metals inhibited the activity, growth, and reproduction of the earthworms [38]. The vermicompost transform rate should be increased by corroborating it with bulking agent materials (e.g. fertilizer, sawdust powder.) in order to boost vital characteristics of earthworms [31].

Examination of the 120-day growth of earthworms in the vermicompost applications revealed that their total live weights were 27.64 g, 18.58 g, 14.32 g, 10.82 g, 6.79 g, and 8.76 g in boxes T8 (control), T5, T3, T4, T6, and T7, respectively.

According to the above data, the earthworms were T2>T3>T4>T7>T6 when comparing with the other doses of the control application. The dose that had the closest physical properties to the earthworms in the control application was box T2. Clitellum structures were found in the earthworms in boxes T8, T2, and T3. The earthworms in boxes T4, T6, and T7 either did not form clitellum structures or were weak.

At the end of the application, it was found that the earthworms in boxes T8, T2 and T3 responded better to physical responses when compared with 90-day period and they were prone to displacement. The colors of earthworms in boxes T4, T6 and T7 were still dark and responded to physical changes better than 120-day period and were prone to displacement. Venter and Reinecke (1988) State that the amount of organic matter present in waste and microfauna in the compost medium determines how long earthworms in compost can survive in the same culture medium [39]. Thus, the period of such studies is determined based on the type of composting materials.

Conclusion

When the amount of heavy metal in the earthworms' bodies in the boxes T8 (control), T4 and T6 was evaluated, the amount of metal in the earthworms' bodies increased in parallel with the increase in the amount of treatment sludge dose in the trial boxes. Zinc intake mostly occurred among heavy metals in earthworm bodies and there was an increase in zinc intake based on time. The heavy metals examined in the study (Cd, Cu, Pb, Hg, Zn, and Cr)—with the exception of Cadmium (<0.24)—increased when compared with the control group for the other metals. The value of Hg was <0.1 on Day 30 day and increased on Day 90. The metal analysis performed on the earthworms in boxes T4 and T6 on Days 90 and 120 revealed that bioaccumulation of the other metals except for mercury increased on Day 120. This can be explained by the increase in earthworm activity upon the addition of nutrients, as well as the indirect increase in metal intake into the earthworms' bodies.

The results of vermicompost analysis indicated that the increase of the treatment sludge dose had a negative impact on the reproduction and sex development of the earthworms. The number of the earthworms which were interacted with 1: 1 (T4) and the increases in treatment sludge dose remained constant on Days 30 and 90 and the decrease in their number occurred on Day 120.

The earthworms' live weight was the highest on Day 30 and decreased on Day 90. This is thought to be associated with the consumption of supportive materials used to test lumbricus and their decreasing amount. Also, the toxic effects of iron-steel industry wastewater treatment sludge, which was low in organic content due to the application, increased with the reduction of nutrients in the environment, in turn slowing down the earthworms' activity. This situation was observed in earthworms in boxes T6, T7, and T4. The number of earthworms remained constant in boxes T2, T3, T4, T6, T7, and T8, and their live weights increased by Day 30. At the end of Day 90, the number of earthworms increased but their weight decreased. At the end of Day 120, the number and weight of the earthworms decreased compared to Day 90. This indicated that increasing wastewater treatment sludge doses including the 1:1 ratio increased the toxic effects on earthworms over time. Homogenized and integrated material was obtained on Day 90 over the 120 day vermicomposting process. Parallel to the nutrition reduction of wastewater treatment sludge (T4) and its increasing dose, the earthworms were slow to react to physical effects (e.g. light, contact.) and their color was darker compared to box T8.

In the light of the data of this study, it indeed seems possible to remove or significantly reduce heavy metals in wastewater treatment sludge produced by the integrated iron and steel industry using different agricultural waste such as cow dung, sawdust, waste tea leaves, and *E. fetida* earthworms. This is the first attempt to examine removing metal from IWS using agricultural waste and earthworms in Turkey.

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