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RESEARCH ARTICLE

BIOTRANSFORMATION OF CHROMIUM BY MEANS OF PHYTOREMEDIATION WITH VETIVER *(VETIVERIA ZIZANIOIDES)*, AND LATER EARTHWORM COMPOSTING WITH RED CALIFORNIAN EARTHWORM (*EISENIA FOETIDA*)

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ABSTRACT

This research studies biotransformation of chromium (Cr) in the tanneries sewage, combining phytoremediation (with *zizanioides*) ad later Earthworm composting (with *Eisenia foetida*) from Vetiver roots with Cr content. The study started by the implementation of phytoremediation in subsurface flow wetland where tanneries sewage flows and it was possible to absorb an initial concentration of 3818mg. kg^{-1} of CR in the sewage to 3735 mg. $kg^{-1}$of Cr in the Vetiver roots. After that, an Earthworm composting was implemented in six beds, with three replicas in each one, in which different quantities of Vetiver roots were added with Cr to the Earthworm composting and the half of them were also added nourishment. Measurements were carried out on days 7, 14, 21 and 28 of dependent variables such as mortality, length, weight, diameter, presence of juveniles and cocoons. In this part of the essay, it was found that *Eisenia foetida* tolerates Cr concentrations and its bioconcentration factor was between 0.143 and 0.274. Furthermore, the acute toxicity was determined CL_{50}, and the chronic toxicity NOEC, NOEC, confidence limits upper and lower to 95% for the concentration values and Cr. As for the decreasing of Cr in the Earthworm composting, 70.9% was found for the best trial. Finally, a model of mortality behavior was determined according to the Cr concentration with correlation of 0.999 and R-squared that explains 99.8% of the mortality variations.

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INTRODUCTION

The increase in the water and soil pollution levels caused by heavy metals pouring as waste of industrial, residential and agroindustrial zones has encouraged the research and development of effective methods for decreasing or eliminating its presence, by means of diverse treatments seeking to modify its great toxic power. The industry of leather tanning has been widely recognized by its great environmental effect and it is known as one of the largest anthropogenic pollutants. One of the most important environmental concerns related to tanneries is the use of Chromium (Cr) in its tanning process, which implies its presence in the sewage and in the leather wastes (Zuriaga-Agustí *et al*., 2015). Tanneries sewage are characterized by a strong color, high values in the chemical oxygen demand (COD), biochemical oxygen demand (BOD), dissolved solids and Cr. Chromium toxicity is one of the main causes of risks for human health; Cr (VI) is severely toxic, mutagenic, carcinogenic and teratogenic(Sharma and Malaviya, 2016).In order to counter the presence of this and other elements, it has been studied its transformation through

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bioremediation, which is a technique that uses living beings to restore contaminated environments as it accelerates biodegradable processes that occur naturally in contaminated ecosystems. Where microbial communities are usually dominated by adapted microorganisms capable of using toxic compounds from the contaminated space (Anza *et al*., 2016). According to (Delgadillo-López *et al*., 2011), the mechanisms involved in the removal of pollutants are of three types: physical (sedimentation, filtration, adsorption, volatilization), chemical (precipitation, hydrolysis, oxidation-reduction or photochemical reactions) and biological (result of microbial metabolism, plant metabolism or bio-absorption processes) (p. 598).The use of plants to treat wastewater and recover contaminated spaces is known as phytoremediation, which takes advantage of the ability of certain plants to absorb, accumulate, metabolize, volatilize or stabilize contaminants such as: heavy metals, radioactive metals, organic compounds and compounds derived from petroleum. The use of phytoremediation depends on the affected resources, the contaminant and the process involved: stabilization, isolation, reduction, degradation, metabolization or mineralization (Cubillos *et al*., 2014). The use of Vetiver has already been studied for the phytoremediation of Cr of residual sludge (Torres *et al*., 2010) and what was initially sought was to check its removal efficiency, then close the bioremediation cycle with red Californian earthworm. More specifically, the study of (Carpena and Bernal, 2007) developed rizofiltration which is the use of roots to absorb and adsorb contaminants from water and other aqueous effluents. Moreover, some experimental results indicate the potential of earthworm compositing using (*Eisenia foetida*) for attenuation of the toxicity and transformation of Chromium (VI) in studies such (Ramírez *et al*., 2011; Espinoza *et al*., 2013) the morphology of this worm, demonstrating that it is highly desirable in the transformation of this type of toxic materials.

Biotransformation by means of earthworms takes advantage of several advantages derived from the activity of certain species of earthworms, which accelerate the decomposition of organic matter of plant and animal origin (Paco *et al*., 2011). Due to its innate biological, biochemical and physicochemical properties, worm humus can be used to promote sustainable agriculture and for the safe management of agricultural, industrial, domestic and hospital waste that can pose a serious threat to life and environment (Pathma and Sakthivel, 2012). During the feeding process, earthworms fragment the residues, increase the microbial activity and the rates of decomposition and/or mineralization of the organic waste, altering the physical and chemical properties of the materials, causing a humidification effect which unstable organic matter is oxidized and stabilized (Méndez and Rodríguez, 2011). This species has a high rate of ingestion and defecation and, as a result of this digestive action, originates on one side, worm humus or earthworm composting with different levels of contamination, depending on the toxic nature of the residue, and on the other, it incorporates. That is to say, bioaccumulate in their tissues part of the ingested (Rodríguez *et al*., 2002) through a process of oxidation-reduction and stabilization of organic matter, mediated by the combined metabolic action of worms and microorganisms. The reduction of Cr^{+6} to Cr^{+3} precipitates the metal under physiological conditions through the use of Cr-resistant microorganisms that perform the bioconversion and is used in the biological treatment of industrial effluents (Vullo, 2003). There are several studies (Cervantes *et al*., 2011; Marco *et al*., 2010), which corroborate the ability of *Eisenia foetida*to accumulate Cr in its tissues, and show that it is able to regulate the amount of metal accumulated proportionally to the amount in the environment. In addition, it was found that earthworms produce changes to the metals they ingest by excreting earth in a certain way with a different state. The presence of Cr in the earthworm is expressed as the bioconcentration factor, and it is usually applied as an indicator of the bioavailability of heavy metals and it is assumed that organisms reach chemical equilibrium in relation to a particular medium or a particular route of exposure (López, 2004). Lower values than one are reported for Cr, Ni and Pb in *Eiseniafoetida* specimens submitted to different doses of sewage sludge (De Matos, 2009).This study presumes that the red Californian earthworm (*Eisenia foetida*), when fed with a compost mixture with the Vetiver roots, which have accumulated Cr, can transform it into a less offensive derivate for the environment. Thus providing a complementary solution to the final disposition problem of the Cr contained in the roots of the Vetiver by effect of phytoremediation.

MATERIALS AND METHODS

In this research, the effect of the two independent variables (trials with two types of earthworm composting beds and six different concentrations of chromium) on the dependent variables (length, weight, diameter, death of earthworms, presence of coconuts and juveniles) was studied. In this design, it was aimed to reduce to the maximum other possible sources of change of the dependent variables and with this to have greater certainty that the changes were due to the manipulated independent variables, which were controlled taking care that they were faithful to the trial established for the earthworm composting conditions and chromium concentration.

The following table shows the organization of the experimental design for each replica.

Table 1. Experimental design for each replica

Trial	Subjects	Treatments (independent variables)	Measurements (dependent variables)
1	RG_{15}	X_1, C_1	O_1, O_2, O_3, O_4, O_5
2	RG_{15}	X_1, C_2	O_1, O_2, O_3, O_4, O_5
3	RG_{15}	X_1, C_3	O_1, O_2, O_3, O_4, O_5
4	RG_{15}	X_2, C_4	O_1, O_2, O_3, O_4, O_5
5	RG_{15}	X_2, C_5	O_1, O_2, O_3, O_4, O_5
6	RG_{15}	X_2, C_6	O_1, O_2, O_3, O_4, O_5
7	RG_{15}	X_1, A	O_1, O_2, O_3, O_4, O_5
8	RG_{15}	X_2, A	O_1, O_2, O_3, O_4, O_5

R = randomization of subjects in order to start the test
G = number or subject groups G_{15}
X, C = treatments, or experimental conditions
X_1 (COMPOSTING+FOOD+VETIVER),
X_2 COMPOSTING + VETIVER,
C_1, C_2, C_3, C_4, C_5, C_6 (CHROMIUM CONCENTRATIONS))
O = final measurement to subjects of the group
O_1 LENGTH, O_2 MASS, O_3 DIAMETER, O_4COCOONS,
O_5 JUVENILES, O_6 DEATHS OF EARTHWORMS
A = chromium absence, indicates that it is the control group.

In the experiment, the external variables were controlled, the control group was present, and the selection of individuals that participated in the experiment was completely randomized, as well as their allocation to each study group.

The hypothesis was the following: the variation in the conditions of the experimental beds (X) and the concentration of Cr in each of them (C), affects the variables dependent on the trial. A probabilistic sampling was carried out since the earthworms that were counted for the trial were equally likely to be selected by simple random sampling. In order to evaluate the effects of treatments (X, C) on subjects, it is established by analyzing the two-factor ANOVA variance with fixed and completely randomized levels of effects; the interest of the design is centered in knowing if these levels differ from each other. The analysis poses two hypotheses: Ho: null hypothesis and H_A: alternative hypothesis. The Ho states that there were no significant effects of the independent variable on the dependent variable; that is to say that the different beds (X) and / or the different concentrations (C) produce the same effect in all the organisms.

Ho $\rightarrow \mu_1 = \mu_2 \rightarrow \mu_1 - \mu_2 = 0$ where $\mu_1 and \mu_2$ are the average of the values at the beginning and at the end of the experiment.

The H_A states that there were differences between the results at the beginning and at the end of the experiment. That is to say that the different beds (X) and/or the different concentrations (C) produce different effect in all organisms. $H_A \rightarrow \mu_1 \neq \mu_2 \rightarrow \mu_1 - \mu_2 \neq 0$ where μ_1 and μ_2 are the means of the values at the beginning and at the end of the experiment. The level of significance of 0.05 or level of 5% was used as the basis for inferring the intervention of non-random factors; that is to say, when $p < 0.05$, the alternative hypothesis is accepted and when $p > 0.05$, the null hypothesis is accepted. In order to compare the average of the populations, it was used the analysis of variance ANOVA of two factors and their interaction with the results of the dependent variables and a factor with the dependent variables to establish specific results. Interpretation for ANOVA when critical F <0.05, the null hypothesis is rejected and the alternative hypothesis is accepted. The project was developed in four phases summarized as follows: Implementation of phytoremediation with Vetiver (*Vetiveriazizanioides*), implementation of experimental beds, study of the effect on the morphology and tolerance to *Eisenia foetida* chromium, and evaluation of the systems tested. The research was carried out outdoors, at the UCEVA campus, at 975 masl, in an arboreal and shaded area with an average annual temperature of 21 ^{0}C (between 17 and 250), in a space protected from the direct sun and rain.

Implementation of phytoremediation with Vetiver (*Vetiveria zizanioides)*

Initially the Vetiver plants are planted at the site where the trial will be carried out to have the material available when the study is to begin at the site and its acclimatization is carried out.

A pilot system was constructed consisting of three tanks connected through a ½-inch hose as follows:

- A 120 L feeder tank with an outlet opening at 25 cm from the bottom of the bowl, where a faucet and a hose are connected.
- A 70 L tank seeding with Vetiver (subsurface flow wetland behavior) with an upper inlet through a PVC pipe with holes to ensure flow distribution and an opposite outlet, with bottom hole in the tank.
- A 20 L collecting tank buried to ensure the flow where the water is stored after passing through the system planted with Vetiver.

The period of acclimatization of Vetiver (*Vetiveriazizanioides*) was one (1) month, divided into two phases: a first phase of adaptation to the gravel and the liquid medium and a second phase of adaptation to the water containing chromium. At the end of the adaptation month, the previously diluted tanning residual water with an initial concentration of 4200 mg.L^{-1} of Cr^{+3} (3918 mg. kg^{-1}), density of 1.072 g. $mL^{-1}$and a flow rate of 2.7 L. day^{-1}. After 40 days, the Vetiver is harvested taking root for further analysis of the total Cr content by atomic absorption spectroscopy and the final concentration of chromium in the collecting tank is also determined by colorimetric analysis with calibration curve with $KCr(SO_4)_2.12\ H_2O$ for Cr^{+3} and spectrophotometer at 540 nm.

Design and implementation of experimental beds

The beds were 8 plastic containers, with the following dimensions: 30 cm high, 32 cm wide and 42 cm long and had 8 containers of the same size (1 under each bed) to collect the leachate. For each bed there were three replicas for a total of 24 beds. The beds were supported on a base made of wood, several perforations were made in the bottom to each one to facilitate the aeration and the exit of the leachates. Each bed was covered with dark-colored polysombra to improve the aeration conditions of the trial and also prevent the entry of pests. Paper was used for the composting, wet leaves, manure and sawdust, which were spread with soil in the bed. The manure used was bovine which had between 10 and 15 days of being produced by the animal. Its consistency was semi-massive, dark green, bearable odor and neutral pH. It was necessary to add some water to stabilize the humidity and control the temperature of the manure. Before starting the earthworm composting, compost conditions were evaluated to verify that it was ready for the worms: humidity between 70% and 80%, pH between 6.5 and 7.5 and temperature between 20 and 25° C.Finally, to know that the compost was ready before beginning the trial, the survival test was performed in which 50 worms were placed in the compost and after 24 hours had already been introduced into it. The methodology developed was basedon these authors (Cuevas *et al*., 2008; Gómez, 2014; Díaz *et al*., 2004; Gámez and Ramírez, 2008).

In order to prepare for the implementation of the test, 1 kg of *Eisenia foetida* is acquired with a earthworm composting from the study area; The purchased worms were acclimatized in the compost for four months and were added weekly to the food in addition to checking the temperature and humidity conditions. Adult worms were selected, verifying that they had clitellum, without appreciable alterations or defects, they were removed the excess of substrate and left without food for 24 hours being ready to initiate the test.

The sowing was carried out by layers as follows:

- Layer one: dry grass about 5 to 7 cm.
- Layer two: compost about 5 cm.
- Layer three: Worm breeding ground (15 per bed) taking care not to expose too much to the sun.
- Layer four only for beds 1, 2, 3 and 7 food (organic litter well chopped over the worm layer); around 7 cm.
- Addition of Vetiver roots with Cr content of the phytoremediation trial. 70, 50 and 40 grams of roots are added to beds 1, 2 and 3 respectively and the same for beds 4, 5 and 6.

After knowing the concentration of Cr in Vetiver roots (mg. kg^{-1}), the concentration of Cr in each bed was calculated, which depends on the amount of compost and food in each case.
In the eight beds and their three replicas were placed the amounts that are presented below:

Table 2. Amounts in beds

Bed	Compost, g	Food, g	Roots, g	Cr Concentration
1	800	200	70	C_1
2	800	200	50	C_2
3	800	200	40	C_3
4	800	0	70	C_4
5	800	0	50	C_5
6	800	0	40	C_6
7 control	800	200	0	0
8 control	800	0	0	0

The conditions of the test were as follows:

The beds were kept at 20 ± 2°C, opened at 7, 14, 21 and 28 days to evaluate the conditions of the worms, pH, temperature and humidity adjusted when necessary. For all the trials at the time of making measurements were taken 10 individuals from each bed. After 7 days, the conditions of the beds were reviewed, the development and death of the worms were evaluated; at 14, 21 and 28 days in addition to verifying their mortality were measured length, mass, diameter, number of coconuts and juveniles and any irregularity was noted. The juvenile worms and cocoons were counted, leaving them in the container for another 28 days under the same conditions, and food was added only at the beginning of this phase in trials 1, 2 and 3 and control. After 56 days, the cocoons and juveniles were counted to establish the final conditions and their viability.

Evaluation of the effects of the Chromium and *Eisenia foetida*range of tolerance

Different values of the initial concentration of total Cr in the substrate were used to evaluate the tolerance of *Eisenia foetida* because it was exposed to the presence of Cr and to the conditions of the different beds with food and without food. With the data of the dependent variables (mortality, length, weight, diameter, number of juveniles and number of cocoons), ANOVA was applied first analyzing the influence of the two independent variables (beds and Cr concentration in the roots) on dependent variables; then ANOVA was applied to identify the individual effect of each independent variable on the dependent variables. The evaluation led in each case to verify whether the null hypothesis is accepted or rejected. With the results obtained in terms of mortality in response to each concentration of Cr, an analysis was performed with PROBIT which could obtained the CL_{50}(concentration of a compound that causes death to half the organisms during the test period).
It was also determined LOEC (lowest concentration in which effect is observed) and NOEC (highest concentration in which no effect is observed), the upper confidence limit in lower to 95%. In addition, using the methodology of (De Matos, 2009) established a region of confidence for the development of future trials according to acceptable levels of mortality; this region was delimited by the average lethal concentration less than twice the calculated standard deviation and at the top by the average lethal concentration plus twice the calculated standard deviation. Finally, it was possible to establish the regression equation that best fits and models the behavior of mortality (dependent variable) with changes in Cr concentration (independent variable) for trials 1, 2 and 3 and 4, 5 and 6.

Evaluation of the systems tested

At the end of the trials, the presence of Cr in the substrate of beds 1, 2, 3, 4, 5, 6 and also in the worms of each bed was determined by atomic absorption spectroscopy. With these values, it was possible to establish that the % of Cr that was diminished with the earthworm composting in the substrate and along with data of mortality, presence of juveniles and cocoons was identified the test that provided the best conditions. Furthermore, the bioconcentration factor of Cr in worms was established using the quotient of the concentration data of Cr in worms and in the initial substrate with which the approximate values of the assimilation of Cr in *Eisenia foetida.*

RESULTS

As for phytoremediation with Vetiver (*Vetiveria zizanioides*), after a total of 70 days of the test, 4200 mg. L^{-1} of Cr^{+3} (3918 mg .kg^{-1}) was achieved at 3735 mg .kg^{-1} of total Cr in The roots of Vetiver which is equivalent to say that 95.3% of the initial Cr was recovered in phytoremediation. Worm measurements were also made on all beds and their replicas; this information is summarized in Table 3.

Table 3. Synthesis of average measurement per worm and for each trial bed

Bed	Substrate Characteristics	mg .kg^{-1} of total Cr in the initial substrate	Mortality	Length, cm	Weight g	Diameter, mm	# of Juveniles	# of cocoons
1	CAR	261.5	7	8.50	0.550	2.94	31	25
2	CAR	186.8	3	9.25	0.558	2.96	33	25
3	CAR	149.4	2	9.25	0.560	3.06	2	27
4	CR	326.8	13	8.75	0.422	2.63	0	0
5	CR	233.4	11	9.00	0.413	3.00	2	7
6	CR	186.8	9	8.00	0.328	2.50	9	4
7	CA	0.0	2	7.89	0.391	3.00	11	5
8	C	0.0	4	8.32	0.406	3.18	8	4

CAR: composting + food + roots with Cr
CR: composting + roots with Cr
CA: composting + food
C: composting

For trials with food (beds 1, 2 and 3), the highest mortality occurred when the initial concentration of total Cr was higher; in terms of length, weight and diameter, these were lower at higher concentration of Cr. For trials without food (beds 5 and 6) this trend was maintained. As for the presence of juveniles and cocoons, trial 4 was zero, which is indicative of the affectation due to the concentration of Cr. In order to the obtained results, an analysis of variance ANOVA was carried out by the comparison of the two independent variables with all the dependent variables together and the synthesis of what it was found is shown in table 4.

Table 4. Two-factor ANOVA and several samples per group

ANALYSIS OF VARIANCE(Beds and Cr concentration with Deaths, Length, Weight, Diameter, Juveniles, Cocoons)				
Origin of variations	F	P	$F_{critical}$	Analysis
Beds (with food and without food)	1,123	0,302	4,351	$F < F_{critical}$ Accepts Ho
Different concentra-tions of Cr	69,13	0,000	2,866	$F > F_{critical}$ Rejects Ho
Interaction (Beds and Concentration)	0,854	0,508	2,866	$F < F_{critical}$ Accepts Ho

From this first result, it is established that the alternative hypothesis is accepted for the use of different concentrations. In other words, that the concentrations have an effect on the dependent variables, whereas in the case of earthworm compostingbeds and the interaction between the beds and the chromium concentration, the null hypothesis is accepted, in which there are no significant differences associated with the different beds and also with the interaction between independent variables. Then, it was compared the effect of the two independent variables with each of the dependent variables and found that the initial trend. It was also performed the separate ANOVA first between the concentration of Cr and each dependent variable and then between the beds and each dependent variable and the results are shown in Table 5.

Table 5. One-factor ANOVA and one sample per group

ANALYSIS OF VARIANCE (Cr concentration and each dependent variable), (Beds and each dependent variable)			
	Cr Concentra-tion	Beds	
	F	F	$F_{critical}$
Mortality	68,6986	4,2478	
Length	68,1957	44,977	
Weight	73,5715	15,676	
Diameter	72,0156	0,7174	4,9646
Juveniles	62,1562	2,2368	
Cocoons	62,2303	4,8530	

In this analysis, it is reaffirmed that the concentrations produce effect in all measured dependent variables; in the case of beds, it can be established that there are no significant differences for mortality, diameter, juveniles and cocoons, but they do exist for length and weight. In addition, the PROBIT analysis was carried out to establish the effect of Cr concentration on *Eisenia foetida*. What was found after 56 days of exposure of the worms to the Cr is presented in Table 6.

Table 6. Results of the PROBIT analysis

Concentrationeffectson*Eiseniafoetida*	Trials 1, 2, 3	Trials 4, 5, 6
CL_{50} mg. kg^{-1} Cr total	342.2	262.6
Confidence range of mg. kg^{-1}Cr total in upcoming trials	220.3 a 679.7	204.1 a 333.8
NOEC mg. kg^{-1}Cr total	15.50	64.13
LOEC mg. kg^{-1}Cr total	149.3	172.3
Confidence limit below 95% mg. kg^{-1}Cr total	280.0	243.6
Limit of confidence higher than 95% mg. kg^{-1}Cr total	740.9	284.8

From the statistical point of view, by means of CENTURION XVI were found several models to represent the behavior of mortality with respect to the concentration of Cr and those that best fit for each type of trial, are presented in Tables 7 and 8.

Table 7. Results of Trials 1, 2, 3

Trials 1, 2, 3

Simple Regression
Mortality vs. Cr Concentration
Dependent variable: Mortality (Deaths/15)
Independent variable: Concentration Cr, mg. kg^{-1}
Square Root-Y Square-X:

$$Y = (a + bX^2)^2$$

Equation of the adjusted model

$$Y = (0.207569 + 0.00000694664\,X^2)^2$$

Where:
Y: No. Individuals dead/15
X: Cr Concentration
Coef. Correlation 0.9999
R-squared 99.82%

Table 8. Results of Trials 4, 5, 6

Trials 4, 5, 6

Simple Regression
Mortality vs. Cr Concentration
Dependent variable: Mortality (Deaths/15)
Independent variable: Concentration Cr, mg.kg^{-1}
X-Reverse Square-Y:

$$Y = \sqrt[2]{\left(a + \frac{b}{X}\right)}$$

Equation of theadjustedmodel

$$Y = \sqrt[2]{\left(1.273\ 99 - \frac{1\ 711\ 29}{X}\right)}$$

Where:
Y: No. Individuals dead / 15
X: Cr Concentration
Coef. Correlation -0.9993
R-squared 99.86%

In figures 1 and 2, the adjusted models.

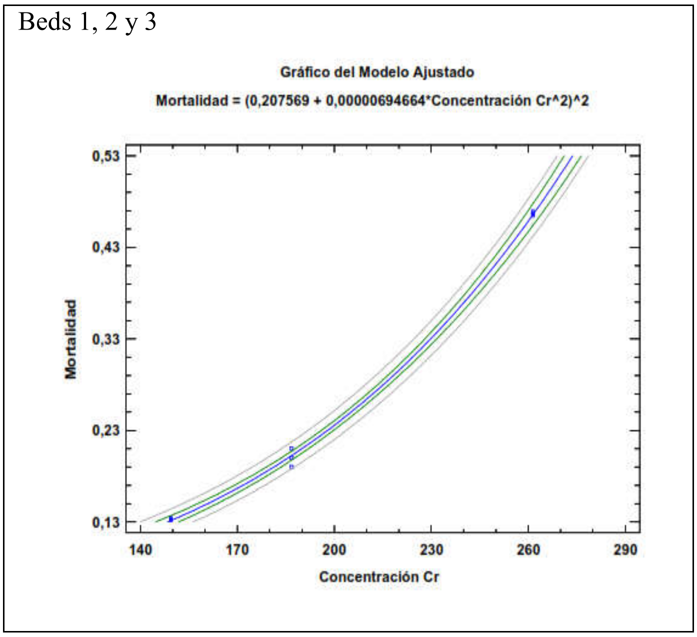

Figure 1. Adjusted model for beds 1, 2 and 3

The final concentration of total Cr in the substrate of all experimental beds and the concentration of Cr in the worms of each bed were also obtained, which determined the bioconcentration factor of Cr in the worms and the percentage of decrease of Cr in the substrate. This information is presented in Table 9.

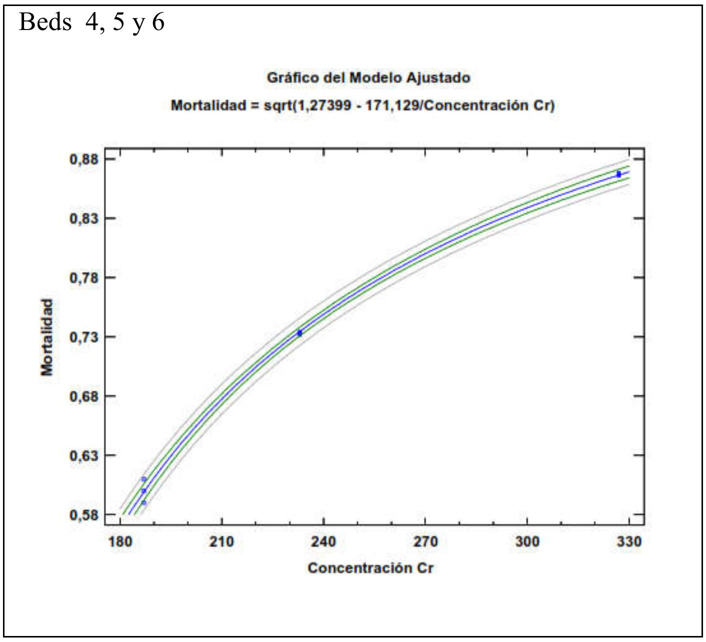

Figure 2. Adjusted model for beds 4, 5 and 6

Table 9. Chromium data in the trial

Bed	A	B	C	D	E
1	261.5	82.8	59.2	0.226	68.3
2	186.8	54.4	47.1	0.252	70.9
3	149.4	42.7	41.0	0.274	71.4
4	326.8	40.2	34.9	0.107	87.7
5	233.4	41.0	40.4	0.173	82.4
6	186.8	20.0	26.8	0.143	89.3
7	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0

A: mg .kg^{-1} of total Cr in the initial substrate
B: mg .kg^{-}of total Cr in final substrate
C: mg .kg^{-}of total Cr in worms
D: Bioconcentration factor of Cr in the worm
E: % Cr decrease in substrate

For beds 1, 2 and 3, it was found that at higher concentration of Cr in the initial substrate (column A), higher concentration of Cr in worms (column C). A similar behavior was found for beds 5 and 6 despite of having different conditions for being a trial without food addition. Bed 4 for having the most aggressive conditions for the survival of the worms, obtained the lowest value in terms of Cr concentration in them. As regards the Cr decrease percentage of the initial substrate (column A) to the final substrate (column B), trials 1, 2 and 3 obtained very close removal percentages. With the same trend, beds 4, 5 and 6 also had similar decrease percentages. In respect of the bioconcentration factors of Cr in the worms all were within the same range within the trials: between 0226 and 0 274 for beds 1, 2 and 3 (compost + roots + food) and between 0.107 and 0.143 in the Beds 4, 5 and 6 (compost + roots). The latter ones were lower because of direct contact with Cr by the worms and their metabolism was affected.

DISCUSSION OF RESULTS

In relation to phytoremediation, it is convenient to implement the rhizofiltration through a subsurface flow wetland planted with Vetiver since good percentages of removal of the Cr in solution are obtained; in this study the phytoremediation managed to retain 95.3% of the initial Cr. In the case of the earthworm compostaing, the best trial yielded 70.9% of the decrease of the Cr concentration in the substrate, which makes it a very good complement to the phytoremediation previously performed. With regard to ANOVA, it is established that there is a significant effect of Cr concentration on all dependent variables and in terms of beds, it is established that they have no significant effect on mortality, diameter, presence of juveniles and cocoons but does have a significant effect on length and weight. And when analyzing the interaction between independent variables, the results show that it is not the interaction between these two variables that causes the changes on the dependent variables. Regarding the values obtained by the PROBIT analysis, the concentration obtained for CL_{50} in beds 1, 2 and 3 as expected was higher than for trials 4, 5 and 6 (342.2 mg . kg^{-1}Cr versus 262.6 mg . kg^{-1}Cr). This, due to the conditions of this trial include in addition to Cr in the roots the possibility of food for the worms with which their conditions of survival will be better. The determination of the range within which concentrations may be located in future trials and which will provide a basis for the experimental design that can be adjusted under trial conditions with Cr and worms yielded a wider range of work (between 220.3 and 679.7 mg.kg^{-1}) for beds 1, 2 and 3 than that obtained for beds 4, 5 and 6 (between 204.1 and 333.8 mg.kg^{-1}) and this is due to the characteristics of these tests. The NOEC value (highest concentration in which no effect was observed, in this case mortality) had a lower value for trials 1, 2 and 3 (15.50 mg.kg^{-1}) because in these trials Cr was mixed with the food as opposed to trials 4, 5 and 6 (64.13 mg.kg^{-1}) where the Cr had more direct contact with the worms. The same trend was observed for LOEC values (lowest concentration in which effect was observed) with a higher value for trials 4, 5 and 6 (172.3) and lower value for trials 1, 2 and 3 (149.3)

Regarding the confidence limits, lower and higher than 95% for the concentration of Cr in mg.kg^{-1}, a broader range (280.0 to 740.9) was obtained in trials 1, 2 and 3 compared to the range of trials 4, 5 and 6 (243.6 to 284.8). Moreover, this trend is maintained with respect to what has already been discussed in terms of the ranges where concentrations can be set for future trials. The equations of the adjusted model to predict mortality as a function of the concentration of Cr in mg.kg^{-1}found are characterized by their high values in the correlation coefficient indicating a relatively strong relation between the variables and as for the statistic R-squared indicates that the adjusted model explains more than 99.8% of the variability in mortality. Removal of Cr in the substrate by the worms at the end of the trials yielded better values of the% decrease for beds 4, 5 and 6 and this because the worms had no option of another food besides the composting. However, when analyzing the aspects that have to do with the tolerance, mortality and conditions of reproduction of worms, none of these three trials turns out to be viable to be a long-term treatment. In addition, the bioconcentration factor of Cr in worms accumulated in 56 days of exposure at different concentrations was determined and, except for the trial 4 that proved to be very aggressive for worms, values between 0.143 and 0.274 were found. It is worth noting that the results indicate that this is the range around which the equilibrium concentration of Cr in the worms rotates and that corresponds with results presented by (Marco *et al*., 2010) in studies with Cr and *Eisenia foetida* values less than one are reported. Regarding the evaluation of the results of beds 1, 2 and 3, it is established that bed 2 is the best option, since it presents the lowest mortality, the longest length, a good value of weight and diameter, the highest number of juveniles, a high number of cocoons and a good Cr% decrease value of the substrate. From trials 4, 5 and 6 there is no continuity or trend in the results that allows one to lean towards one of them; even the results of test 4 were unfavorable. As it presented the highest value of mortality, there were no juveniles or coconuts and although the percentage of removal of Cr from the substrate was the highest, it is not a viable trial. It is noteworthy that the values of length, weight and diameter of worms in beds 1, 2 and 3 were higher than those obtained for their respective controls, and in the case of trials 4, 5 and 6, a behavior is not clearly established.

Conclusions

- The phytoremediation trial showed to be a very good option for the biotransformation of Cr, retaining in its roots 95.3% of the initial Cr.
- The earthworm composting of Vetiver roots with Cr content allowed to reach Cr decrease percentages in the substrate for the most viable trial of 70.9%.
- It was identified that the changes in the measurement of the dependent variables were caused by the different concentrations of Cr in beds and not by the interaction of the different beds and concentrations.
- For the *Eiseniafoetida* tolerance tests for the presence of Cr, CL_{50}, NOEC, LOEC and confidence limits were found for the two types of test (with food and without food) that determine beds 1, 2 and 3 as the most viable trials.
- It was found that bed 2 had the best results in terms of worm development, number of deaths, presence of juveniles and cocoons.
- It was possible to establish that the Cr bioconcentration factor in worms was inversely proportional to Cr concentration in the initial substrate in beds 1, 2, 3, 5 and 6.
- Even under extreme conditions of high concentration Cr, *Eisenia foetida* has the ability to bioconcentrate the Cr in its body with values between 0.143 and 0.274, which could become a reference number for comparison with other trials involving Cr and *Eiseniafoetida*.
- The behavior model found for the different trials was characterized by correlation coefficients of ± 0.999 and the R-squared statistic explains more than 99.8% of the changes in mortality.

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