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Vermicomposting process apllied in tannery waste and after evaluation of Cr in onion cultive.

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ABSTRACT

Tanneries are an important piece to garments industry and economy, however, the wastewater produced by these matrix may contain aggressive chemical components, heavy metals such as Chromium, to the environmental and consequently to humans. The chemical treatment for the tanneries wastes are very expensive. In this sense, the vermiculture, the organic waste material degradation process with action of micoorganisms and earthworms, can be a cheaper alternative. In spite of this, the objetive of this paper was evaluate the earthworms use (*Ensenia andrei, Eudrilus eugenia, Perionyx excavatus*) to perform vermicomposting process in tannery sludge and after use of the humus in onion plantation. For this, several waste/soil concentration were tested to the best condition of earthworms transform the waste in the humus. After, the humus containing Cr was used in onion plantation. The samples, after growing, were harvested, cleaned, measured and dried. The roots and shoots were separated for Cr bioavailability analyses. Statistical analysis were made. As a result, it was possible vermicomposting process using tannery waste, transforming the waste in a fertilizer and onion cultive was made using different proportion of humus and substrate. A reduction of Cr concentration in waste using humus from *Ensenia andrei* earthworm has been detected, and also a reduction of the concentration in the plant's leaves. In conclusion, it is possible to reuse tanneries wastewater after vermicomposting process and the best concentration substrate:humus to onions plantation was 60:40, respectively. The vermiculture was considered an efficient process to reuse the tannery waste and there was a reduction concentration of Cr bioavailability in the onions leaves.

INTRODUCTION

Tannery industries contribute with the country's economy, but has been extensively examined because during the process, it need adition of a lot of chemical and their wastewaters are characterized by pH elevation with high chemical oxygen demand (COD), biochemical oxygen demand (BOD), and presence of chromium (Central Leather Research Institute, 2008). Chromium in form of chromium sulphate is used as tanning agent. (Srivastava *et al.*, 2007). Cr can be toxic to living organisms, depending the concentration, and it can reach human beings through food chain which can cause many diseases and problems for humans health (Iyer and Mastorakis, 2010). The major of tannery industries usually discharge their wastewater effluents to large wastewater treatment plants using chromium precipitation process. This treament is expensive, and some papers have been showing some treatment to reduction of this metal in their wastewater effluents, such a physicochemical methods available for the treatment of tannery wastewater (Malaviya and Singh, 2011);

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(Montañes *et al*., 2014); (Srivastava *et al*., 2007), but they have one or other shortcomings. Some papers show the treatment of reduction of chromium using biological tretament with microorganisms (Kovacevic *et al*., 2000); (Alibardi and Cossu, 2016); (Sharma and Adholeya, 2011). In this sense, vermicomposting has been a cheap stabilization process of organic matter using interaction between microorganisms and earthworms (Villar *et al*., 2016); (Domínguez, 2004). This process has been successfully applied on the treatment of several wastes (Villar *et al*., 2016), (Bhattacharyaa *et al*., 2012), (Huang *et al*., 2014), transforming in a bioproduct used for the organic fertilizer for agricultural applications (Tajbakhsh *et al*., 2011); (Mainoo *et al*., 2009); (Huang *et al*., 2013). The vermicomposting can be used for removal some elements (Morand *et al*., 2011), including heavy metals (Gogoi *et al*., 2015). But there is no paper showing the heavy metals after vermicomposting process used in vegetables. Then, the aim of this paper was evaluate the earthworms use (*Ensenia andrei, Eudrilus eugenia, Perionyx excavatus*) to perform vermicomposting process in tannery sludge and after use of the humus in onion plantation, for evaluation of Cr bioavailability in the vegetable.

MATERIAL AND METHODS

Samples:

Tannery waste was adquired in the final process of the local tannery industry, localized in the Vale do Taquari Region, RS, Brazil. The waste was characterized about C, N, K, P and heavy metals and reserved for further analysis.

Firstly, it was necessary evaluated the best proportion of soil and tannery waste for the earthworms made the vermicomposting process. For this, test using plastic boxes were used, containg 1 kg of different proportion: 0:100 ; 20:80; 40:60; 50:50; 60:40; 80:20 and 100:0 of tannery waste: soil, respectively.

The soil used for mix tannery waste for the vermicomposting process was classified such as red latossolo.

Behavioral avoidance tests were made with the 3 earthworms species: *Ensenia andrei, Eudrilus eugeniae, Perionyx excavatus.* Avoidance tests were performed following the guidelines established by ISO 17512-1 for earthworms (ISO, 2008). Earthworms with well-developed clitellum and 10-12 day old were selected for the test. The earthworms were maintained in laboratory cultures under a photoperiod of 16 h light and 8 h dark at 20 ± 2 ºC. Rectangular plastic containers (length 20 cm; width 12 cm; height 5 cm) were used, respectively, for all species earthworms. In both tests, containers were divided with cardboard in two sections that were filled on the side: amounts of different proportion of soil: tannery waste previoulsy described above and the other side it were filled with just soil red latossolo. Five replicates per combination were performed. After soil addition the cardboard division was removed and 10 earthworms, previously washed and dried with absorbent paper, were carefully placed on the midline of each container. To keep individuals from escaping and to reduce water loss, the test containers were covered with a lid (Matos-Moreira *et al*., 2011). For test containers with earthworms, a few holes were drilled in the lids to allow aeration. The tests were run for 48 h at 20 ± 2 ºC with a photoperiod of 16 h light and 8 h dark. At the end of the exposure time, the cardboard division was reinserted on the middle line of each container and individuals of both sections were counted.

Humus production:

The best condition of behavioral avoidance tests for all species it was 20:80 of tannery waste:soil, respectively. The total tests for the humus production occurred in 18 plastic boxes (20 x 12 x 5 cm). In each boxes it was containing: 200 g of tannery waste and 800 g of soil with cow manure and 20 earthworms of each species. Then, this condition was apllied for the vermicomposting process for 60 days. The soil and the tannery waste mix were analysed about chromium total in the inicial and in the final of vermicomposting.

Onion cultive using humus by tannery waste:

After process of vermicomposting with soil and the tannery waste mix, the humus was used in onion (*Allium fistulosum* L) cultive. For this, there was prepared a mix containing soil and cow manure and leaves (substrate) and the humus of tannery waste after vermicomposting, in accordance with the Table 1.

Table 1: Treatment with proportions of substrate:humus for onion cultive

Treatment	Worm specie	Proportion substrate:humus, respectively
T1Ea	*Ensenia andrei*	100:0
T2Ea	*Ensenia andrei*	80:20
T3Ea	*Ensenia andrei*	60:40
T4Ea	*Ensenia andrei*	40:60
T5Ea	*Ensenia andrei*	20:80
T6Ea	*Ensenia andrei*	10:90
T7Ea	*Ensenia andrei*	0:100
T1Ee	*Eudrilus eugeniae*	100:0
T2Ee	*Eudrilus eugeniae*	80:20
T3Ee	*Eudrilus eugeniae*	60:40

T4Ee	*Eudrilus eugeniae*	40:60
T5Ee	*Eudrilus eugeniae*	20:80
T6Ee	*Eudrilus eugeniae*	10:90
T7Ee	*Eudrilus eugeniae*	0:100
T1Pe	*Perionyx excavatus*	100:0
T2Pe	*Perionyx excavatus*	80:20
T3Pe	*Perionyx excavatus*	60:40
T4Pe	*Perionyx excavatus*	40:60
T5Pe	*Perionyx excavatus*	20:80
T6Pe	*Perionyx excavatus*	10:90
T7Pe	*Perionyx excavatus*	0:100

TEa: treatment using *Ensenia andrei*
TEe: treatment using *Eudrilus eugeniae*
TPe:treatment using *Perionyx excavatus*

The onion seed were adquired by commercial local. It were selected and put in the plastic vessels containing 25 g of the mix of proportion substrate:humus, respectively. The experiment was conducted in a greenhouse. The process of growing was about 60 days.

During the experiment, samples of this mix (substrate and humus proportions) were colected and Cr was analysed in the 1° and 60° days. In the final of the 60 °C, the plants were harvested, cleaned, measured with a caliper, weighed (for the wet weight determination) and dried (at 65°C) for 24 h (for the dry weight determination). The roots and shoots were separated for further Cr analysis.

Sample preparation for Chromium determination:

All chemicals were analytical-grade reagents, and ultrapure water (MilliQ, Millipore, USA) was used for the preparation of solution. For Cr determination 0.5 g of dried sample was digested with 6 mL of HNO_3 (Merck) and 2 mL of HF (Merck) in closed vessel of microwave oven (Anton Paar, model Multiwave PRO). Each sample was analyzed in triplicate. The samples were digested in a microwave digestion system, according the following program: step 1 (time: 10 min, temperature until: 120 °C), step 2 (time: 10 min, temperature in 120 °C). The digested samples were filtered and then transferred to a polypropylene tubes whose total volume was made up to 50 mL by adding ultrapure water. The samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer, model Nexion 300x) The detection limits of ICP-MS was 0.06 ng/L for Cr. In order to satisfy the defined internal quality controls (IQCs), each sample was made to run, including blank and standard addition. Standard stock solutions containing 10 mg/L of Cr (Perkin Elmer), were prepared and used for the calibration curve (Shaheen *et al*., 2016). Statistical analysis were made using ANOVA (on way) and Tukey test with $p<0.05$.

RESULTS AND DISCUSSION

The table 2 shows the characterization soil and tannery waste used in the vermicomposting process.

Table 2: Vale do Taquari (utisol) Region physical and chemical characterization soil and tannery waste.

Parameter	Soil	Tannery sludge
Organic Carbon	0.89 g kg^{-1}	2.08 g kg^{-1}
Total Chromium	84.2 mg kg^{-1}	9772 mg kg^{-1}
Density	1.21 g cm^{-3}	0.75 g cm^{-3}
pH	7.1	6.8
Humidity	26.2%	54.4%
Cadmium	1.01 mg kg^{-1}	<DL*
Lead	9.24 mg kg^{-1}	0.83 mg kg^{-1}
Copper	84.9 mg kg^{-1}	17.5 mg kg^{-1}

*DL: Detection limit of the Flame atomic absorption Spectrophotometry equipment (DL to total chromium 0.01 mg kg^{-1})

The results shows high Cr concentration in tannery waste. The soil parameters concentration were similar to native soil (Lozano-García *et al*., 2016), or with poor soils regions (Inboonchuay *et al*., 2016).

The Table 3 shows OC, N total and pH characterization before and after the best vermicomposting proportion.

Table 3: Organic Carbon, Total N and pH analysis of the samples after vermicomposting process in different treatments with *Eisenia andrei, Perionyx excavatus e Eudrilus eugeniae* earthworms.

Parameter	Control		*Eisenia andrei*		*Perionyx excavatus*		*Eudrilus eugeniae*	
	0 days	60 days	0 days	60 days	0 days	60 days	0 days	60 days
OC[1]	71±1	64±1	71±1	60±2	71±1	67±1	71±2	66±1
Total N[2]	0.3±0.01	0.4±0.01	0.3±0.01	0.3±0.01	0.4±0.01	0.3±0.01	0.4±0.01	0.4±0.01
pH	6.5±0.2	6.5±0.2	6.5±0.1	7.4±0.2	6.5±0.1	7.4±0.1	6.5±0.1	6.8±0.1

[1]Organic Carbon, quantified in mg kg^{-1}; [2]Total Nitrogen, quantified in mg kg^{-1}

The result were in acordance with the literature, these species are easily adapted in different media and there a loss of carbon and nitrogen total after vermicomposting process because of the fixing the N and C in the substrate in form of stable elements to further use in fertilizers (Domínguez, 2004).

Table 4 shows the results of Cr concentration before and after the vermicomposting process.

Table 4: Cr determination before and after the vermicomposting process with 20: 80 tannery waste: soil, respectively using three earthworms species.

Days	Control soil			*Eisenia andrei*			*Perionyx excavatus*			*Eudrilus eugeniae*		
	0	30	60	0	30	60	0	30	60	0	30	60
Total Cr (mg/Kg)	86±2	82±4	82±3	998±6	760±7	535±9	1070±9	835±8	575±5	1080±7	855±9	705±8

It was observed reduction of chromium concentration in the final of the process of vermicomposting. The best reduction was using vermicomposting with *Ensenia andrei* eathworm. It was in accordance with Domínguez, 2004, because the specie Ensenia andrei has facilities to adaptation in different wastes and produce a good fertilizer product.

For onion cultive using substrate:humus proportion, Figure 1 shows Cr concentration in the leaves, using vermicomposting of different species.

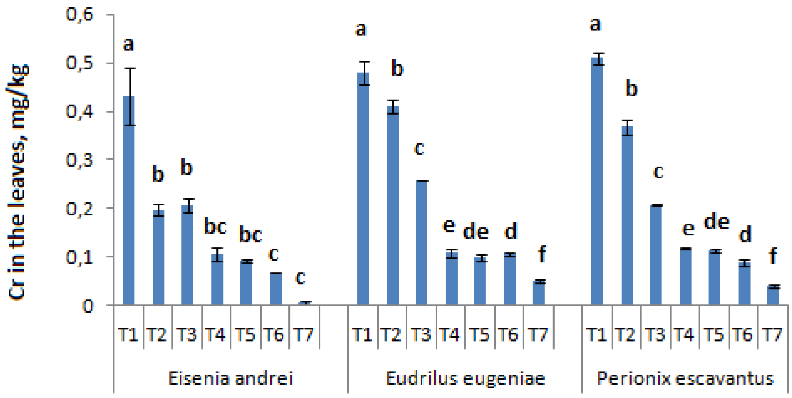

Fig. 1: Cr concentration in the leaves, using vermicomposting of different species.

TEa: treatment using *Ensenia andrei*
TEe: treatment using *Eudrilus eugeniae*
TPe:treatment using *Perionyx excavatus*

In according the results, it was notice that from to T2 test, using substrate by *Ensenia andrei*, there was Cr concentration by half reduction in the leaves. In comparation of the substrate used with *Eudrilus eugenae* and *Perionix escavantus*, from T3 there were reduction by half in comparation with T1. The Figure 2, shows Cr concentration in the onion roots.

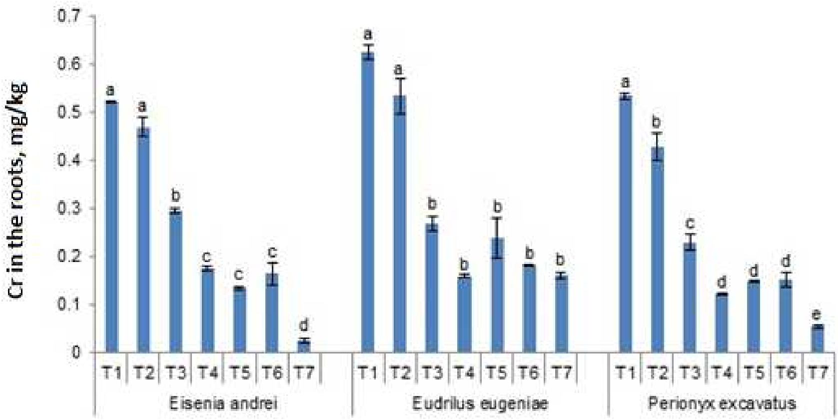

Fig. 2: Cr concentration in the roots, using vermicomposting of different species

TEa: treatment using *Ensenia andrei*
TEe: treatment using *Eudrilus eugeniae*
TPe:treatment using *Perionyx excavatus*

It was observed the Cr high concentration in T1 and according increasing the humus in the subtrate, there was Cr reduction. In T3 treament, Cr decrease by a half Cr concentration in comparation with T1. It probability occurs because the earthworm could reduce the Cr mobile fraction. Furthermore, these results indicated that vermicomposting played a positive role in stabilizing Cr in the treatment of tunnery wastes. These results were in accordance with works using vermicomposting to stabilizing heavy metals (Lv *et al*., 2016; Soobhany *et al*., 2015).

There was a Cr reduction concentration in onion cultive, when it was increasing the humus proportion in the soil. Presumably by forming organic-bound complexes with the Cr. In despite of other onion characterization (pH, height, wet and dry weight determination) there is no statistical difference using from T1 to T7 tests.

Conclusion:

The present study inferred that it was possible reaproved tannery waste using vermicomposting process, because it contents nutrients, and it reduces Cr bioavaliable fraction for the onion cultive. The best treatment for use onion cultive was from T3 (60:40 substrate:humus, respectively). The vermicomposting was considered an efficient process, tranforming the waste in a fertilizer.

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