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Removal of Aluminium, Lead and Nickel from Industrial Sludge via Vermicomposting Process

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Abstract: Increasing waste generation in developing countries has alarmed authorities on waste disposal issues. Therefore, various alternatives have been looked into to reduce waste disposal onto landfill. Among others alternative methods are the bioremediation which may allow the conversion of putrescible wastes into value added products such as vermicompost and optimizing this sludge waste to becoming an organic fertilizer. In general the conducted research will be focussing on the suitability of the vermicomposting technique in treating the electronic industrial waste sludge that contains some metals namely Al, Ni and Pb. A particular attention will be given in assessing the accumulation effects of Al, Ni and Pb and its toxicity behaviour on the vermicomposting agent that is *Eisenia foetida* during the treatment process. There was four vermicomposting containers used with different metal sludge percentage (20%, 50%, 80% and100%). Analysis of selected metals concentration in the *Eisenia fetida's* body tissues and in the vermicompost (a product of vermicomposting process) from each vermicomposting container was conducted within 56 days and after 56 days. The toxicity effects of the accumulated metals were evaluated via dry weight of *Eisenia fetida*. Results exhibit that the toxicity behaviour in *Eisenia fetidas* were increased with retention time and percentage of metals sludge concentrations. Through vermicomposting process using *Eisenia fetida* revealed about 97% of Pb^{2+} successfully been removed from the sludge compare to only 86% and 72% for Ni^{2+} and Al^{3+} respectively. In conclusion this fundamental research work gives some positive remarks on the potentiality of vermicomposting technique in removing metals compound from the electronic industrial sludge.

Key words: Vermicomposting · Metals · Accumulation · Toxicity · Eisenia fetida

INTRODUCTION

Many articles reported severe damages on the environment that was due to pollutants discharged by both small and large scale industries. Most of the pollution cases associated with toxic and hazardous materials sludge. The illegal dumping could happened purposely and sometimes unintentially due to lack of enforcement by an authorized agency. Pertaining to this issue there are many established and approachable treatment technology for toxic and hazardous sludge specifically that contained metals compound.

The technology varies from chemical and biological or combination of both processes. The promising technique that can be applied to treat the industrial sludge is known as vermicomposting by a specific composting worm. This technique has been tested on some of the industrial sludge, organic wastes, sewage and wastewater treatment sludge for the biodegradation and decomposition purposes [1-3].

Previous study stated that *Eisenia fetida* can easily decompose kitchen leftover together with animal waste and agricultural biomass that was used as a feeding substrate in the vermicast [4].

In contaminated soil remediation the analysis of heavy metals toxicity in earthworm species was usually conducted according to fractionation (using sequential extractions), mobility (toxicity characteristic leaching procedure ((TCLP) and oral bioavailability (Ruby's physiologically based extraction test (PBET)) [5] .

A few studies had been conducted in assessing and evaluating some of the metals accumulation such as Zn, Cu, Pb and Cd either in plant tissue, earthworm or in the microbiological community and in the soil fertility [6- 8].

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Most of the studies focussing on the contaminated sewage and soil by those metals or in an attempt to evaluate the direct impact of those synthetic metals solution on the enzymatic activity of substrate [9].

This study aim firstly to demonstrate the ability of composting worms that is *Eisenia fetida* in accumulating the Al^{3+}, Ni^{2+} and Pb^{2+} which exist in the dewatered sludge from electronic industry's wastewater treatment plant as well as removal efficiency of the metals content from the sludge. Indirectly the toxicity behaviour of the *Eisenia fetida* when exposed to different tested metals sludge concentration shall be evaluated.

MATERIALS AND METHODS

Samples Background: The heavy metals sludge samples were collected from one of the electronic industrial factory in Penang State of West Malaysia.

Acclimatization of Vermicomposting's Worms (Eisenia Fetida): All vermicomposting worms used in this study was having same age (mature worms) and the same weight as well around 1g ± 0.75. The worms were washed using deionized water and dried up with the normal tissue paper prior to taking their body weight then after that the weighted worms was added in the composting using OECD procedure [10].

The acclimatization period was needed prior to the actual experiments using four containers with different ratios of sludge to sheep manure and agricultural wastes as an initial procedure. This should allows the worms to adapt with the new vermicomposting environment and conditions by so call training the worms to leave in the vermicomposting materials that also contained heavy metals sludges. The acclimatization process was conducted in the vermicomposting container size of 33.5 cm x 24 cm x 10 cm that was divided into two sections.

The first side was the mixtures of the sheep manure and agricultural wastes. For the started all the selected worms was added in the first side of the container (sheep manure + agricultural wastes) only. The other side of the vermicomposting container was leave solely with heavy metals sludge. The acclimatization container was covered with drapery and with micro holes. The acclimatization container was left in the laboratory environment and conditions similar to the proposed experiments for a period of two months. Observation of the acclimatization container and process was observed every day. It was found that within two months of the acclimatization process gradually the selected worms were moving to the other side of the acclimatization container which contains only heavy metals sludge just after one week of acclimatization periods and remained there until a month. After a month all the selected worms moved into the heavy metals sludge side and remained there ever after. After two months leaves in the heavy metals sludge it was observed that some worm cocoons appears. Thereafter these worms were used in the proposed experiments with different ratios of heavy metals sludge to a mixture of sheep manure and agricultural wastes.

Set up Procedure for Experimental Vermicomposting Container: There were four plastic containers dark in colour was used as vermicomposting bin with the size of 23cm x 16cm x 5cm and covered with drapery with micro holes. Different ratio of heavy metals sludge to a mixture of sheep manure and agriculture wastes was used in each container as follows 20:80%, 50:50%, 80:20% and 100:0% respectively. About 50 acclimatized worms of *Eisenia fetida* species (scientific name) and commonly known as Tiger worms was added to each four set up container (reactors). The other set up containers which follow the similar preparation as the samples container was prepared as control container but without worms. The pH value in all vermicomposting bins was fixed between 6 to 8 and the studied temperature was between 29°C to 35°C. The experiments were conducted inside the laboratory environment as illustrated in Table 1.

Vermicomposting Experimental Procedure: The initial content of heavy metals in sludge samples studied, vermicomposting materials and acclimatized vermicomposting worms was analyzed for Al^{3+}, Ni^{2+} and Pb^{2+} prior to proposed vermicomposting process.

Table 1: Vermicomposting container contents.

	Reactors							
Feed Substrate	Cont.1 (1:4)	R 1(1:4)	Cont.2 (1:1)	R.2(1:1)	Cont.3 (4:1)	R.3(4:1)	Cont.4 (1:0)	R.4(1:0)
Sheep manure in g	400	400	250	250	100	100	0	0
Sludge in g	100	100	250	250	400	400	500	500
Worms' number	-	50	-	50	-	50	-	50

Cont. = control R. = Reactor

This procedure is importance to record the initial concentration of Al^{3+}, Ni^{+} and Pb^{+} that contain in all stated materials. So that the evaluation of the vermicomposting process effectiveness can be compared afterwards. The experimental vermicomposting process was prepared as described in *2.3* and deionized water was applying everyday to maintain the moisture contents up to 75%. The tested *Eisenia fetida* worms were feed every three days with the same mixtures of sheep manure and agricultural wastes around half of the worms' weight. Theoretically the daily uptake for the worms is about 1/3-1/2 of their total body mass weight and excrete about 75% their waste known as vermicompost as by product. The addition of this sheep manure and agricultural wastes mixture was done in order to keep the same concentration of heavy metals in side worms' gut as well as to maintain the good ratio of C:N in the studied vermicomposting bin [11, 2]. The concentration of Al^{3+}, Ni^{2+} and Pb^{2+} was analyzed in each vermicomposting bins inclusive control container from 0 day before proceeded with the vermicomposting treatments. The measurement was carried out for every 7 days interval and for a period of 28 days [11]. The same measurement was done for temperature and pH. The pH range was maintained by adding the egg shells to the studied vermicomposting bin. The period of 28 days exposure was important to investigate the toxicity effect of the heavy metals accumulation in the vermicomposting's worm's body tissue as well as dry weight of the exposed vermicomposting's worms before and after 56 days of the vermicomposting process [12]. Under ideal conditions normally *Eisenia fetida* will need approximately 55-85 days for sexual maturity [13, 14]. All the experimental materials used in this study were made by plastic to avoid any additional heavy metals contents in the studied samples.

Heavy Metals Analysis

Vermicompost and Heavy Metals Sludge: Heavy metal analyses were carried out using flame atomic absorption spectrophotometer. The calibration curves were prepared separately for all the metals by running different concentrations of standard solutions. The instrument was set to zero by running the respective reagent blanks. Heavy metal analysis for both vermicompost and heavy metal sludge was followed APHA Standard Method for Water and Wastewater Analysis method number 3030E [15]. Atomic Absorption Spectrophotometer (AAS) brand Perkin Elmer 100 and 200 was used for heavy metals concentrations measurement. pH for both vermicompost and heavy metals sludge were analyzed by taking 10 g each of both dry samples to 30 mL deionized water and shake it on 175 rpm for 2 hours then leave it for 15 minutes before measured the solution on HACH pH meter brand Senion 3.

Heavy Metals Accumulation in Vermicomposting's Worms: The concentrations of Al^{3+}, Ni^{2+} and Pb^{2+} in the *Eisenia fetida*'s tissues body was measured every 7 days for a period of 28 days. The analysis procedures followed digestions method proposed by Katz and Jennies [16] then analyzed by AAS brand Perkin Elmer 100 and 200.

Effect of Heavy Metals in Vermicomposting's Worms: The accumulation of Al^{3+}, Ni^{2+} and Pb^{2+} in the *Eisenia fetida's* body tissues before and after 56 days exposure in the studied vermicomposting bin was measured. The dry weight of 12 *Eisenia fetida* represented the whole population of *Eisenia fetida* in each vermicomposting container was measured before and after 56 days in order to observe any toxicity effect due to heavy metals sludge used as feedstock. By using dry weight of the exposed vermicomposting's worms the extrusive relationship can be obtain [12].

Statistical Analysis: Average values of three replicates were taken for each determination. All the samples characterization was conducted in triplicate to obtain mean values. One way analysis of variance (ANOVA) and T- test models was selected to analyze the heavy metals removal efficiency via vermicomposting process for the entire composting period of 56 days with 95% Confidence Interval of the Difference.

RESULTS AND DISCUSSIONS

Table 2.0 depicted the industrial sludge characteristics the metals content in the industrial sludge in comparison with international standard limits such as United State Environmental Protection Agency for sewage sludge (US EPA), UK for sewage sludge and Environmental Quality Criteria in Canada for the allowable limit of metals contents in the Industrial soil. It was found that concentrations and availability of heavy metals in raw studied sludge could be ranked as follows $Al^{3+} > Ni^{2+} > Zn^{2+} > Pb^{2+} > Cu^{2+} > Cd^{2+}$. Most of the metals content was not comply with the stipulated standard limit for metals content in soil or sewage sludge of US EPA, UK and Environmental Quality Criteria in Canada except for Mn, Fe and Cr [17, 18].

Table 2: Characteristics of the electronic sludge.

The characteristics of the sludge										
pH	Moisture (%)	Al (mg/kg)	Ni mg/kg	Pb (mg/kg)	Z (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Cr (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
6.72	78	7200	839.6	422	619	66.37	239.8	1.7	208.4	110.3

Table 3: ANOVA removal concentrations between control and reactors individual for each metal.

	ANOVA				
	Sum of Squares	df	Mean Square	F	P
Concentrations of Al Between controls	2973.382	4	743.346	0.000	1.000
Concentrations of Al Between Reactors	2.025E7	4	5063625.157	3.183	0.044
Concentrations of Pb Between controls	21.732	4	5.433	0.000	1.000
Concentrations of Pb Between Reactors	820051.455	4	205012.864	14.782	0.000
Concentrations of Ni Between controls	21.732	4	5.433	0.000	1.000
Concentrations of Ni Between Reactors	820051.455	4	205012.864	14.782	0.000

At present, Malaysia does not have specific regulation on the management and remediation of soil and groundwater contaminated sites. With regards to several contaminated land problems that have been highlighted, for example the illegal dumping of aluminium dross at Felda Bukit Gatom in Labis Johor occurred in 2006 (i.e. which has become a national issue and public outcry due to ammonia vapour). Department of Environment (DOE) has currently undertaken their own extensive study to develop the standard and this is followed by SIRIM Bhd, who currently drafting some standards on remediation of contaminated land based on the standard from American Society for Testing Materials (ASTM). Malaysia will have to keep up with the “pace” set by its ASEAN neighbours, such as Singapore, Thailand and Vietnam that have some form of regulations to control soil and groundwater pollutions [4].

After an exposure period of 28 days, the concentrations of Al^{3+}, Ni^{2+} and Pb^{2+} concentration in the sludge was measured and compared between vermicomposting reactors and controls reactors. Exposure period of 28 days was selected with referring to [12, 19, 20] reported that heavy metals normally will show adverse effect to the earthworm or composting worm in the long term exposure period. It is important to understand this phenomenon in order to sustain the system stability [20]. After 28 days it was found that the concentrations of the tested metals in the remaining sludge decreased significantly ($p<0.05$) with time for all vermicomposting reactors whereby all control reactors remain with initial concentrations. This is in agreement with [21] stated that control reactor was not affected after10th week composting period compared to reactors with heavy metal dosages.

Figure 1a summarized the aluminium removal in all vermicomposting reactors for all the treatment ratios. Reactor 4 (100% metal sludge or pure sludge) depicted the best removal of aluminium ($F = 3.183$) denote that the difference degree between the vermicomposting reactors with different ratios of metal sludge to agricultural biomass is about ± 3.183 as tabulated in Table 3.0. This already considered a significantly difference ($p = 0.04$) between the vermicomposting reactors for aluminium removal.

In the other hand the concentrations of heavy metals in the worms’ body tissue increasing significantly ($p <0.05$) that means the worms able to accumulating the metals in their bodies Figure 1b. The concentrations of (Al) in the worms’ body were increased and container No. 4 had the highest concentrations that were a good demonstration for decrease the (Al) concentrations in container No. 4. The symbol (t) means the degree of the difference between them as you seen in Table No. 3 there is no other studies work on the Al in the vermicomposting and you can said this is the first time.

In the same reactors the (Ni) results was the same for (Al) that means the worms could remove the (Ni) from sludge significantly ($p <0.05$) and accumulating it in their body that made the concentrations became increasing in the worms and best removal is in container No.4 also, but in container No.1, 2, 3 the removal percentage it was equal, The significant between the four reactors was 0.000 see Table No. 3. The results of Ni in vermicomposting after (28 days) are summarized in Figures No. 2a and 2b.

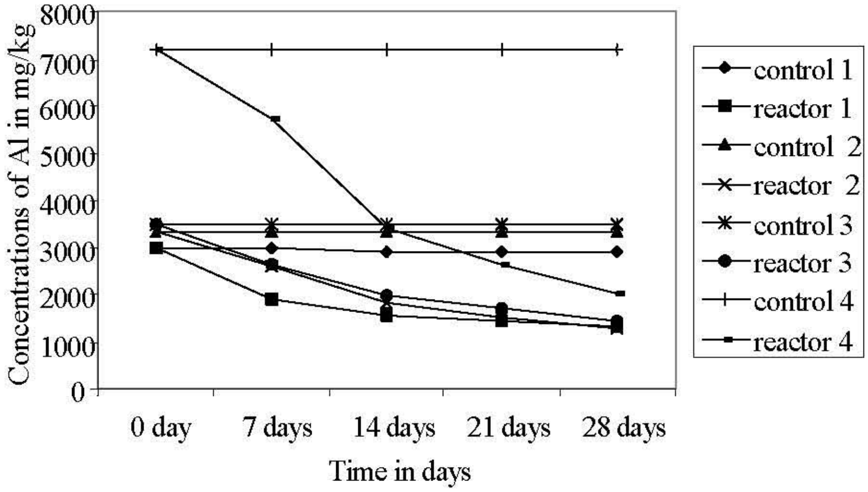


Fig. 1a: Concentrations of (Al) in the four reactors and controls in mg/kg.

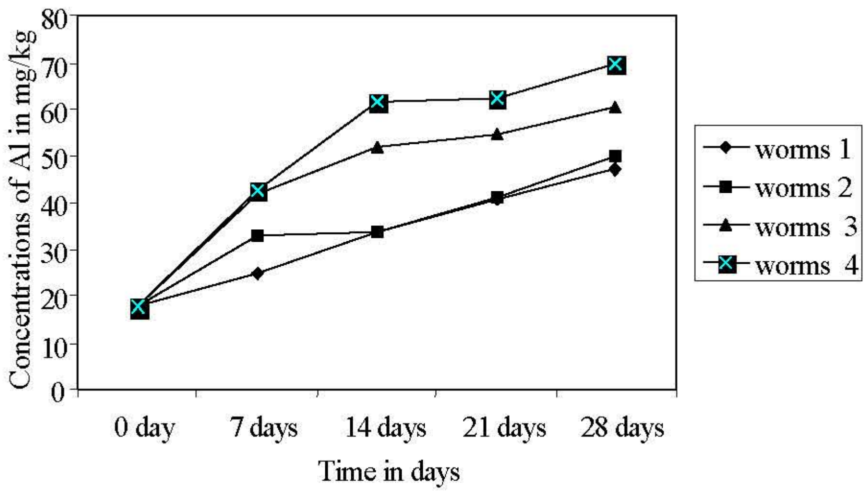


Fig. 1b: Concentrations of (Al) in the worms' body tissue in four reactors in mg/kg.

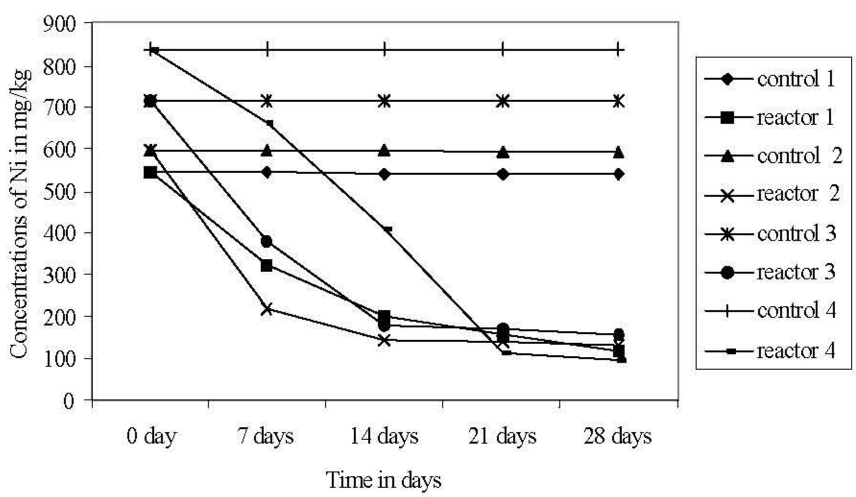


Fig. 2a: Concentrations of (Ni) in the four reactors and controls in mg/kg.

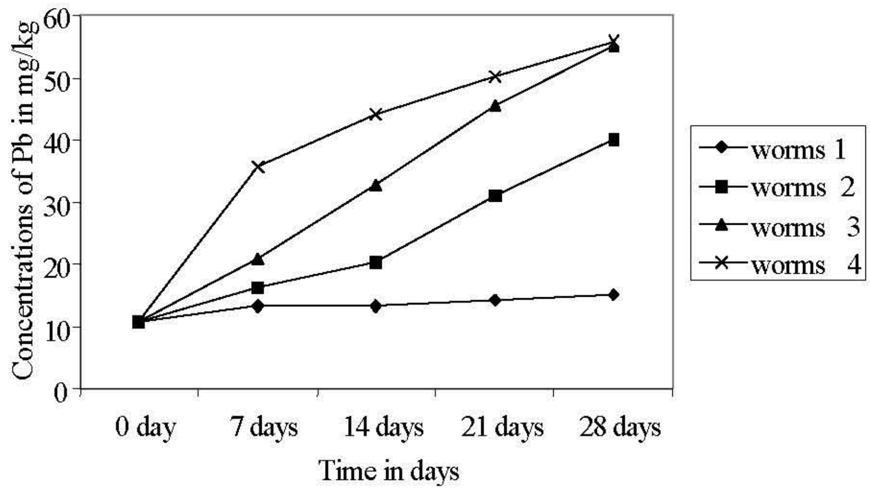


Fig. 2b: Concentrations of (Ni) in the worms' body tissue in four reactors in mg/kg.

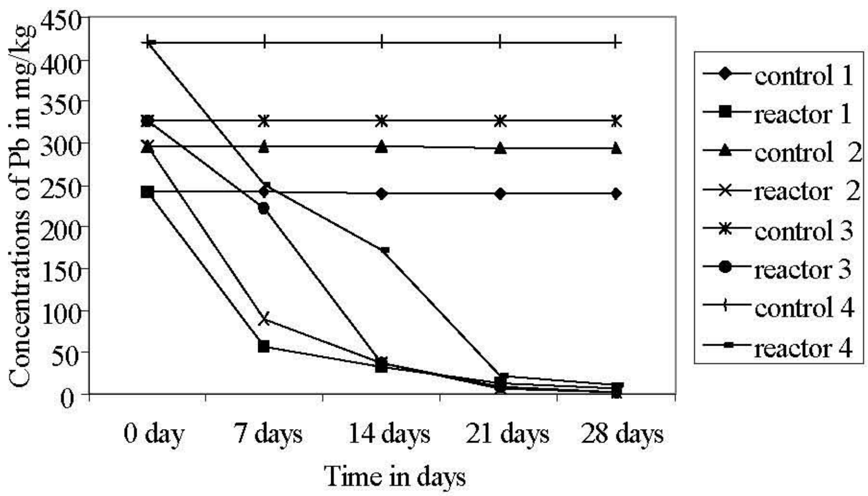


Fig. 3a: Concentrations of (Pb) in the four reactors and controls in mg/kg.

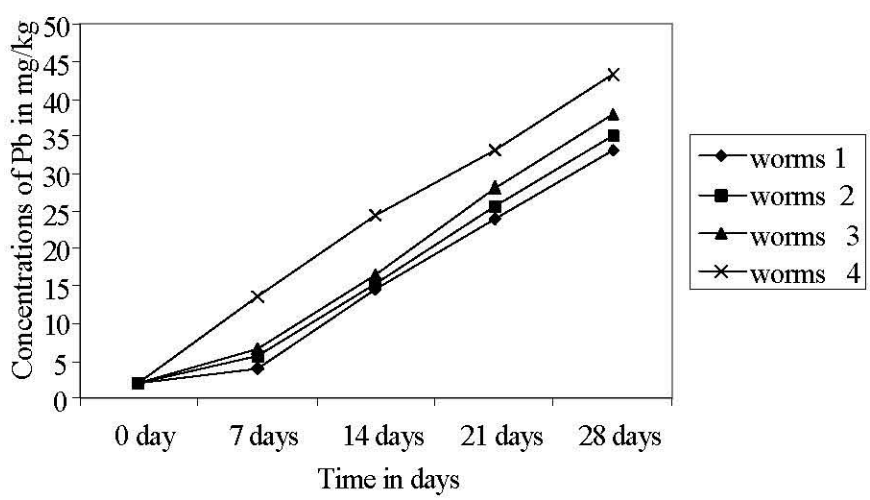


Fig. 3b: Concentrations of (Pb) in the worms' body tissue in four reactors in mg/kg.

Table 4: ANOVA removal concentrations between controls and reactors between all metals.

	ANOVA				
	Sum of Squares	df	Mean Square	F	P
Concentrations of Al,Ni,Pb in the Reactors					
Between all metals	1.168E7	4	2920362.461	1.458	.228
Concentrations of Al,Ni,Pb in the controls					
Between all metals	1226.479	4	306.620	.000	1.000

Table 5: T-test accumulation of heavy metals in worm's body tissue in four reactors.

	Test Value = 0					
One-Sample Test	T	df	P	Mean Difference	Lower	Upper
concentration of Al in the earthworms body tissue in reactor 1	4.519	5	0.006	40.62000	17.5146	63.7254
concentration of Al in the earthworms body tissue in reactor 2	4.406	5	0.007	44.10500	18.3755	69.8345
concentration of Al in the earthworms body tissue in reactor 3	5.027	5	0.004	54.04500	26.4093	81.6807
concentration of Al in the earthworms body tissue in reactor 4	4.864	5	0.005	60.75667	28.6463	92.8670
concentration of Ni in the earthworms body tissue in reactor 1	7.516	5	0.001	15.25000	10.0345	20.4655
concentration of Ni in the earthworms body tissue in reactor 2	3.749	5	0.013	30.70000	9.6523	51.7477
concentration of Ni in the earthworms body tissue in reactor 3	3.706	5	0.014	42.65000	13.0629	72.2371
concentration of Ni in the earthworms body tissue in reactor 4	4.282	5	0.008	48.86667	19.5333	78.2000
concentration of Pb in the earthworms body tissue in reactor 1	2.883	5	0.034	20.80000	2.2567	39.3433
concentration of Pb in the earthworms body tissue in reactor 2	2.918	5	0.033	22.65000	2.6967	42.6033
concentration of Pb in the earthworms body tissue in reactor 3	3.040	5	0.029	23.91667	3.6916	44.1417
concentration of Pb in the earthworms body tissue in reactor 4	3.520	5	0.017	29.08333	7.8431	50.3236

This results is better when it is compared with previous study, when [22] exposed *Esienia Fetida* to artificial soil with different concentrations of(Ni), all the worms died when the concentration of (Ni) in the soil 1000 mg/kg and in 700 mg/kg no significant was obtained in the (Ni) concentrations, from this study the researchers reach to conclusion that all the worms can survive in 85 mg/kg of (Ni), while in this work the concentration of (Ni) in the sludge is 839.6 mg/kg after acclimatized the worms to live in this kind of toxic sludge. [20], studied the accumulation of (Ni) in *Esienia Fetida* for 28 days from exposed these worms to the artificial soil with different concentrations of (Ni) 64-370 mg/kg, they obtained when the concentration increase in the soil the accumulation of this metal in the worms body tissue increase also and decreasing in the compost, the accumulation amount for Ni in the worms for 28 days it was 0.9 mg/kg while in this work the accumulation for Ni in pure sludge reactor is 85 mg/kg.

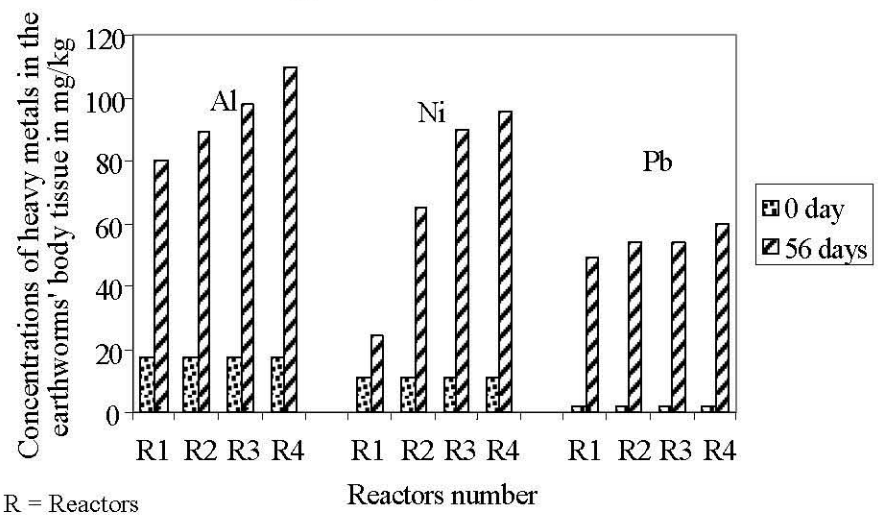
The Pb removal percentage was so nearly in concentrations that mean the worms can remove the Pb equal in all the containers and with different ratio of sludge. In the same containers the concentrations of Pb in the earthworms' body tissues are increase with time when the temperature and pH are fixed. The results are summarized in Figures 3a and 3b.

The significant between the reactors was $0.00<0.05$ see Table No. 3. In previous study the researchers treat the artificial soil spiked with different ratios of soluble and less soluble Pb compounds by *Lumbricus terrestris* for 60 days this kind of worms can accumulate soluble Pb better than the less soluble Pb compounds [23]. Other study investigate the concentrations of Pb for four years in different kinds of natural soils when treat it with *Esienia Fetida* they found the Pb removal in the soil with tannery sludge better than the soil was mixed with fertilizer because the sludge contain a good amount of minerals for worms [24] .

No significant difference in removals was observed between the reactors (p= 0.228)for the heavy metals that means the worm ability to remove the heavy metals is good in all reactors they were decrease in heavy metals concentrations inside the reactors see Table 4.

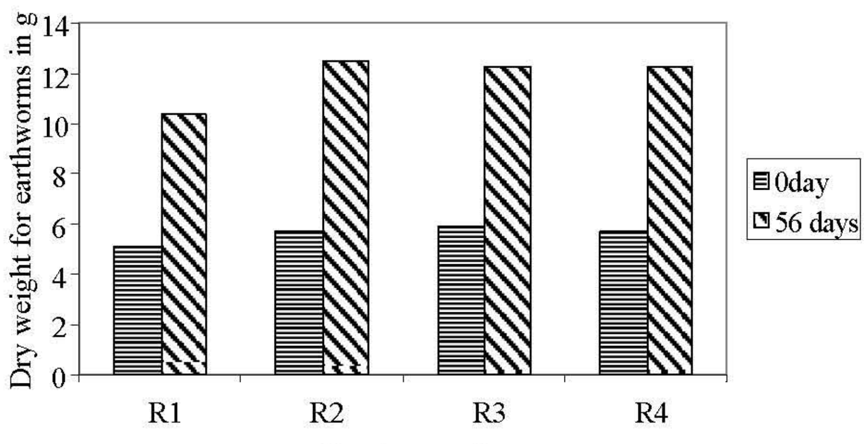
After exposed the worms to the sludge for 56 days the accumulation was measured by determined the concentrations of heavy metals in the worms' body tissues before and after 56 days exposure. The results are summarized in Figure 4 and the significant for all reactors is less than < 0.05, the accumulating in the worms body in R3> R4> R1> R2 for the (Al), R4> R3> R2> R1 for (Pb^{2+}) and for Ni^{2+} is R1> R4> R2> R3 as in (t) value.

When the concentration of heavy metals increased in the worm's body that means the toxicity increased also in their bodies, see Table 5. The accumulation of heavy metals compared with another study for 60 days [19, 25] it was best than the other studies.



R = Reactors

Fig. 4: The accumulation of heavy metals in four reactors and mg/kg before and after 56 days.



R = Reactors

Fig. 5: Dry weights for the earthworms' body in g before and after 56 days.

The dry weight for the worms can be considered as indirect toxicity parameter in the worms body, the dry weight of (12 earthworms) before and after 56 days in composting were measured, the dry weight increased with time and when the concentration of heavy metals in composting were increased. The Results are summarized in Figure 5. The dry weight of earth worms increases with increasing the pollution in the reactors but it is highest in the container No. 2 when the ratio of sludge to the composting is 1:1 that means the toxicity was increased with exposure time to sludge in the worms' body [12, 25].

This result is also supported by the observations of [25, 26] where Cu is regulated in a much narrower range than Zn in earthworms. This capacity of earthworms to store and redistribute heavy metals especially Cu and Zn in their bodies might lead to a balance between uptake and excretion which will help them to survive to a certain extent in metal contaminated site.

CONCLUSION

The vermicomposting with different ratio of sludge in four reactors with *Eisenia fetida* the removal of the heavy metals from the vermicomposting in (28 days) was studied the concentrations of (Al, Ni, Pb) are decrease with time in the composting and increase in earthworm's body tissues when the pH and temperature are fixed the result was significant 0.00<0.05. After exposure (56 days) the accumulations was measured by study the concentrations of (Al, Ni, Pb) in worms' body tissue before and after 56 days the accumulation became high. The toxicity in earthworms' body tissues were increased with time and with increase the pollution in the reactors but the toxicity is highest in the reactor No. 2 when the ratio of sludge to the composting is 1:1. By measured the dry weight of 12 earthworms before and after 56 days when the pH and temperature are fixed. The best removal percentage it was for the Pb 97% then for Ni 86% in the last for Al 72%. The contribution here the electronic sludge treatment with vermicomposting technique.

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