International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(6): 185-189

Received: 16-09-2018
Accepted: 17-10-2018

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Characterization of Bantala tannery sludge and its vermicompost

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Abstract
Wastes generated from different tanneries are subjected to high risk of contamination due to the presence of toxic Cr (VI) that poses a serious threat to the environment and human's wellbeing. It is very important that tannery waste in the form of sludge should be managed in an environmentally sound manner. The present endeavor had been undertaken to characterize the Bantala tannery sludge sample with respect to its chemical, microbiological and biochemical properties. Data revealed that the sludge was a rich source of plant nutrient with high enzymatic activities except its high concentration of chromium. Vermicomposting of the tannery sludge mixed with cow dung at different proportions (1:1 and 3:1) using *Eisenia fetida* suggested that the vermicompost could be a good source of plant nutrient with effective enzymatic activities and reduced concentration of chromium. The stability and maturity study of the vermicompost suggested that the vermicompost with ratio 1:1 was stable and mature compost than the ratio 3:1. The result inferred that vermicomposting of the tannery sludge could be a suitable way to reduce the high concentration of chromium of the raw tannery sludge.

Keywords: Tannery sludge, Vermicomposting, Stability and maturity, *Eisenia fetida*

1. Introduction
The leather industry has always been a very important part of country's economy due to its high demand in daily life. The process of tanning of hides includes several mechanical and chemical stages. This generates huge amount of solid wastes and wastewater that leads to the deposition of toxic chromium salts used during tanning. (Familec *et al*., 2011) [13]. According to the data received from different studies, approximately 200 kg of leather is manufactured from 1 tone of wet-salted hide. This amount constitutes about 20% of rawhide weight. More than 600 kg of solid waste is generated during the transformation of rawhide into leather. Thus, solid wastes containing protein and fat that constitute more than 60% of rawhide weight are disposed to the environment by leather factories without turning them to good use (Ozgunay *et al*., 2007) [27]. This is a matter of concern for the environment. Worldwide, tanneries use the conventional systems for treatment of the mixture of all effluents. Hence, the production approach makes it possible to meet the environmental regulations. But the high cost of the various treatment processes makes the implementation scarce, mainly in the developing countries (Nacheva *et al*., 2004) [22, 24].
The Bantala leather complex which is 20 Kms from Kolkata, India, on its south-eastern periphery is a living hell. The smell of the chemicals used to treat leather hangs heavy and sickens one to nausea. The water canal gets choked with rotting animal hair, fat and the omnipresent plastic. The visible canal water often gets crimsoned with blood, dyes or chromium and even shines in grimy bubbles.The burning and boiling of shaving dust, flesh linings and trimming are often used to serve as fertilizers and fish feeds. The chromium content in these can pollute surface water and also can leach down to contaminate ground water. The supply of water laden with chemicals and salts in the surrounding farming lands has devastatingly reduced paddy yield (Bera, 2013) [9]. Data regarding the characterizations of the Bantala tannery sludge and its suitable utility for agricultural purpose is very scarce.
Characterization of chromium rich tannery waste related to its chemical composition, total organic carbon and extraction of salt present in the waste has been done on waste supplied by a leather industry from the city of France, South-eastern Brazil (Abreu and Toffoli, 2009) [1]. Leather wastes and semi-processed leather trimmings and shavings provided by the

Environmental protection agency of the Valencian Government, Spain have been characterized to determine its moisture level, reactivity, toxicity, etc., (Sempere *et al*., 1996).Chemical analysis of the waste generated from manufacturing of garment sheep skins, shoe upper goat skins, shoe upper hides and sole leathers from different factories of Turkey has been done to obtain data that can be used for new management and evaluation. (Ozgunay *et al*., 2007) [27].

Vermicomposting could be a possible way of treatment of the chromium contaminated tannery sludge. A study was carried out where animal fleshing (ANFL) generated as solid waste from tannery industries was vermicomposted using the epigeic earthworm *Eisenia fetida*. The results obtained from the study showed that the earthworm *Eisenia fetida* was able to convert ANFL into nutrient-enriched products. (Ravindran *et al*., 2008) [28].

It was of immense need to start a comprehensive study on different profiling of tannery wastes of Bantala tannery to obtain data for management and evaluation methods and to come out with possible remediation for a protective and healthy environment and maintenance of the agricultural yield. Thus, the present study was carried out with the objective of chemical and microbiological characterization of the tannery waste and to make an effort to reduce the chromium concentration of the raw tannery sludge through the process of vermicomposting.

2. Materials and methods

2.1 Collection of Sample from Bantala Leather Complex

The study site was situated at Calcutta Leather Complex, Bantala, East Kolkata, West Bengal, India (22.5073°N and 88.5329°E). Four plots of the area were selected for the present study that had been dumped with the solid tannery sludge. Portions of the sludge were collected from the four plots (W1, W2, W3 and W4) during December 2016. The sludge samples were brought to the laboratory in the field moist condition. The samples were made deprived of the unwanted plant debris and plastic or other unwanted materials and were sieved (<2mm) prior to physical and microbiological studies. Part of the sludge samples were dried and powdered prior to chemical profiling of the solid tannery sludge. Each of the analysis was made in four replicates.

2.2 Vermicomposting of Tannery Sludge with cow dung

The vermicompost was prepared at the Agricultural Experimental Farm (22º22´ N and 86º26´E) of the Calcutta University, Baruipur, South 24 Parganas, West Bengal, India. The collected tannery sludge from the four plots was compiled together, watered to retain 60% moisture and were kept overnight. Urine free cowdung was obtained from farmer's cattle shed adjacent to the experimental farm. The moist tannery sludge sample was mixed with cow dung at 3:1 and 1:1 ratio (tannery sludge: cow dung) respectively. Two different vermicomposting pits of 0.75 X 0.75 X 0.25m^3 sizes were made under covered shade at the farm and were filled with mixture of tannery sludge and cow dung in the stated ratio for pre decomposition. The mixtures were kept and watered regularly for 30 days. They were turned over on daily basis to eliminate the pungent gases before the release of the earthworms. Healthy, juvenile earthworms *Eisenia fetida* were collected from the stock culture of the Experimental Farm, Baruipur, University of Calcutta which were released on the bed at the rate of 20 earthworms / square feet. The appropriate moisture (60%) was maintained during this vermicomposting process by periodical sprinkling of an adequate quantity of water till the maturity of final product (Ahmed *et al*., 2007; Ravindran *et al*., 2008; Singh *et al*., 2011) [2, 28, 32].

Samples from two different pits (VC 1:1 and VC 3:1) were collected for its characterizations. It was observed that the process of composting was completed within 60 days after predecompostion of the waste.

2.3 Chemical Properties

The pH and EC of the samples was measured using a digital pH meter and EC meter (Systronics) in 1:2.5 (w/v) aqueous solutions for all the samples (deionized water). Total organic carbon (TOC) was estimated by the method of Walkley-Black (Walkley and Black, 1934) [33]. Total Kjeldahl nitrogen (TN) was measured using the method described by Jackson (1958). Available phosphorus was measured according to Olsen *et al.* (1954) [26]. Total chromium was estimated through tri-acid digestion and determined by atomic absorption spectrophotometer (AAS) (Instrument: Varian AA240FS; Flame atomizer. Software: Work sheet Oriented AA software; Version 5.1 pro). Homogenized sample of 10g were taken in digestion tube and 20ml tri acid mixture of nitric acid, perchloric acid, sulphuric acid (HNO_3:$HCLO_4$:H_2SO_4 :: 10:4:1) were added to the sample. Digestion was done at 150ºC for 1hr and then the temperature was raised to 250ºC for digestion until appearance of white fumes. The sample was cooled and filtered through Whatman No. 1 filter paper along with a small amount of distilled water (Haroun *et al*., 2007 and 2009) [15, 16]. Available micronutrients of the samples which included Fe, Mn and Cu were measured by the DTPA extracting solution (0.005 (M) DTPA, 0.01(M) $CaCl_2$, and 0.1 (M) TEA followed by AAS analysis of samples.Soil of 10gm was weighed and 20ml of the DTPA extractant was added to it. The solution was under continuous shaking for 2hrs on a horizontal shaker and filtered through Whatman no.42 filter paper.10 standards using DTPA as the matrix for each element was prepared with a range 0 to 3 ppm for copper and from 0 to 20ppm for iron and manganese. Readings were taken first for the standards and then measured the element from the filtrate by AAS (Instrument: Varian AA240FS; Flame atomizer. Software: Work sheet Oriented AA software; Version 5.1 pro). Calcium and magnesium was determined by versenate titration method. Flame photometric method was used to determine available potassium (Amir *et al*., 2008) [7].

2.4 Microbiological and Biochemical Properties

The dilution plate technique was implemented to record the total count of a composite sludge sample in nutrient agar plates. The method was also done for the determination of total nitrogen fixers count in Jensen's medium (Döbereiner, 1995) [11] and phosphate solubilizer's count in Pikovskaya agar medium (Aneja, 2003) [8]. Microbial biomass carbon (MBC) was determined by the fumigation extraction method using a correction factor (K_{Ec}) of 0.38 according to Vance *et al*. (1987) [31]. The basal soil respiration (BSR) was measured according to the method of Alef (1995a) [4], modified in the following way: Weighed 2g of moist sludge sample and vermicompost samples (1:1 &3:1) and kept within the respiratory flasks. 5ml of 0.1 (M) NaOH solution was taken in a vial and inserted into the flasks and incubated the whole setup at 22 ºC for 24hrs within the BOD incubator. After 24hrs of incubation taken out the vials containing NaOH and quantitatively transferred to a 50ml conical flask with 10 ml distilled water. 5ml of $BaCl_2$ solution was added to it

followed by 3 drops of phenolphthalein indicator. Titrated the samples with 0.05(M) HCl and observed the change in colour. Fluorescein diacetate hydrolysing activity (FDHA) of the sludge samples and vermicompost was measured by shaking the samples for 3hrs at 24°C followed by spectrophotometric analysis using a UV-vis Spectrophotometer at 490nm (Alef, 1995b) [5]. B-glucosidase activity was recorded following the method of Alef and Nannipieri (1995) [3, 6]. Urease and the acid and alkaline phosphatase activities of the sludge samples were also estimated through the respective enzyme activity estimation methods (Tabatabai, 1994) [30].

2.5 Stability and Maturity analysis of Vermicompost

2.5.1 Respiration study

The respiration of the vermicompost samples was determined by the method of (Alef, 1995a) [4]. About 2g of vermicompost sample from each ratio (1:1 and 3:1) was taken into respiration flask and the same procedure was followed as stated earlier in BSR estimation.

2.5.2 Seed Germination Study

Samples from both the ratios of vermicompost were mixed with deionized water 1:10 (w/v) followed by filtration. 10 seeds of paddy *(Oryza sativa*) and 8 seeds of gram (*Cicer arietinum*) were placed in 8cm diameter petridishes lined with filter paper containing 6ml of compost water filtrate. Same procedure was followed for control using 6ml distilled water instead of compost water extract. The plates were placed in an incubator at 25 ºC for 48 hours in a seed germinator. The percentage of relative seed germination (RSG), relative root growth (RRG) and germination index (GI) were calculated as follows (Hoekstra *et. al*., 2002).

$$\text{RSG} = \frac{\text{Number of seeds germinated in vermicompost extract}}{\text{Number of seeds germinated in control}} \times 100$$

$$\text{RRG} = \frac{\text{Mean root length in vermicompost extract}}{\text{Mean root length in control}} \times 100$$

$$\text{GI}(\%) = \frac{\text{RSG} \times \text{RRG}}{100}$$

2.6 Statistical Analysis

Assigning different lots of tannerysludge as treatment factor, analysis of variance (ANOVA) was carried out by Completely Randomized Design (CRD) using SPSS 11.0 statistical package. The least significance difference (LSD) test was applied to evaluate the significance of difference between individual treatment factors. The treatment means were compared by Duncan's multiple range tests at 0.05P.

3. Results and discussion

3.1 Comparative analysis of the physico-chemical parameters of Tannery sludge and Vermicompost

The physico-chemical analysis (Table 1) suggested that the raw tannery sludge depicted an alkaline pH and high EC that indicated towards high salinity stress. The data showed quite an appreciable amount of organic carbon content and high amount of the macronutrients i.e. total nitrogen, available potassium, phosphorous, calcium and magnesium. The physico-chemical analysis of the vermicompost suggested that the samples at both the ratios (1:1 and 3:1) showed neutral pH and a lower EC depicting a lower salt stress than the raw sludge sample. The relative value of total organic carbon had increased but the total nitrogen percentage had decreased that might be due to the amendment of cow dung. The C/N ratio of the raw tannery sludge was less than 10 due to the presence of higher total nitrogen which indicated towards its stability in its raw state. The C/N ratio of both the vermicompost was less than 10 which confirmed the organic waste mineralization and compost maturity (Shak *et al*., 2014). But the stability and maturity analysis of vermicompost through seed germination test gave a confirmatory result in favour of VC 1:1 as mature compost than VC 3:1. Available phosphorus and potassium content was of higher amount in VC (1:1 & 3:1) than the raw tannery sludge. In comparison to both the ratio (1:1 & 3:1) of vermicompost, it was seen that in all cases VC (1:1) showed higher value for the nutrient parameters than VC (3:1).

Table 1: Physico-chemical properties of the Tannery sludge and Vermicompost

Sludge Sample	pH (1:2.5)	EC (dSm^{-1})	TOC (%)	TN (%)	C/N ratio	Avl.P (mg/kg)	Avl. K (mg/kg)	Ca (meq/kg)	Mg (meq/kg)
W1	8.5^{a*}	6.7^{ab}	7.7^{a}	2.49^{ab}	3.09	63^{a}	131^{c}	62.52^{d}	21.27^{c}
W2	8.1^{b}	7.1^{a}	6.9^{b}	2.15^{c}	3.20	52^{b}	139^{c}	70.85^{c}	25.85^{b}
W3	8.0^{b}	6.3^{bc}	7.8^{a}	2.73^{a}	2.85	63^{a}	185^{a}	78.52^{a}	28.87^{a}
W4	8.1^{b}	5.9^{c}	6.5^{b}	2.19^{bc}	2.96	63^{a}	151^{b}	73.50^{b}	19.17^{d}
Vermicompost									
VC (1:1)	7.56	3.5	9.32	1.91	4.87	102	235	55.5	48.5
VC (3:1)	7.28	3	8.52	1.90	4.48	85	198	46.3	55.5

*Figures denoted by same alphabets are statistically similar at 5% Probability level by DMRT

3.2 Comparative analysis of the micro nutrients and heavy metal of Tannery sludge and Vermicompost

The micronutrient content of the sludge sample (Table 2) that included iron, manganese and copper was relatively high. Hence, it could be inferred that the tannery sludge had high containment of plant nutrients. But the excessive high content of total chromium than the standard limit (45mg/L) (Bera, 2013) [9] made it detrimental for use in the agriculture system. Data revealed that preparation of vermicompost with the tannery sludge had depicted a reduction in the total chromium content than the raw tannery sludge. Both VC (1:1) and VC (3:1) could reduce the total chromium content but the value of total chromium in VC (1:1) was lower than VC (3:1). Thus, the vermicompost generated might be implemented in the agricultural field after further field study due to its high nutritional effectiveness as plant manure but the chromium concentration needed to be decreased at a suitable level (Ahmed *et al*., 2007) [2].

Table 2: Characterization of the micro nutrients and heavy metals of the Tannery sludge and Vermicompost

Sample	DTPA Extractable			Total Cr (mg/g)
	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	
W1	16.39^{ab*}	73.45^{b}	0.71^{b}	21.64^{b}
W2	16.34^{ab}	70.49^{b}	0.69^{c}	23.70^{a}
W3	17.77^{a}	78.18^{a}	0.84^{a}	21.47^{b}
W4	14.65^{b}	64.85^{c}	0.75^{b}	18.92^{c}
Vermicompost				
VC (1:1)	12.65	10.09	0.24	8.51
VC (3:1)	14.11	15.69	0.27	10.3

*Figures denoted by same alphabets are statistically similar at 5% Probability level by DMRT

3.3 Comparative analysis of the microbiological and biochemical parameters of Tannery sludge and Vermicompost

The microbiological and biochemical analysis (Table 3) suggested that the tannery sludge was a suitable habitat for high number of microorganisms. The total count study of the tannery sludge samples after 48hours of incubation suggested relatively higher growth of microorganisms. The rate of growth of nitrogen fixers was also relevantly high but the growth of phosphate solubilizer was quite negligible. The high value of MBC and BSR indicated towards effective microbial activity. The presence of such microbial activity was confirmed by the higher values of different enzymatic activity of the sample that included FDHA which indicated towards the presence of the enzymes protease, lipase and esterase. The data also revealed higher level of urease, acid and alkaline phosphatase and β-glucosidase.The microbiological and biochemical analysis of the Vermicompost suggested that the vermicomposting process for the tannery waste had relatively increased its microbial content and their activities. The total count, nitrogen fixers and the phosphate solubilizer's count had shown a high rise in growth. The data indicated towards high affectivity of the compost as a source of plant nutrients in the agricultural field.

Table 3: Microbiological and biochemical properties of the Tannery sludge and Vermicompost

Sample (Tannery waste)	Total count ($x10^6$)	N_2 fixer count ($x10^5$)	P-Solubilizer Count ($x10^5$)	MBC (µg g^{-1})	BSR (µ g CO_2 -C g^{-1} h^{-1}at 22°C)	FDHA (µ g fluorescein g^{-1} h^{-1} at 24°C)	Enzyme activities			
							Urease (µ g urea hydrolyzed g^{-1} h^{-1} at 37°C)	Phosphatase (µ g pnp g^{-1} h^{-1} at 37°C)		β-glucosidase (µ g pnp g^{-1} h^{-1} at 37°C)
								Acid	Alkaline	
W1	65^{b*}	37^{c}	25^{b}	784^{b}	1.46^{b}	699^{b}	121^{b}	480^{b}	775^{b}	136^{b}
W2	60^{c}	42^{b}	21^{b}	699^{c}	1.22^{c}	685^{b}	110^{c}	470^{b}	765^{b}	112^{c}
W3	69^{a}	49^{a}	35^{a}	858^{a}	1.56^{a}	766^{a}	135^{a}	540^{a}	855^{a}	162^{a}
W4	61^{c}	31^{d}	15^{c}	621^{d}	1.11^{c}	601^{c}	106^{c}	430^{c}	687^{c}	96^{d}
Vermicompost										
VC (1:1)	125	62	34	508	1.44	1236	536	512	842	242
VC (3:1)	102	51	31	542	0.94	1120	485	457	742	276

*Figures denoted by same alphabets are statistically similar at 5% Probability level by DMRT.

3.4 Stability and maturity analysis of the Vermicompost

The data for stability and maturity of the vermicompost at the ratio 1:1 and 3:1 is represented in Table 4. The respiration rate of vermicompost at both the ratio was less than 2 mg CO_2-C gm^{-1} vermicompost C $day^{-1.}$ Thus, the vermicompost was considered to be very stable one (Epstein, 1997). In our study for the maturity test of the vermicompost with rice and gram seeds the GI value of the vermicompost ratio 1:1 was found to be greater than 70%. In case of vermicompost with ratio 3:1 the GI value was less than 70% for both types of seeds which indicated the presence of higher level of phytotoxic substances in the vermicompost ratio 3:1. Hence, the vermicompost of the tannery sludge with the ratio 1:1 might be considered as a mature vermicompost while vermicompost ratio 3:1 under study was not suitably mature compost (Helfrich *et al*., 1998).

Table 4: Stability and maturity of Vermicompost

Sample VC	Respiration (mg CO_2-C gm^{-1} vermicompost C day^{-1})	RSG (Percent of relative seed germination) (%)		RRG (Relative root growth) (%)		GI (Germination index) (%)	
		Rice	Gram	Rice	Gram	Rice	Gram
VC (1:1)	1.44	90	87.50	97.14	89.18	87.42	78.03
VC (3:1)	0.94	80	75.00	84.28	76.21	67.42	57.15

4. Conclusion

The physico-chemical, microbiological and biochemical characterization of Bantala tannery solid waste would unfold a new avenue for further research as there was very scarce data available regarding the Bantala Tannery. The use of the tannery sludge as an effective organic amendment in the agricultural field was subdued because of its high chromium (VI) content. Our study showed a prospective containment of plant nutrients in the raw tannery sludge along with enriched microbial enzymatic activities but the only problem was its high concentration of chromium. The results after the process of vermicomposting indicated towards reduction in the chromium concentration and relative enhancement of plant nutrients and its microbial activities. Thus, the study concludes that vermicomposting of the raw tannery sludge with cow dung could be a suitable way to reduce the high chromium concentration of the raw tannery sludge.

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