Closure Report

File Number : EMR/2016/002609

Project Title : Utilization of textile industry sludge through application of vermitechnology: An in-sight on metal accumulation potential of earthworms

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Total Sanctioned Amount : 57,60,600 (INR)

Total Released Amount : 55,48,000 (INR)

Start Date of the Project: 30 Mar, 2017

Date of completion: 29 Mar, 2020 (36 months)

Approved Objectives :

• To convert toxic textile industry sludge and effluents through vermicomposting technology for better nutrition to crop plants.
• To evaluate the metal remediation efficiency of E. fetida and M. posthuma from metal contaminated textile sludge and effluents
• To navigate the in-situ movement of heavy metals in earthworm and vermicast during vermicomposting through fluorescence-tagging and identification of novel metal binding proteins in earthworm intestinal cells
• To study the impact of vermiremediated textile sludge on soil health and crop production through field experimentation

Deviation made from original objectives (If Any) :

Not any

Ph.D. Produced/ Likely to be : 1

Technical Personnel Trained : 1

Total Expenditure : 54,00,000 (INR)

Concise Research Accomplishment :

Sustainable remediation of potentially toxic metals through application of vermitechnology in two types of textile industries (silk and cotton processing) wastes was targeted in this project. Both the textile sludge (TS) materials were highly rich in toxic metals like Cd, Cr, Zn, Cu, and Pb. Initially, feedstocks for earthworms were prepared by mixing TS with cow dung in various proportions. Then Eisenia fetida and Metaphire posthuma was used as vermicomposting agents; however, M. posthuma population sharply reduced in the vermireactors with high mortality or fleeing of the added specimens. This may be due to high oxidative stress with elevated DNA methyl transferase activity and considerable tissue damage in M. posthuma. Therefore, we replaced this species with Eudirlus eugeniae, which satisfactorily grew in the TS based feedstocks as like as E. fetida. However, TS+CD (1:1) mixture found to be most conducive among other feed-mixtures for E. fetida and E. eugeniae. Although high adaptability of earthworms in metal rich condition is well known, their growth, reproduction, and internal adjustment in TS like complex industrial wastes have rarely been studied. Encouragingly, organic C stabilization and significant increment in nutrient availability (N, P, K, Ca, and S) was recorded in silk industry sludge. Moreover, significant gain in NPK content was recorded due to vermicomposting with cotton sludge. Furthermore, about 10-12 folds reduction in Cd, Cr, Pb, and Zn contents in the vermi-processed feedstock with proportional increment in metal accumulation in earthworm guts was clearly evidenced. At this stage, we were interested to learn more about metal accumulation mechanism by this species. We used fluorescence probed cadmium for navigating the metal in vermibeds as well as in earthworm body. We know that earthworms efficiently detoxify metals by a small (~ 13 kDa) cysteine rich protein, metallothionein (MT). However, MT expression and the extent of metal accumulation often do not correlate; but the reason behind such incoherence is yet unclear. Our present observation shows that earthworm (E. fetida) exposure to cadmium rich ambience significantly triggers expression of a high molecular weight protein (HMWP) (150 kDa) among others. Moreover, fluorescence spectroscopic analysis and ICP-OES based studies clearly showed that Cd readily binds to this HMWP. Eventually, immunofluorescence staining and confocal microscopy exhibited that the HMWP translocate the bound cadmium in chloragogenous tissue where it is neutralized. Purification of this protein, determination of its N-terminal sequence, and bioinformatics analysis unveil it to be a glutamic acid rich new protein. Hence, this apparently novel HMWP is an important heavy metal binding protein in earthworm.

SNo.	CO-PI Details

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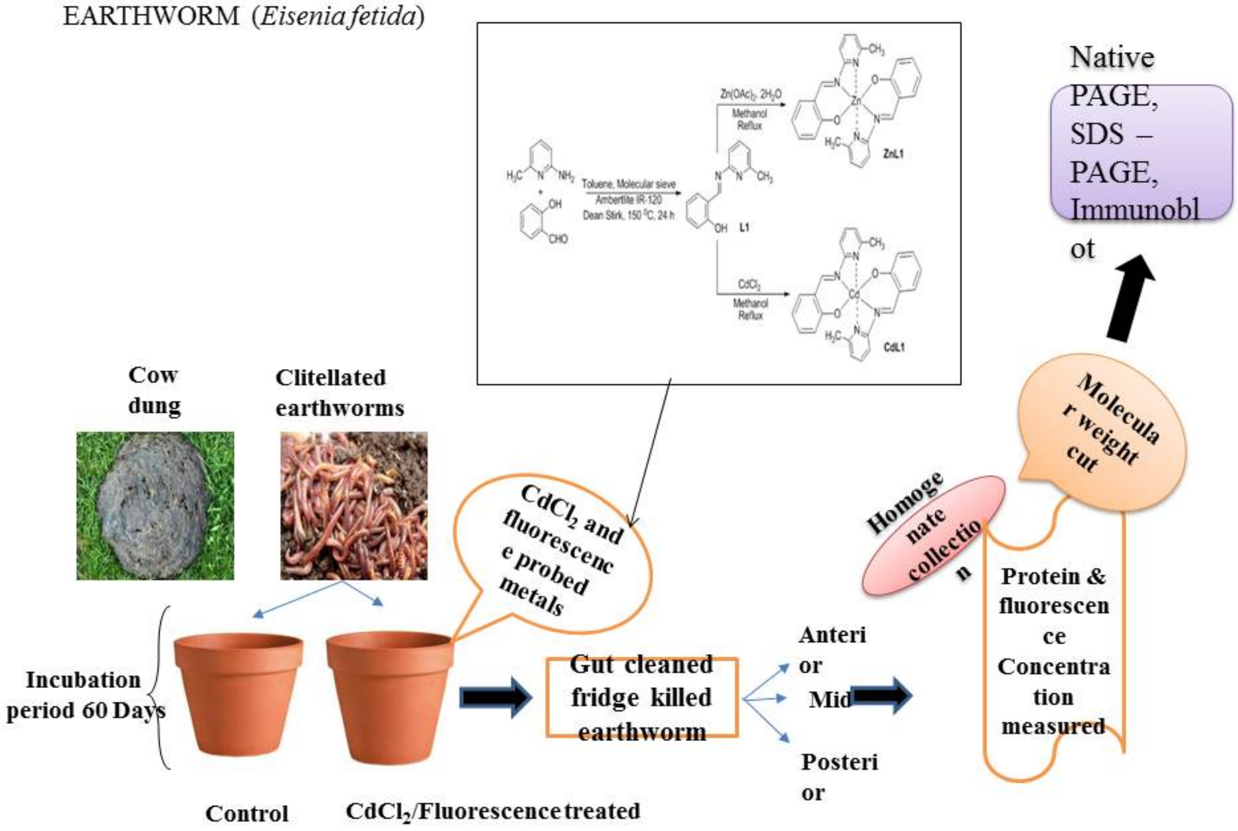
Closure Details

Experimental/ Theoretical Investigation carried out

Experimental methods for achievement of objective 1 and 2: Collection of raw materials (textile sludge/effluents, cow dung, and earthworms) and feedstock preparation- The silk processing sludge and effluents (SPES) was collected from the sludge storage ground and the discharge channels of the treatment facility of the Fabric Plus Ltd., Assam, India. Moreover, cotton textile sludge (CTS) was obtained from the sludge deposit premise of Orient Processor Ltd., Guwahati; a cotton thread processing industrial unit. Huge quantities of oil and dye loaded spinning-loom waste generated from different processing stages of silk yarn are referred to as sludge here; which is mostly free from the dye-rich effluents. The water effluents generated in the dying process are separated from the sludge; eventually treated via physical and chemical treatment routes to maintain hazardous dyes/chemicals in the effluents within critical limits of environmental standards. However, none of the industrial units take adequate measures to remove potentially toxic metals in the wastes. Therefore, the main focus of this work was to assess the metal accumulation potentials of the selected earthworm species in SPES and CTS. The sludge is usually burnt in open rather than following any treatment. Necessary gears (gloves, masks, eye-wear, etc.) were used while collection of sludge and effluents in biohazard bags/containers. Then, the effluents were mixed with the sludge @ 1 L kg-1 to form the mass of the SPES. The general physico-chemical characteristics of the sludge, effluents and the SPES are presented in table 1. Well grown (250-350 mg) clitelleted specimens of three earthworm species (Eisenia fetida, Metaphire posthuma, and Eudrilus eugeniae) were collected from the departmental vermiculture unit of Tezpur University, Assam for subsequent use. The taxonomic positions of the worm species were confirmed with the help of the Zoological Survey of India. Design of vermireactors and experimental set up- Circular truncated-cone shaped and perforated earthen reactors of 5 L capacity [0.05 m (lower radius) × 0.1 m (upper radius) × 0.3 m (height)] was used for the experiment. The feedstocks were prepared of different combinations of homogenized mixtures of the SPES and CD and allowed for pre-composting for 4-5 days. As such, pre-composting is practiced to evade the temperature rise and also to provide initial startup energy to the earthworms during vermicomposting (Hussain et al., 2018). Subsequently, 4 kg of each feedstock was poured into the reactors and incubated separately with E. fetida, Metaphire posthuma, and E. eugeniae specimens @ 10 worm kg-1 of the material. The rate of earthworm incorporation (i.e. 10 worms kg-1 of substrate) has previously been standardized and reported in many of our publications (Goswami et al., 2013; 2014; Sahariah et al., 2014; Das et al., 2015; 2016). A series of aerobic composting reactors with similar feedstock compositions were prepared for comparison. Each vermicomposting and composting reactors were repeated thrice and they were sprinkled with deionized water to sustain the moisture level within 40-50% for both composting and vermicomposting reactors. Moreover, the feedstocks were turned twice daily to ensure uniform aeration throughout the incubation period (60 days). The whole set of reactors (vermicomposting and composting) was replicated thrice and the study was continued for 60 days during April to May in 2017 and 2018 respectively. The ambient temperature was recorded between 27°C to 33°C during the period of the study. The details of feedstock composition are given as below: Vermicomposting Aerobic composting Eisenia fetida Eudrilus eugeniae Metaphire posthuma Es1- Only TS Ed1- Only TS Mp1- Only TS C1- Only TS Es2-TS+CD (1:1) Ed2- TS+CD (1:1) Mp 2- TS +CD (1:1) C2- TS+CD (1:1) Es3- TS+CD (3:1) Ed3- TS+CD (3:1) Mp 3- TS +CD (3:1) C3- TS+CD (3:1) Es4- Only CD Ed4- Only CD Mp 4- Only CD C4- CD Here, TS or 'textile sludge' signify both SPES and CTS. However, we have conducted separate experiments for both SPES and CTS. The changes in the physico-chemical properties of the feedstocks were periodically recorded by drawing samples at 0, 30 and 60 d from each replicate. Nutrient parameters and metal analysis in various feedstocks and earthworm intestines The temporal dynamics in compost quality attributes (pH, cation exchange capacity (CEC), total organic C (TOC), total N (TN), available P and available K, available S, and available Ca) were assessed following standard procedures (Page et al., 1982). Moreover, the changes in diethylene triamine penta-acetic Acid (DTPA) extractable metals (Fe, Mn, Zn, Cu, Cd, Cr and Pb) were measured by following Lindsay and Norvell (1978). The gut accumulated metal concentrations were determined in the SPES exposed earthworm specimens at the end of the study period (60 d). Earthworms were freeze-killed after gut cleaning by keeping them overnight on a moist filter paper without food. Subsequently, the freeze-killed earthworm specimens were digested in HNO3–HClO4 acid mixture and metal concentrations were assessed in ICP-OES (Berman, 1980). Stress, genotoxicity, and tissue damage in sludge exposed earthworm species- It was necessary to select the most efficient and adaptive earthworm species in textile sludge based feedstocks for determining the standardized vermitechnology prescription for large scale utilization of the specific industrial wastes. Stress enzyme (catalase and superoxide dismutase (SOD)) assay was performed to find out the mechanism by which earthworms respond to the heavy metal stress. Earthworms subjected to different feedstocks were sacrificed for the analysis. They were collected, gut cleaned and analyzed for Catalase, Superoxide dismutase and lipid peroxidation following suitable protocol. For catalase a method developed by Abie et al. (1974) was followed. SOD activity was accessed by its ability to inhibit the antioxidation of hematoxylin into hematin and subsequent changes in the absorbance at 556 nm (Mastin et al, 1987). Comet assay was performed following the method developed by Singh et al. (1988). Firstly, the basal slide was prepared by coating microglial slide with LMPA (Low melting point agar) and NMA (Normal melting agar). Than for cell isolate preparation, tissue was taken in 1ml cold HBSS containing 20 mM EDTA/10% DMSO. 5 -10 l removed and mixed with 75 l LMPA. Followed by electrophoresis of the embedded slide under alkaline condition pH >13. Electrophoresis was carried out with power supply 24 volts and current 300 miliampere for 30 minutes. Than the slides were placed in a draining tray and drop wise coated with neutralizing buffer and let sit for 5 minutes. Slides were stained with Ethidium Bromide and then dipped in distilled water for excess stain removal. Subsequently cover slip was placed and the slides were drained by keeping for 20 min in cold 100% ethanol or cold 100% methanol for dehydration. Air dried the slides and placed them in an oven at 500C for 30 min. Than the DNA damage was evaluated using 40x objective on a fluorescence microscope. Simultaneously, a group of textile sludge treated earthworms were gut cleaned, killed by freezing, and used for histological assay (Sharma and Satyanarayan, 2011). Worm tissues were primarily fixed in Bouin's fluid for a day and subsequently dehydrated for 10 minutes in graded alcohol solutions and then in xylen solution before embedding in paraffin. Eventually, 5 µm sections of worm body with the help of the Microtome cutter, mounted on albumin coated slides, and stained following hematoxylin-eosin staining technique. In addition DNA methyl transferease (DNMT) activity was measured in sludge exposed earthworms to assess the species wise variation at gene level. Firstly, the earthworm DNA was isolated using HiPura TM Multisample DNA Purification Kit HIMEDIA MB554-50PR and then DNA methylation was assessed using DNMT Activity Quantification kit (Colorimetric). Ecotoxicity and pollution assessment in the finished vermicompost- These assessments were performed in SPES and CTS based feedstocks of vermicomposting and composting reactors in comparison with similar municipal solid waste mediated feedstocks as secondary control. Pollution index (PI) is a helpful model to predict the cumulative pollution potential of the constituent heavy metals in terrestrial bodies (Equeenuddin et al., 2013; Mondal et al., 2017). The data on heavy metal content from the different vermicomposted, composted, and untreated wastes (SPES, CTS, MSW) were used in the model to calculate the PI (equation no. 3). PI=(Fe300+Mn300+Zn300+Pb300+Cd100+Cr100)/6 ……………………………………… (1) The numerical values viz. 100 and 300 represent the tolerance limit of the respective metals, expressed in mg kg-1 (Mondal et al., 2017; Sahariah et al., 2015). Higher pollution potential is represented by PI>1 while PI=1 and PI1 indicate moderate and low pollution potential respectively (Kabata-Pendias and Mukherjee, 2007). The contamination index (CI), ecological risk index (ERI) and geo-accumulation index (Igeo) of the vermicomposted, composted, and untreated wastes (SPES, CTS, MSW) was enumerated. The following mathematical relations (equation 4, 5, and 6) were utilized for calculating these indices (Yang et al., 2009; Mondal et al., 2017). CI=C_nB_n ……………………………………………………………………………… (2) ERI=((T_i×C_n))B_n ………………………………………………………………. (3) The ecotoxicity levels of vermicomposted, composted, and untreated wastes (SPES, CTS, MSW) were determined on the basis of CI and ERI. The ratio of the total concentrations of different metals and their concentrations in reference site expresses the CI. The computation of ERI was done by deriving the ratio of the cumulative changes in metal concentrations in a substrate and reference values (i.e Bn). I_geo=log_2 C_n(1.5B_n)…………………………………………………………………....(4) The Igeo helps in predicting the possible accumulation of different metals in future. Here, Cn indicates metal concentration in the vermicomposted, composted, and untreated wastes (SPES, CTS, MSW) and Bn is the concentration of the respective metals in the uncontaminated soil. Ti represents the change in metal concentration as per reference values (e.g. Ti for Fe, Cd, Cr, Mn, Pb, and Zn were 1, 2, 5, 1, 5, and 1 respectively) and a constant value of 1.5. The concentrations of the studied metals (Fe, Cd, Cr, Mn, Pb, and Zn) in a nearby pristine soil were taken as reference value for the respective metals for computing the above mentioned equations (Soil and Land Use Survey of India (SLUSI) Ann. Report, 2013). Methodology and experimental for achievement of the objective 3 Experimental set-up- A schematic flow chart of the experimentation has been given in Fig. 1. We selected Eisenia fetida as test organism because of its wide acceptability as a vermicomposting agent and consistent efficiency for remediation of textile sludge and effluents. We used a previously tested fluorescent tagged cadmium complex (hereafter Fl-Cd) for addressing the metal binding mechanism in the earthworm species and subsequent purification of high molecular weight metal binding proteins. The Fl-Cd complex was synthesized following the protocol of elaborated in our previous paper (Goswami et al., 2016). The substrate was formed by adding uniform mass (2 kg) of urine free cow dung in earthen pots. The Cd concentration in the cow dung was below the detection limit of the ICP-OES. Therefore, CdCl2 and Fl-Cd was separately spiked in the earthen pots in a manner that the concentration of added cadmium was uniform (135 mg kg-1) for both the substrates. Thus, the amount of spiked cadmium (135 mg kg-1) was same for both the substrates. A set of Cd free cow dung substrate was maintained as control. Afterwards, earthworms were introduced in all the substrates at 15 worm kg-1. Water was sprinkled and the substrate was churned thoroughly at 2-3 days interval for maintaining favorable moisture (40-50%) and temperature (28- 32oC). Plastic plate was placed beneath all the reactors for the leachate collection and eventual recycling of the same with ~100% efficiency. The study was conducted for 2 months with four replicates for precision and confirmation of the results. Cadmium estimation in substrate and earthworm gut: The total cadmium concentration in the substrate and in the earthworm body was monitored at the end of the incubation period (60 days) (Berman, 1980). Three specimens were collected from each replicate of control and Fl-Cd treated samples for investigation of the gut metal content. The worm specimens were kept for gut evacuation for removal of their intestinal debris; subsequently the worms were freeze killed and cut in three different sections (anterior, mid, and posterior). The first (anterior), second (mid), and third (posterior) cut were made at segment no. 22, 40, and 70-75 respectively. Each section of the sacrificed earthworm samples were digested in (HNO3–HClO4) di-acid mixture (1: 6) and Cd concentrations were determined by ICP-OES (OPTIMA 7300 DV). The bioaccumulation factor was calculated using the formula given below (Giesy et al., 1977): Bioaccumlation factor (%)= (Cd concentration in earthworm body)/(Cd concentration in Cd free substrate)×100………………….(4) Identification and purification of Cd binding protein: Our aim was to search some hitherto unknown metal binding proteins and we hypothesized that the proteins could be larger than 100 kDa based on our previous findings (Goswami et al., 2016). Initially, the protein contents in three sections of worm body (anterior, mid, and posterior) were measured following Bradford28. Then, the obtained sections were homogenized with 1X PBS and subsequently centrifuged for 10 minutes at 10000×g. The supernatants were collected and subjected to Amicon YM-100 filter devices (Millipore, Bedford, MA) for separating out the above 100 kDa protein fractions. The Amicon YM-100 tube along with the supernatant was centrifuged at 14000×g for 20 minutes. The >100 kDa fraction was collected in the upper compartment of the column, eventually transferred to a separate tube and centrifuged at 1,000 × g for 2 minutes at 4oC.The supernatants were analyzed for protein content and subsequently undergone gel exclusion chromatography using Sephadex G-75 column (size: 31 × 0.75 cm). Protein content and fluorescence spectrum were measured in all the gel excluded fractions (1 ml each) collected at fixed time interval. The gel exclusion chromatography of >100 kDa proteins was repeated five times not only for precision and confirmation, but also for obtaining adequate amount of proteins for further purification. The protein concentration in all the column eluted fractions was determined in a UV-VIS spectrophotometer (Cary 60) after calibrating the same with bovine serum albumin (BSA) standards (Renny et al., 2013). On the basis of the fluorescence spectra and protein contents of the gel eluted fractions (treated and control), 15 to 19 number fractions were pooled together for control, $CdCl_2$, and Fl-Cd treated worms respectively and the molecular mass of the gel excluded fractions were tentatively determined following standard methods (Whitaker, 1963; Marshall, 1970). Electrophoresis and Western blotting: Sephadex G-75 column eluted pooled (fraction 15-19) protein fractions were run on 7.5% NATIVE-PAGE and 10% SDS-PAGE respectively to purify with greater precision and confirmation of the molecular weight of the metal binding protein. Following this, the gel eluted protein samples (approx. ~150 kDa) of control, $CdCl_2$ treated, and Fl-Cd treated worms were transferred to PVDF membranes (Millipore, Bedford, MA) with the help of Semi-Dry Trans-Blot SD Cell (Bio-Rad Laboratories). The membranes were first incubated with primary antibody raised in mice through foot pad route at 1:1000 dilutions followed by secondary antibody conjugated with alkaline phosphatase at 1:2,000 dilutions using SNAP ID apparatus (Millipore). The protein bands in the immunoblots were detected by using 5-bromro 4-chloro 3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT). Purification of proteins extracted from polyacrylamide gels: The desired protein was excised carefully from the gel matrix with the help of a scalpel. Passive elution extraction method was used to elute the desired protein from polyacrylamide gel. The excised gel pieces were placed in clean screw-cap culture or micro-centrifuge tubes; 0.5-1 ml of elution buffer (50 mMTris- HCL, 150Mm NaCl, and 0.1mM EDTA: pH 7.5) was added in each sample for complete immersion of the gel pieces. The gel pieces were crushed using a clean pestle and later incubated in a rotary shaker at 30°C overnight. Next day, the samples were centrifuged at 5,000- 10,000 × g for 10 minutes and the supernatants were estimated for protein contents and Cd content as well through ICP-OES. Moreover, the fluorescence spectrum was checked at each stage to detect the Fl-Cd complex in fluorescence spectrophotometer (Cary Eclipse). N-Terminal sequencing of proteins and identification: Purified protein was run in 7.5% SDS-PAGE and transferred to PVDF membrane. Neatly excised protein band was sequenced on PPSQ-33A automated peptide/protein sequencing system (Shimadzu) in Regional Centre for Biotechnology, DBT, New Delhi, following Edman degradation method. Edman’s sequencing of protein yielded N-terminal sequence of 15 amino acids residues. These 15 residues of peptide were used for protein identification in the BLAST searched algorithm. Microscopic assessment of Cd binding: Immunofluorescence staining: To localize high molecular weight protein in the CT of earthworm, de-paraffinized and hydrated sections from the earthworm gut were washed as per protocol and incubated in blocking buffer (0.5% Triton X-100 and 1% bovine serum albumin in PBS) for 1 h at room temperature and then probed overnight at 40C with anti-HMWP polyclonal primary antibody diluted with blocking buffer. After three washes in PBS, slides were incubated with the secondary antibody (fluorescein isothiocyanate [FITC] conjugated goat anti-mouse (IgG) in diluted blocking buffer for 1 h at room temperature in the dark). Slides were then washed four times in PBS. Vectashield mounting medium (Vector Labs; www.vectorlabs.com) with 40,6-diamidino-2-phenylindole dihydrochloride (DAPI) was applied for counter staining and DABCO (Sigma, Aldrich) to mount glass cover slips onto the slides. Immunofluorescence was detected with fluorescence inverted microscope (Leica DMi8; www.leica-microsystems.com) and confocal microscope (Leica DMi8) mounted digital camera. Statistical analysis Two-way ANOVA were performed with three observations per cell to appreciate the temporal changes in different chemical attributes (pH, TOC, TN, C/N ratio, available P and available K, available S and available Ca) and microbial counts during vermicomposting. In addition, the impacts of various biocomposting systems on metal accumulation potential of earthworms and all metal binding protein purification studies were analyzed using one-way ANOVA. The least significant difference (LSD) test was also performed for all the attributes to compare the performance of composting and vermicomposting reactors.

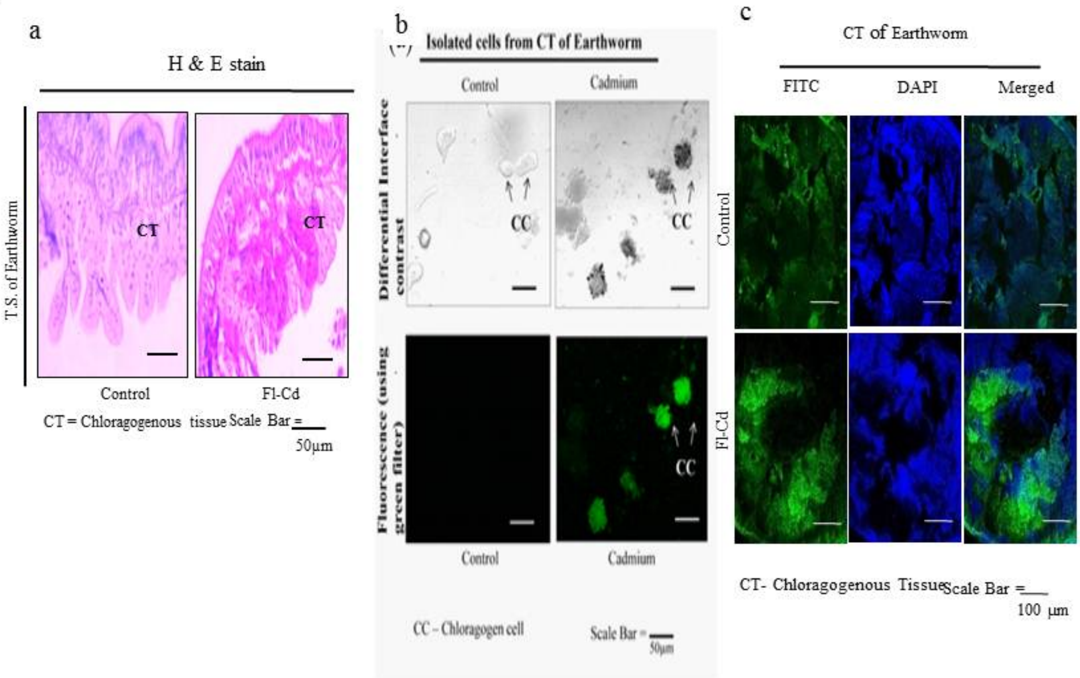
Fig 1: EXPEIRMENTAL PLAN FOR METAL DETOXIFICYING PROTEIN ISOLATION IN EARTHWORM (*Eisenia fetida*)



Detailed Analysis of result

• Achievement of objectives 1 & 2 Physico-chemical characteristics of silk processing effluents and sludge (SPES) and cotton textile sludge (CTS) – The data on physico-chemical properties of the two textile sludge are presented in supplementary item 1. Both the SPES and CTS were mixture of spent fibers and dyes; however, proportion of dye solutions was greater in SPES and CTS. Bulk density of SPES was significantly lower than CTS indicating highly porous nature of the waste. Both the materials were alkaline in nature but alkalinity in CTS was more extreme than in SPES (pH: SPES - 7.8±0.5; CTS – 9.1±0.46). High occurrence of base forming cations (Ca and K) and low S content are the probable reasons for such high alkalinity. TOC was noticeably higher in the SPES than CTS; while total N (TN) level was similar in both SPES and CTS. These results were in good agreement with few earlier reports (Kaushik and Garg, 2003; Bhat et al., 2013). However, concentrations of Mn, Zn, Cr, Pb, and Cu were considerably high in both SPES and CTS as compared to the permissible limits set by the Central Pollution Control Board, India (CPCB, 2017). Earthworm fecundity and ecotoxicity response in textile sludge based during composting and vermicomposting reactors – According to the approved objectives of the project, we started the vermicomposting experiment with Eisenia fetida and Metaphire posthuma. However, between 10-20th day of incubation the population of Metaphire posthuma drastically reduced in TS dominated feedstocks (supplementary item 2). All earthworms fled by 20th day from the Mp1 feedstock. The reducing population of the feedstocks were periodically observed and recorded at 0, 10, 20, and 30th day in each replicate. Hence, it was understood that the TS based feedstocks were insufferable for M. posthuma; therefore, we have taken Eudrilus eugeniae as a replacement of M. posthuma for the study. Interestingly, growth and reproduction of both E. fetida and E. eugeniae were satisfactory in TS (i.e SPES or CTS) based feedstocks over a period of 60 days. Eisenia fetida population increased by 2.16 and 1.4 folds in SPES+CD (1:1) and SPES+CD (3:1) vermibeds respectively. On the other hand, the increment of Eudrilus eugeniae was 1.4 and 1.16 folds respectively in the same feedstocks. Similar results were also obtained in CTS based reactors (Supplementary item 2). Although Eisenia fetida showed greater tolerance and adaptability to SPES or CTS based feedstocks as compared to Eudrilus eugeniae, both the earthworms escaped in significant numbers from the pure SPES or CTS (Es1 and Ed1) feedstock after 30 days. Interestingly, Eisenia fetida showed marginal recovery in growth potential after 40 days of incubation in SPES or CTS based substrates; their growing density was also greater than Eudrilus eugeniae. Overall, it appeared that metal rich TS (textile sludge) based feedstock might be variedly toxic to the three earthworm species and Eisenia fetida was the most tolerant among the three species. Hence, at this stage, we were interested to evaluate the toxicity response of the studied earthworm species. Elevated activity of oxidative stress relieving enzymes (catalase and superoxide dismutase (SOD) was evidenced in all three species in only sludge feedstocks (supplementary item 3). In contrast, activity of these enzymes sharply reduced in earthworms grown in TS+ cow dung (1:1) feedstock. This indicated that mixing TS with cow dung in 1:1 ratio can greatly substantiate oxidative stress in earthworms. Similar trend was also recorded for DNA methyl transferase (DNMT) activity. In fact, elevation in DNMT activity indicates suppression of transcription at gene level; thus the results indicate that high occurrence of toxic metals in TS probably induced genotoxicity in earthworms. This was further confirmed through comet assay, which showed that the level of DNA degradation was significantly higher in TS exposed earthworms than the untreated specimens (supplementary item 4). Moreover, that histological analysis (supplementary item 4) revealed that loss of structural integrity in chloragogenous tissue and its detachment from the epithelial lining of lumen took place in TS treated earthworm specimens. However, among the three earthworm species level of oxidative stress and genotoxicity was lowest in Eisenia fetida followed by Eudrilus eugeniae. Changes in TOC, total N (TN), available P, and K during vermicomposting of textile sludge (TS) – The data on the changes in TOC, TN, avl. P, avl. K during vermicomposting and composting of the TS based feedstocks is presented in Supplementary item 5. In general temporal decrease in TOC corresponding with the increment in TN was evidenced in all the feed stocks as compared to the initial value (Supplementary item 5). TOC reduced more sharply in Eisenia mediated vermibeds as compared to Eudrilus and composting system. The accelerated decomposition in E. fetida mediated system was probably due to the voracious feeding habit coupled with high excretion rate of the species (Kale, 1993; Domínguez and Edwards, 2010). At the end (60 d), TOC reduction was significant in cow dung + TS (1:1) for both types of the sludge (SPES and CTS). Total nitrogen significantly increased by 1.71 (Es3) to 5.02 (Es1) folds under Eisenia systems followed by Eudrilus [1.49 (Ed2) to 3.23 (Ed4) folds] and composting [1.26 (C3) to 2.62 (C4) folds] systems. Among the various feed stocks, the increment in TN level was most prolific in SPES/CTS+CD (1:1) followed by SPES/CTS+CD (3:1). Generally, the decrease in TOC and increase in TN result from CO2 loss and microbial mineralization during organic matter decomposition process. Phosphorus availability was initially within low to medium range in the SPES or CTS based feed stocks which significantly increased due to vermicomposting (Supplementary item 3). At the end of (60d), bioavailability of P was significantly greater in Ed3 followed by Es2, Es3 and C3 and C1 in SPES based feedstocks (p0.001; LSD=1.94). The trend was quite similar in CTS based feedstocks. K content was exorbitantly high in both SPES and CTS (Supplementary item 1). Interestingly, bioavailability of K receded by more than five folds in the feedstocks over time in both (E. fetida and E. eugeniae) vermicomposting systems (Supplementary item 5). This may due to leaching of the readily solubilized K fractions owing to the reduction in CEC of the substrates. Overall, we could conclude that vermicomposting can be a useful proposition for stabilization and balance of nutrient elements in textile sludge; which in turn shall increase the recyclability of the toxic industrial waste. Changes in metal availability in feedstock and accumulation efficiency of the earthworm species during vermicomposting – The temporal changes in concentrations of various metals in the composting and vermicomposting feedstocks and accumulation in earthworm guts have been presented in Fig. 2 and Supplementary items 6-8. Overall, initial concentrations of all the studied metals were extremely higher in CTS as compared to SPES. Levels of PTM (Cd, Cr, Pb, Cu, and Zn) in the SPES and CTS based feed stocks gradually reduced over time under both composting and vermicomposting. However, the extent of reduction in PTMs was remarkably higher in under vermicomposting than composting. For example, 30-53 % Cd reduction in composting beds was noted whereas metal reduction was 70-99% in the SPES mediated vermi-beds at end. Similarly, Cr concentration decreased by 3-17.3 folds due to vermicomposting of SPES. At the end, significantly low Cr concentration among the SPES based feedstocks was recorded in Es1 and Es2 followed by Es3, Ed3, Ed1 and Ed2 (p0.001; LSD =0.046). This is interesting because Nguyen et al. (2016) observed increment in Cr concentration in textile sludge after vermicomposting by Perionyx excavatus. Contrarily, our study reveals that Cr reduction was greater in Eisenia and Eudrilus mediated vermicomposting than that of aerobic composting. Generally, the metal detoxifying efficiency of vermicomposting systems greatly varies depending on the worm species and the feedstock composition (Nannoni et al., 2011; Soobhany et al., 2015). Moreover, the amount of Zn and Pb sharply decreased in SPES due to vermicomposting (p0.001). Correspondingly, both the earthworm species accumulated Pb, Cd, Zn, Cu, and Cr in substantial amount in SPES based feedstock, which may be the major reason of metal reduction after vermicomposting of SPES. We also obtained similar results with CTS. In general, Cd reduced by 1.1 to 3.3 folds due to vermicomposting of CTS; whereas 0.3 to 1.2 folds reduction in Cd was recorded under composting. Moreover, about 2.5 to 3.5 folds reduction of Pb was recorded in the vermibeds with significant accumulation in worm body. However, Cr reduction was sharper than Cd; the metal reduced by 3.5 to 11.5 folds due to vermicomposting (Fig. 2). Interestingly, concentrations of less toxic micronutrient elements (Fe, and Mn) considerably increased in SPES under both composting and vermicomposting (Supplementary item 6). In contrast, about 37-62% and 90-95% reduction of Fe and Mn respectively was evidenced due to vermicomposting of CTS. Correspondingly, considerable level of metal accumulation was recorded by both Eisenia and Eudrilus was evidenced. However, the extent of accumulation significantly varied depending on feed mixtures. Overall, accumulation of Cd, Cr, Cu, Pb, Zn, Mn, and Fe was significantly greater in only TS followed by TS+CD (1:1) feedstocks (Fig. 2 & Supplementary item 9). This may be due to substantially higher occurrence of all the studied metals in the raw CTS as compared to the SPES material. However, such unique selectivity of the earthworms in regard to metal accumulation has been reported by many workers (Nannoni et al., 2011; Goswami et al., 2014; 2016). Various factors like chemical species requirements, concentrations, interspecific variations in dietary intake, and hazard potential of the metals etc. determine selective accumulating affinity of the earthworm species. Ecotoxicity analysis of vermicomposted textile sludge (SPES and CTS) in comparison with municipal solid waste (MSW)- The pollution evaluation indices were computed in SPES and CTS along with MSW before and after vermicomposting and composting (Supplementary item 10). The purpose of such comparison was to assess the environmental quality of the vermicomposted textile sludge; here the data for MSW has been used as positive control. The Pollution index (PI) [Eq. (1)] values greater than 1 (PI>1) signify potential threat for the environment and human health (Sahariah et al., 2015). PI was significantly higher in untreated CTS than SPES and MSW (Supplementary item 9). However, PI sharply reduced after vermicomposting and composting of all the three wastes and we recorded lowest PI in vermicomposted MSW followed by vermicomposted CTS. Contamination index (CI) [Eq. (2)] reduced by 2.81 (MSW) to 3.2 (CTS) folds after vermicomposting. Correspondingly, sharp reduction in ecological risk index (ERI) [Eq. (3)] and geoaccumulation index (Igeo) was evidenced in vermicomposted SPES and CTS (Supplementary item 9). After analyzing these indices, it was encouraging to realize that environmental and ecological compatibility of vermicomposted textile sludge was overall greater than vermicomposted MSW. Hence, we could ascertain that textile sludge and cow dung mixture @ 50:50 ratio can be useful proposition for textile industries to recycle their solid wastes into environmentally benign useful fertilizer for agriculture employing Eisenia fetida or Eudrilus eugeniae as vermicomposting agents. • Achievement of objectives 3 Cadmium reduction in $CdCl_2$ and fluorescence probed Cd complex (Fl-Cd) spiked feedstock and accumulation in earthworm – Two forms of Cd ($CdCl_2$ and Fl-Cd) exposure were given to E. fetida specimens in the vermicomposting vessels for a period of 60 days. The variations in bioavailable and bound Cd fractions were measured in the feedstocks (Fig. 3a). Concurrently, the temporal changes in the Fl-Cd concentrations were measured in earthworm body on the basis of fluorescence intensity (Fig. 3b). In addition, the total accumulated Cd was estimated in earthworm body (Fig. 3c). The water soluble and exchangeable fractions of cadmium sharply reduced by 76-97% in Fl-Cd and $CdCl_2$ spiked substrates in presence of earthworms (Fig. 3a). Similar trend was also observed for the carbonate bound fraction whereas the reduction of Fe-Mn bound form was marginal in the treated substrates. Interestingly, the organic matter and mineral bound forms steadily increased over time in Fl-Cd and $CdCl_2$ spiked substrates. Although under oxidizing condition organic matter can slowly degrade and eventually release the bound metals, the residual bound form remains insoluble for several years. Interestingly, the total cadmium concentration (sum total of all fractions) significantly reduced by 41-42% in Fl-Cd and $CdCl_2$ spiked substrates. These results suggest that good amount of cadmium was also sequestered in worm biomass and also in agreement with other recent findings (Soobhany et al., 2015; Goswami et al., 2018; Mondal et al., 2020). At this stage, we were interested to study the fluorescence spectral pattern in the substrate and in worm body. Application of fluorescent probes for monitoring the biological systems either through imaging or by fluorescence emission spectrum are established techniques (Enamullah et al., 2015). Hence, the technique that we used allowed us to determine the amount of Cd accumulated by the worms and the total amount retained in the substrates. We found that the typical signature of Fl-Cd compound was prominent at the initial stage of incubation, which sharply deteriorated at 30th day of incubation and followed the same pattern of reduction till the end of the incubation period (Fig. 3b). Overall, fluorescence intensity in the Fl-Cd treated substrates reduced by 3-4 folds over 60 days; while the concentration of Fl-Cd greatly enhanced (4-5 folds) in earthworm body as determined from the intensity based standard curve (Fig. 3d). On the 60th day, we also observed that earthworms could significantly accumulate cadmium in both the Fl-Cd and $CdCl_2$ spiked vessels (Fig. 3c). However, small amount fluorescence emission was also observed in the earthworms reared in the control vessels. Earthworms are known to exhibit auto-fluorescence (Wampler and Jamieson, 1980); therefore, the detected emission in the controlled worms might be assigned to such inherent property of the organisms. Upon measuring the amount of Cd accumulated in the anterior (segment no. 1-22), mid (segment no. 23-40), and posterior sections (Fig. 3c); we found that Cd concentration was highest in the posterior section as compared to the mid and anterior sections. Correspondingly, we recorded remarkably greater bioaccumulation factor (BaF) in the treated earthworms compared to the control ($p=0.000$, LSD=1.02) as reported previously by other workers (Soobhany et al., 2015; Goswami et al., 2018; Paul et al., 2018) (Fig. 3e). Purification and identification of high molecular weight proteins- Total protein concentration was significantly higher in Fl-Cd and $CdCl_2$ exposed worms as compared to the control (Fig. 4a & Supplementary item 11a); while protein concentration was remarkably greater in the posterior section than the other two sections due to Cd treatment (Fig. 4b). Interestingly, these observations were well corresponded to the Cd accumulation pattern as discussed above. Overall, the protein level was about eight folds greater in the Fl-Cd and Cd-Cl2 treated earthworms as compared to the control ($p=0.000$; LSD=0.13) (Fig. 4b). We repeated the experiment several times with different earthworm specimens and found identical results. Now, it was necessary to separate out above 100 kDa size proteins to remove all possibilities of isolating MT or MT-like proteins; which are commonly much smaller in size. Eventually, gel exclusion chromatography was performed with above 100 kDa protein fractions. On an average, the fractions collected between 15 to 19 ml elution volumes have shown greatest absorbance with corresponding signature of the fluorescence peak in the Fl-Cd treated samples (Supplementary item 11b). We could separate at least four proteins by gel exclusion chromatography, which were expressed only in the Cd treated earthworms. The approximate molecular weight of such proteins varied between ~140 to 230 kDa (Supplementary item 11c). We performed both SDS and native polyacrylamide gel electrophoresis (PAGE) to observe the number of peptide associated with such proteins (Fig. 4c and 4d). The major goal of this study was to find the character of the Cd induced high molecular weight proteins (HMWP) in earthworms. At this juncture, it was intriguing to note that Cd exposure induced HMWP expression; which eventually chelated the metals in worm body. Interestingly, a monomeric protein of 150 kDa molecular mass was found to be highly induced by Fl-Cd treatment (Fig. 4d). We therefore attempted to purify the most effective one of those gel-eluted fractions on the basis of their Cd binding potential. The density of the protein bands purified from Fl-Cd and CdCl2 exposed earthworms was considerably greater than the control (Fig. 4d). Interestingly, we found 17-18 folds greater accumulation of cadmium in the SDS-PAGE eluted ~150 kDa protein as compared to the untreated worms (p=0.000; LSD=11.41) (Fig. 4e). Correspondingly, the signature peak of the Fl-Cd complex between 450-550 nm in gel eluted protein (~150 kDa) strongly substantiated our hypothesis (Fig. 4f). However, we failed to detect any fluorescence peak between 450-550 nm in control worms. We then raised antibody against purified HMWP (150 kDa protein) in mice; after examining the cross reactivity of the anti-HMWP antibody, we used this in the fluorescence probed tracking studies and microscopic analysis. The densitometric analysis of immunoblot also indicated about four-fold greater upregulation of ~150 kDa HMWP compared to the control (Fig. 4g). Microscopic studies on the protein bound metal deposition in chloragogenous tissue and cells and N terminal sequencing- The H & E stain based histological analysis of showed that the chloragogenous tissue (CT) of Fl-Cd treated worms was denser and compact as compared to the control; suggesting a Cd induced alteration of the CT (Fig. 5a). Interestingly, Cd localization in chloragogenous cells of the Fl-Cd treated worms was vividly observed through fluorescence microscopy (Fig. 5b). Hence, the anti-HMWP antibody was further used for its localization in treated and control worms for confocal microscopy. Chloragogenous cells were isolated by using proteolytic enzymes from the tissue. This was followed by determination of Cd localization in them; which exhibited considerable accumulation of Cd in these cells (Fig. 5c). By using fluorescence probed anti-HMWP antibody in confocal study we were convinced that ~150 kDa HMWP has robustly accumulated Cd in response to Cd treatment and transported the metal into the CT (Fig. 5c). The N-terminal sequencing of the SDS raised purified protein performed through Edman's method yielded a sequence of 15 amino acid residues (Fig. 5d). However, no definite homology or partial homology with other proteins was found in the database of the BLAST searched algorithm. Overall, this research reveals a hitherto unknown pathway of cadmium detoxification in earthworms and endeavor also ended up with a purified and characterized new metal binding protein that is induced in earthworms when the animal is exposed to toxic metal rich conditions.

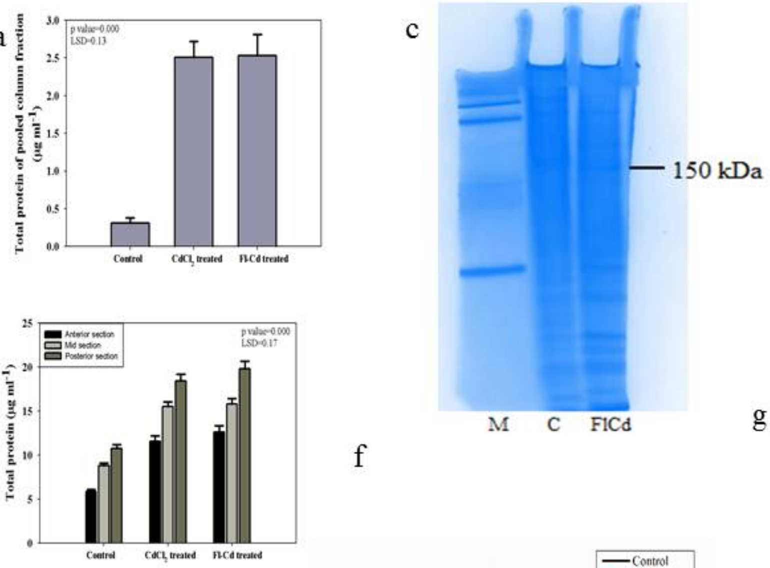
Fig. 5



d Protein (~150KDa) sequence of the N-terminus region:

Ser Gly Gly Glu Glu Thr Asn Val Pro Leu Glu Ile Gln Glu Val

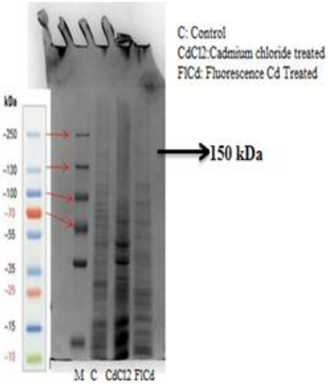
Fig. 4a



b

c

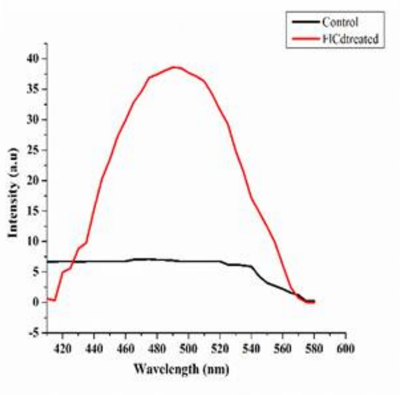
d



e

Cd concentration in gel eluted proteins (ICP-OES)	Native-PAGE (~ 150 k Da) Cd ng mg^{-1}
Control	0.06
Cd-Fl. treated	1.93

f



g

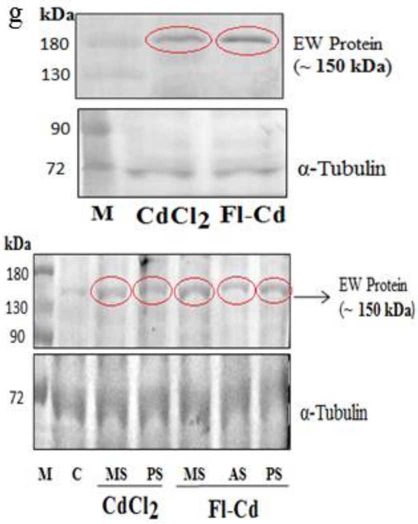
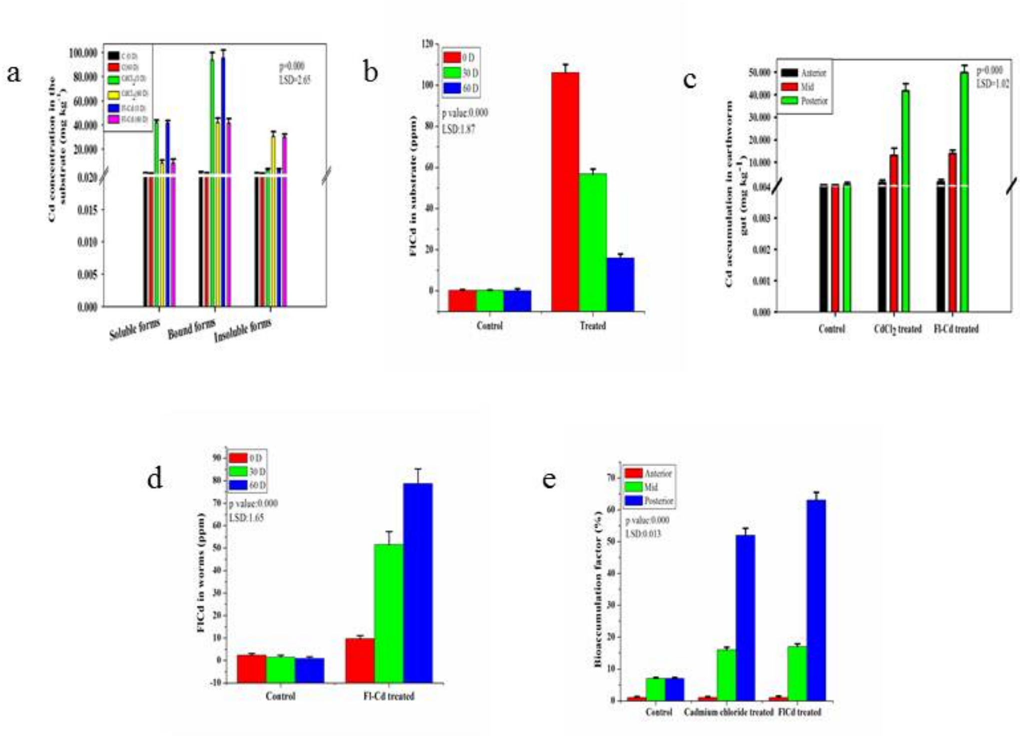
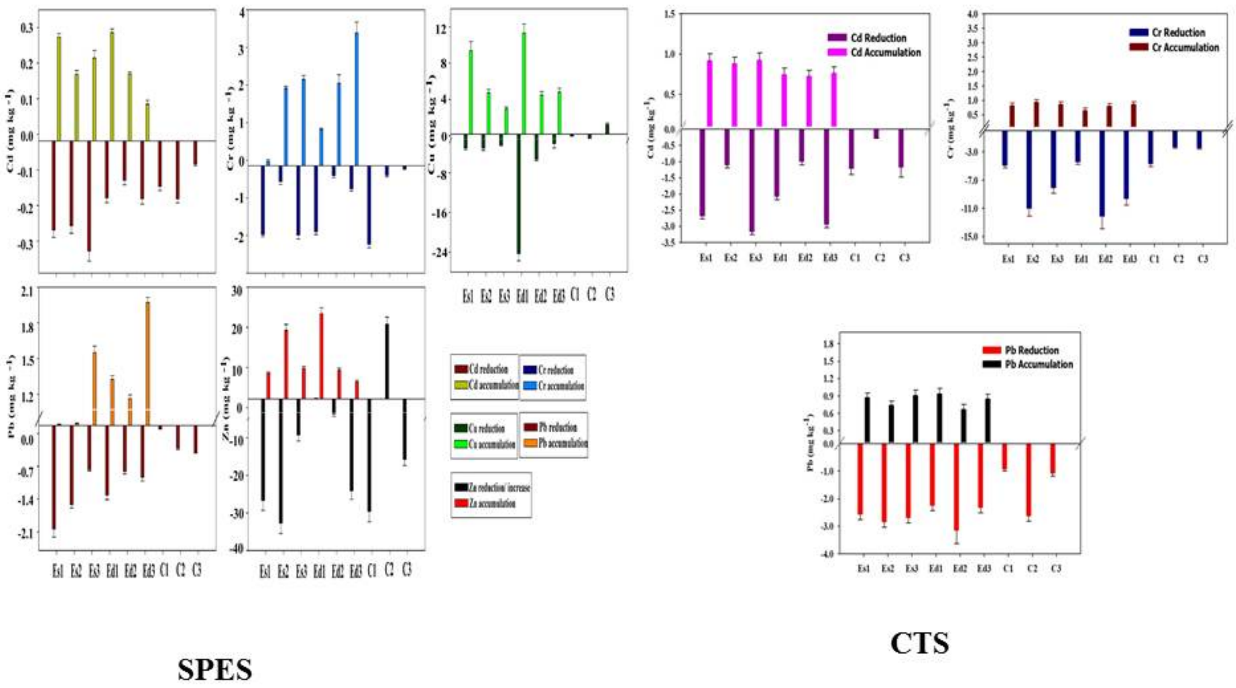


Fig. 3



a
p=0.000
LSD=2.65
Cd concentration in the substrate (mg kg-1)
Soluble forms
Bound forms
Insoluble forms
b
0 D
30 D
60 D
p value:0.000
LSD:1.87
FlCd in substrate (ppm)
Control
Treated
c
Anterior
Mid
Posterior
p=0.000
LSD=1.02
Cd accumulation in earthworm gut (mg kg-1)
Control
CdCl2 treated
Fl-Cd treated
d
0 D
30 D
60 D
p value:0.000
LSD:1.65
FlCd in worms (ppm)
Control
Fl-Cd treated
e
Anterior
Mid
Posterior
p value:0.000
LSD:0.013
Bioaccumulation factor (%)
Control
Cadmium chloride treated
FlCd treated

Fig. 2 Pattern of reduction/accumulation of heavy in vermicomposted TS (SPES and CTS) and earthworm gut at the end of 60 days.



Conclusions

The primary objective of this project was to assess the feasibility of vermitechnology for conversion of toxic textile sludge (TS) into nutrient rich compost. We have targeted two types of textile sludge (silk and cotton processing sludge) for vermicomposting with three different earthworm species (Eisenia fetida, Metaphire posthuma, and Eudrilus eugeniae). After a detailed study we could conclude that a palatable feedstock for earthworms can be prepared by mixing TS with cow dung (CD) in 50:50 proportions. The results indicated that Eisenia fetida most efficiently transformed TS into eco-friendly organic fertilizer with high nutrient (N, P, K, Ca, and S) value and significantly low contents of toxic metals (Cd, Cr, Pb, Zn etc.). According to the second objective of the research, we evaluated metal remediation efficiency of earthworms. The results revealed that TS was highly toxic for M. posthuma; while Eisenia fetida exhibited high adaptability with profuse reproducibility. We also verified the prospect of E. eugeniae in regard to metal remediation. This species also performed satisfactorily for transformation and metal remediation TS based feedstock. Subsequently, we studied the underlying mechanism of metal remediation vis-à-vis detoxification using fluorescence tagged cadmium complex and taking Eisenia fetida as test organism in order to address the third objective of the project. Eventually, we detected few non-metallothionein metal binding proteins; out of which we isolated and purified a 150 kDa protein. This monomeric protein consistently expressed in worm intestine upon cadmium exposure and chelated the metal in substantial amount. Moreover, immunofluorescence staining and confocal microscopy confirmed that the protein bound cadmium was deposited in chloragogenous tissue of earthworm. We purified this protein and undergone N terminal sequencing of 15 amino acid residues. The sequencing output revealed that glutamic acid might be the most dominant residue of the polypeptide. We also failed to detect strong similarity of this protein with known proteins in the database after a thorough bioinformatics study. Hence, it appeared that we could isolate a new metal binding protein in Eisenia fetida.

Scope of future work

Earthworms are fantastic animal to study. Their diversity, utilization, and evolution under the rapidly changing environmental conditions would be an interesting re for research. The protein we purified from earthworm intestine warrants in-depth future research. So far, we could explore only fifteen amino acid residues of the protein. But it is necessary to unveil the whole structure of the protein and identify the specific gene that regulates the expression of the protein. Moreover, the efficacy of vermiconverted textile sludge need to studied in agriculture on large scale basis.

List of Publications (only from SCI indexed journals) :

Title of the Paper	List of Authors	Journal Details	Month & Year	Volume	Status	DOI No	Impact Factor
Vermi-sanitization of toxic silk industry waste employing Eisenia fetida and Eudrilus eugeniae: Substrate compatibility, nutrient enrichment and metal accumulation dynamics	Sarmistha Paul, Subhasish Das, Prasanta Raul, Satya Sundar Bhattacharya	Bioresource Technology (International)	Jul-2020	266 (267-274)	Published	10.1016/j.biortech.2018.06.092	7.539
Vermiremediation of cotton textile sludge by Eudrilus eugeniae: Insight into metal budgeting, chromium speciation, and humic substance interactions	Sarmistha Paul, Linee Goswami, Ratul Pegu, Satya Sundar Bhattacharya	BIORESOURCE TECHNOLOGY (International)	Jul-2020	(123753)	Published	10.1016/j.biortech.2020.123753	7.539
Epigenetic regulations enhance adaptability and valorization efficiency in Eisenia fetida and Eudrilus eugeniae during vermicomposting of textile sludge: Insights on repair mechanisms of metal-induced genetic damage and oxidative stress	SarmisthaPaul, LineeGoswami, RatulPegu, SubhenduKumar Chatterjee, SatyaSundar Bhattacharya	BIORESOURCE TECHNOLOGY (International)	Dec-2021	(126493)	Published	https://doi.org/10.1016/j.biortech.2021.126493	9.642
Assessing the ecological impacts of ageing on hazard potential of solid waste landfills: A green approach through vermitechnology	Sarmistha Paul, Moharana Choudhury, Utsab Deb, Ratul Pegu, Subhasish Das, Satya Sundar Bhattacharya	JOURNAL OF CLEANER PRODUCTION (International)	Jul-2020	(117643)	Published	http://doilorg/10.1016/j.clepro.2019.117643	7.246
Metal induced non-metallothionein protein in earthworm: A new pathway for cadmium detoxification in chloragogenous tissue	Nazneen Hussain, Subhendu Kumar Chatterjee, Tushar Kanti Maiti, Linee Goswami, Subhasish Das, Utsab Deb, Satya Sundar Bhattacharya	JOURNAL OF HAZARDOUS MATERIALS (International)	Jul-2020	401 (123357)	Published	https://doi.org/10.1016/j.jhazmat.2020.123357	9.038

List of Papers Published in Conference Proceedings, Popular Journals :

Title of the Paper	List of Authors	Journal Details	Month & Year	Volume	Status	DOI No	Impact Factor
Not Available							

List of Patents filed/ to be filed :

Patent Title	Authors	Patent Type	Country/Agency Name	Patent Status	Application/Grant No.
Not Available					

Equipment Details :

Equipment Name	Cost (INR)	Procured	Make & Model	Utilization %	Amount Spent (INR)	Date of Procurement

Equipment Name	Cost (INR)	Procured	Make & Model	Utilization %	Amount Spent (INR)	Date of Procurement
Microtome cutter with paraffin bath chamber	1,42,000	Yes	Caltech	90	1,42,000	13 Jan, 2020
Homogenizer	5,66,000	Yes	GENAXY	95	5,66,668	13 Jan, 2020
Fluroscence spectrophotometer	11,94,000	Yes	Agilent Corp.	90	12,44,400	13 Jan, 2020
Lyophilizer	7,07,000	Yes	Eyela	95	7,17,000	13 Jan, 2020
Table top centrifuge	3,99,000	Yes	GENAXY	95	3,99,960	13 Jan, 2020

Plans for utilizing the equipment facilities in future:

The procured equipment will be used by all faculties and research scholars of the department

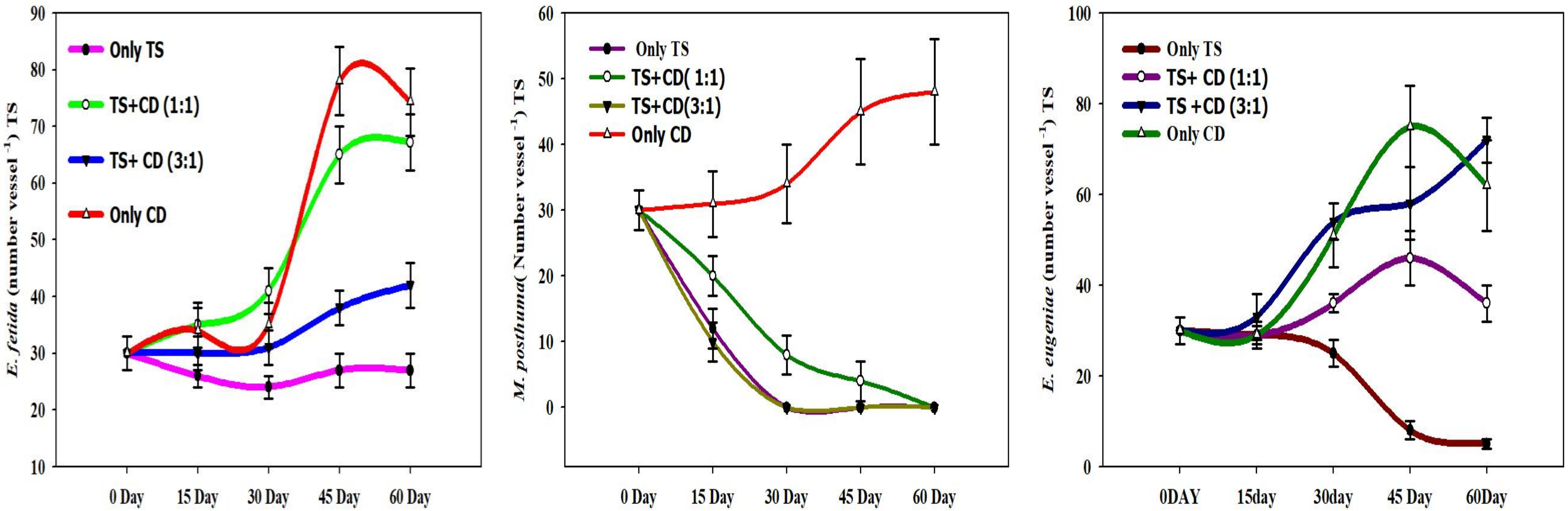
Supple item 1

Physico - chemical characteristics of the silk processing effluents and sludge (SPES) and cotton textile sludge (CTS). Values represent mean ± standard deviation

Parameters	SPES	CTS	Inland* Surface	Public* Sewer
pH	7.8 ± 0.5	9.1± 0.5		
Electrical conductivity (µS cm^{-1})	1.5 ±0.2	1.35±0.4		
Bulk density (g cc^{-1})	0.08±0.02	0.89±0.4		
Available K (mg kg^{-1})	588.2±45.6	1127.66±96.3		
Available Ca (mg kg^{-1})	92±7.2	206.87±11.8		
Available P (mg kg^{-1})	96.44±6.4	101.20±10.7		
Available S (mg kg^{-1})	8.09±0.4	932.80±19.0		
Total organic C (%)	3.8±0.2	0.15±0.4		
Total Kjeldahl N (%)	2.34±0.2	2.05±0.5		
Cd (mg kg^{-1})	0.25±0.1	0.11±0.01	2.0	1.0
Cr (mg kg^{-1})	1.38±0.12	0.03± 0.01	0.1	2.0
Mn (mg kg^{-1})	224.82±20.1	190.88±12.7	-	-
Pb (mg kg^{-1})	3.21±0.39	1.33±0.38	0.1	1.0
Zn (mg kg^{-1})	58±4.2	108.88±8.7	5.0	15.0
Cu (mg kg^{-1})	14.77±1.2	10.58±0.1	3.0	3.0

**Textile sludge discharge standards for metals in Inland and Public sewer water as per Central Pollution Control Board, India (CPCB, 2017).*

Supple item 2 Temporal change in the population of *Eisenia fetida*, *Metaphire posthuma*, and *Eudrilus eugeniae* under different feed mixtures.



Supple item 3

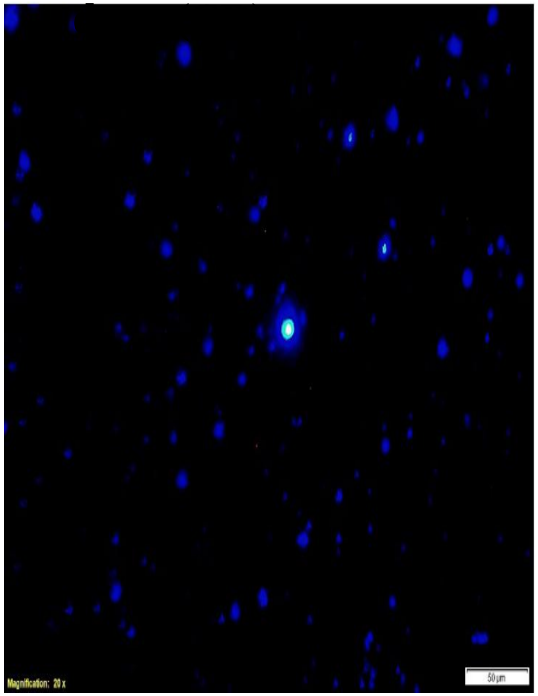
DNA methyltransferase and oxidative stress relieving enzyme (catalase and superoxide dismutase) in textile sludge (TS) exposed earthworm species. Values represent mean ± standard deviation

Treatments	Catalse activity/ mg protein	Super Oxide Dismutase activity/ mg protein	DNA methyltranferase activity/hr/µg
Cow dung (*Eisenia fetida*)	70.42±1.56	2.86±0.21	298.0±24.3
Cow dung (*Metaphire posthuma*)	82.01±5.43	4.13±0.36	416.0±35.6
Cow dung (*Eudrilus eugeniae*)	70.61±4.65	2.97±0.16	323.1±27.8
Only TS (*E. fetida*)	298.59±22.03	11.03±0.98	1964.2±123.6
Only TS (*M. posthuma*)	432.83±33.44	16.32±1.01	8064.3±70.1
Only TS (*E. eugeniae*)	304.22±26.13	11.62±0.87	2880.2±17.1
1:1 (TS + Cow dung) (*E. fetida*)	121.97±9.42	5.66±0.23	1018.4±24.3
1:1 (TS + Cow dung) (*M. posthuma*)	178.45±11.35	7.14±0.44	3188.5±27.6
1:1 (TS + Cow dung) (*E. eugeniae*)	117.41±9.65	5.76±0.48	1818.1±78.2

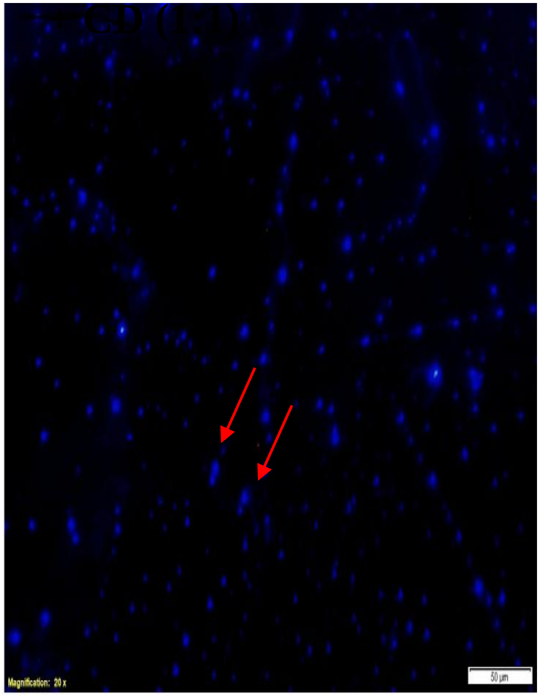
Supple item 4

COMET ASSAY

***E. fetida* DNA in cow**



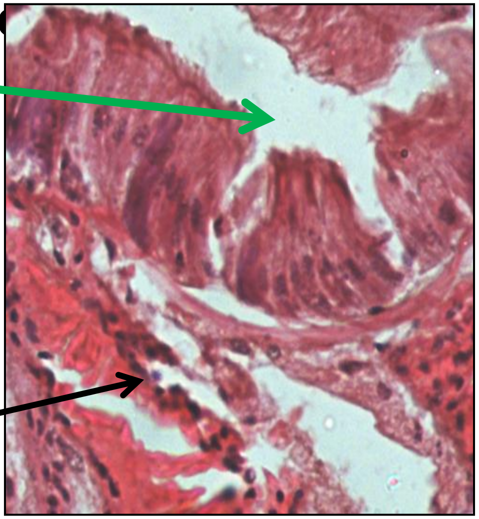
***E. fetida* DNA in Textile Sludge**



HISTOLOGY

***E. fetida* in cow**

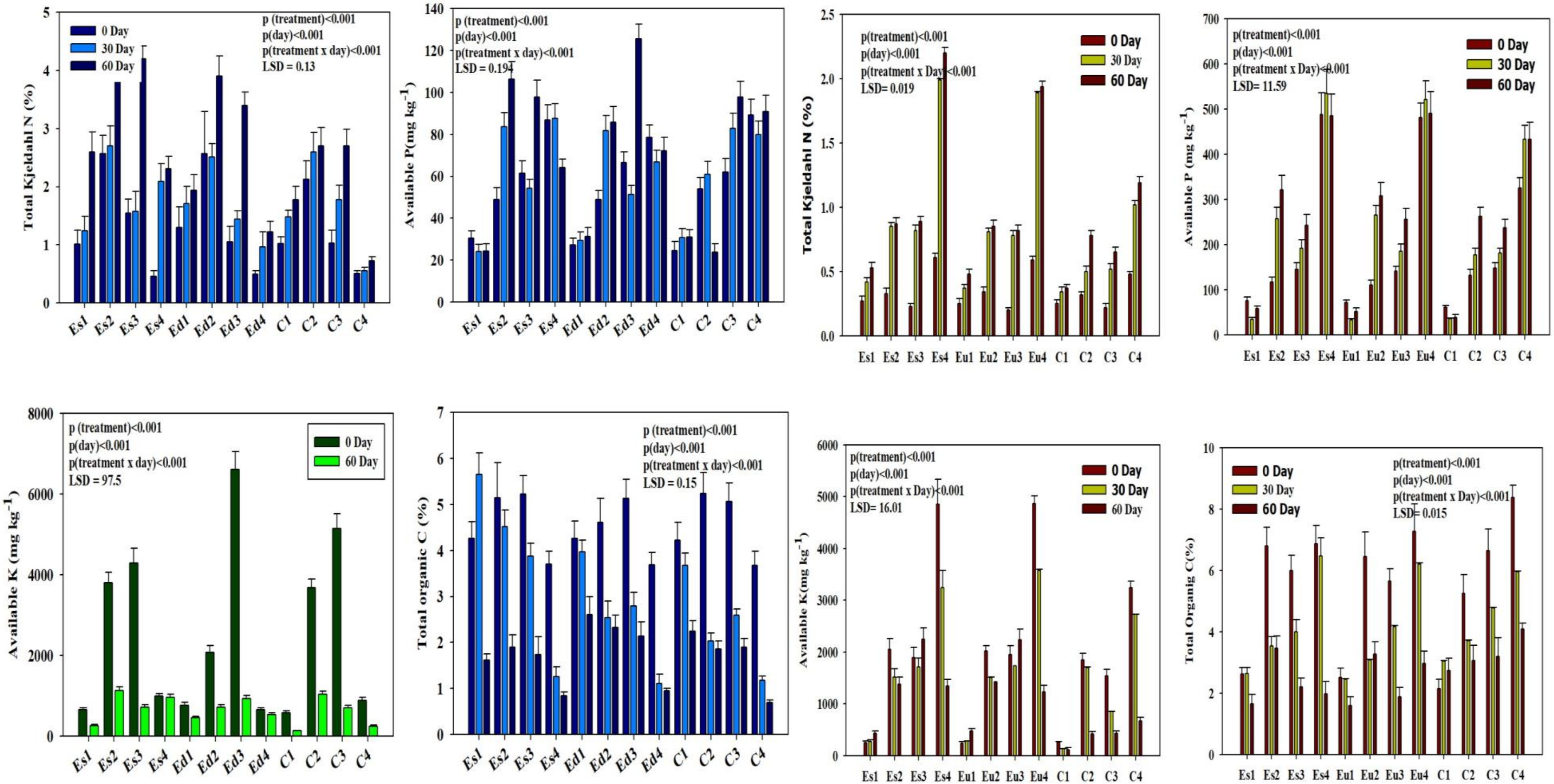
***E. fetida* in Textile Sludge +**



Lumen

Chloragogenous tissue

Supple item 5 Changes in total organic C, total Kjeldahl N, available P and K in the composted and vermicomposted TS mixtures.



Supplementary item 6

Changes in Fe, Mn, and Cu content in the silk sludge and cow dung mixtures under various treatments (Mean±SD)

Treatments	Fe (mg kg^{-1})			Mn (mg kg^{-1})			Cu (mg kg^{-1})		
	0 Day	**30 Day**	**60 Day**	**0 Day**	**30 Day**	**60 Day**	**0 Day**	**30 Day**	**60 Day**
Es1	61.0±5.20	124.2±11.3	297.9±22.3	191.3±15.6	259.6±23.4	338.8±27.9	14.7±0.23	11.5±0.13	11.3±0.17
Es2	101.5±7.30	121.3±10.3	525.5±19.4	227.8±21.0	392.6±32.4	782.4±57.2	7.28±0.13	5.34±0.18	3.95±0.23
Es3	123.7±10.4	133.9±10.5	263.4±21.0	283.9±17.3	410.5±36.3	554.4±42.6	7.25±0.16	7.12±0.36	4.63±0.27
Es4	42.9±3.70	61.9±4.92	148.7±12.3	30.1±1.68	30.7±1.69	46.7±3.51	4.04±0.24	1.61±0.04	1.61±0.06
Ed1	64.7±5.30	141.5±11.5	543.2±39.0	198.4±16.7	311.8±29.2	312.1±26.1	32.8±1.67	20.5±1.25	8.54±0.54
Ed2	122.9±10.7	117.1±10.3	423.9±38.4	354.1±28.3	457.9±38.9	553.6±40.2	10.4±0.89	6.98±0.39	4.97±0.39
Ed3	165.5±3.72	180.5±15.6	270.1±26.3	207.7±16.3	369.1±28.5	490.4±38.6	5.69±0.42	5.51±0.05	3.27±0.17
Ed4	41.6±3.15	85.9±6.31	140.0±9.53	32.6±2.85	39.9±2.41	47.0±3.92	3.97±0.23	3.39±0.21	1.99±0.10
C1	66.7±5.62	182.9±16.2	196.7±11.4	218.2±19.3	255.9±20.3	288.4±19.3	11.0±1.15	6.61±0.32	10.9±1.24
C2	132.1±11.7	134.6±10.6	479.1±39.3	298.3±22.8	325.6±28.3	663.0±47.3	6.94±0.39	4.84±0.29	6.28±0.38
C3	145.7±12.4	152.2±10.8	308.9±23.7	224.8±20.3	294.8±22.4	301.0±28.7	5.06±0.48	3.74±0.19	6.07±0.26
C4	63.1±5.21	53.9±3.20	72.8±4.31	21.9±1.38	37.7±2.61	50.8±3.71	2.92±0.12	2.12±0.17	1.52±0.06
p (T)	<0.001			<0.001			<0.001		
p (D)	<0.001			<0.001			<0.001		
p (T × D)	<0.001			<0.001			<0.001		
LSD	7.70			12.3			2.25		

**T= treatment, D= Day, LSD= Least significant difference*

Supplementary item 7

Changes in Cd, Cr, Pb, and Zn content in the silk sludge and cow dung mixtures under various treatments (Mean±SD)

Treatments	Cd (mg kg^{-1})			Cr (mg kg^{-1})			Pb (mg kg^{-1})			Zn (mg kg^{-1})		
	0 Day	30 Day	60 Day	0 Day	30 Day	60 Day	0 Day	30 Day	60 Day	0 Day	30 Day	60 Day
Es1	0.29±0.02	0.08± 0.01	0.07±0.02	1.66±0.3	0.16±0.01	0.06± 0.01	3.86±0.21	1.58±0.21	1.81±0.21	56.9±4.33	37.0±2.16	33.2±2.18
Es2	0.25±0.03	0.20± 0.02	0.03±0.03	0.43±0.02	0.16±0.02	0.05± 0.02	1.96±0.09	1.45±0.09	0.39±0.09	51.6±4.15	36.5±2.67	22.9±1.79
Es3	0.28±0.01	0.18 ± 0.01	0.002±0.01	1.73 ±0.1	0.14±0.01	0.12± 0.1	1.58±0.11	1.07±0.11	0.71±0.11	37.6±2.17	43.0±2.79	37.9±2.18
Es4	0.10±0.01	0.05± 0.01	0.03±0.02	0.09±0.01	0.07±0.04	0.02± 0.02	0.34±0.01	0.28±0.01	0.05±0.01	43.2±3.68	29.7±1.47	32.8±2.16
Ed1	0.21±0.02	0.10± 0.01	0.06±0.02	1.63 ±0.2	0.43±0.2	0.10± 0.03	3.91±0.02	1.58±0.02	2.53±0.02	52.8±3.11	39.1±2.79	52.9±3.21
Ed2	0.23±0.01	0.20± 0.01	0.13±0.03	0.34 ±0.03	0.23±0.1	0.10± 0.02	1.42±0.12	1.18±0.12	0.51±0.12	46.4±2.69	25.7±1.89	44.1±2.78
Ed3	0.25±0.01	0.23± 0.03	0.10±0.03	0.63± 0.10	0.25±0.1	0.09± 0.01	1.63±0.11	0.80±0.11	0.61±0.11	37.6±2.18	33.3±1.89	25.9±1.88
Ed4	0.11±0.02	0.07± 0.02	0.02±0.02	0.04±0.01	0.02±0.01	0.01± 0.03	0.46±0.02	0.22±0.02	0.08±0.02	41.7±3.15	25.9±1.67	46.3±3.16
C1	0.30±0.03	0.18± 0.02	0.18±0.01	1.98 ± 0.20	0.72±0.24	0.15± 0.02	3.36±0.23	3.10±0.23	3.31±0.23	61.6±3.62	38.5±2.13	25.4±1.58
C2	0.28±0.02	0.22± 0.01	0.13±0.02	0.37±0.04	0.22±0.03	0.15± 0.03	1.63±0.12	1.30±0.12	1.18±0.12	37.8±1.57	22.8±1.58	62.9±4.21
C3	0.20±0.02	0.19± 0.02	0.14±0.01	0.17±0.03	0.14±0.21	0.11± 0.02	1.63±0.98	1.13±0.98	1.10±0.98	32.4±2.16	21.7±1.87	27.4±1.45
C4	0.10±0.01	0.08± 0.01	0.06±0.02	0.02±0.01	0.01±0.01	0.01± 0.01	0.16±0.01	0.03±0.01	0.15±0.01	39.5±2.16	31.0±2.18	40.1±2.87
p (T)	<0.001			<0.001			<0.001			<0.001		
p (D)	<0.001			<0.001			<0.001			<0.001		
p (T × D)	<0.001			<0.001			<0.001			<0.001		
LSD	0.008			0.046			0.097			1.229		

**T= treatment, D= Day, LSD= Least significant difference*

Supplementary item 8

Changes in Fe, Mn, Cu and Zn content in the Cotton Textile sludge and cow dung mixtures under various treatments (Mean±SD)

Treatments	Fe (mg kg^{-1})			Mn (mg kg^{-1})			Cu (mg kg^{-1})			Zn (mg kg^{-1})		
	0 Day	**30 Day**	**60 Day**	**0 Day**	**30 Day**	**60 Day**	**0 Day**	**30 Day**	**60 Day**	**0 Day**	**30 Day**	**60 Day**
Es1	2590±254	2590.03±201	1423.64±101	859.73±82.3	754.657±69	664.66±60.1	70.55±6.1	38.89±3.1	34.74±2	294.87±21	216.68±20.3	155.52±13
Es2	1642±142	699.86±65.3	1029.23±97.3	924.35±90.4	574.0396±48	221.07±18.3	56.50±4.1	24.39±2.2	14.15±1	157.15±12	145.98±12.4	37.33±2.8
Es3	2070±199	735.54±60.1	781.71±59.30	997.32±95.3	430.355±40	165.602±14	52.16±4.3	20.61±1.4	12.91±1	246.98±21	167.60±14.6	132.72±11
Es4	490.40±387	235.36±20.1	216.70±18.20	490.2 ±38.3	268.59 ±29.1	199.02 ±17	14.07±0.9	5.17±0.3	4.18±0.3	121.59±10	89.66±7.10	60.12±5.2
C1	2590.03±223	2132.51±199	1463.40±144	762.55±71.2	689.78±59.6	579.1±55.2	96.80±8.5	46.92±3.5	33.07±2	297.59±24	190.23±17.9	142.84±12
C2	1948.57±148	1644.56±157	1389.67±112	983.86±93.4	651.96±61.4	305.25±29.3	62.38±5.4	29.95±2.1	6.11±0.4	304.81±29	160.75±15.4	73.08±6.1
C3	2536.00±213	1066.34±98.3	1040.31±98.3	1123.95±100	600.68±58.6	144.11±13.5	69.27±5.6	25.60±2	18.60±1.1	305.59±27	160.77±14.3	112.79±7.3
C4	366.42±342.3	310.30±29.30	121.03±10.10	313.079±31	230.714 ± 21	103.965± 10	12.62±1.1	6.40±0.4	2.88±0.18	159.20±12	69.54±5.40	50.45±4.2
p (T)	<0.001			<0.001			<0.001			<0.001		
p (D)	<0.001			<0.001			<0.001			<0.001		
p (T × D)	<0.001			<0.001			<0.001			<0.001		
LSD	1.09			27.18			1.55			7.29		

**T= treatment, D= Day, LSD= Least significant difference*

Supple item 9

Accumulated heavy metal content (Cd, Cr, Cu, Fe, Mn, Pb, and Zn) in gut of *Eisenia fetida* and *Eudrilus eugeniae* after 60 days of incubation (Mean±SD)

Treatments	**Cd mg/L**	**Cr mg/L**	**Cu mg/L**	**Fe mg/L**	**Mn mg/L**	**Pb mg/L**	**Zn mg/L**
Es1	0.27±0.01	0.10± 0.02	9.07± 0.40	20.3± 1.25	0.24±0.04	0.02±0.01	5.15±0.24
Es2	0.17±0.01	1.80± 0.05	4.49± 0.28	4.00± 0.25	0.01±0.001	0.04±0.01	13.7±0.001
Es3	0.22±0.02	2.01± 0.09	2.71± 0.17	16.2± 1.25	1.49±0.01	1.44±0.02	6.15±1.49
Es4	0.07±0.01	1.47± 0.04	2.10± 0.11	10.3± 1.22	0.01±0.001	0.01±0.001	4.98±0.001
Ed1	0.28±0.01	0.84±0.03	11.0± 0.98	24.2±1.29	1.74±0.003	1.23±0.05	17.0±1.26
Ed2	0.17±0.01	1.92±0.20	4.00± 0.24	58.6±4.27	10.1±0.02	1.08±0.04	5.81±0.38
Ed3	0.10±0.01	3.09±0.25	4.00± 0.31	27.6±1.66	4.66±0.03	1.83±0.05	3.43±0.27
Ed4	0.03±0.01	0.57±0.02	2.00± 0.17	17.9±0.89	0.92±0.002	0.05±0.001	2.90±0.07
p (T)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	0.009	0.098	0.344	1.53	0.378	0.027	0.544

Supple item 10 –Pollution risk evaluation in composting and vermicomposting beds

Ecotoxicological indices	SPES	CTS	MSW	*p*
Pollution risk index (PI)	1.24±0.09b	1.52±0.07a	1.26±0.31b	<0.01
Contamination index (CI)	67.3±1.12c	79.33±5.8a	68.2±2.24c	<0.01
Ecological risk index (ERI)	79.8±21.1a	88.4±17.3a	158.7±17.1b	<0.03
Geoaccumulation index (I_{geo}) (Cr)	2.89±0.26a	5.3±0.28a	2.77±0.18b	<0.05
Geoaccumulation index (I_{geo}) (Cd)	1.60±0.22b	2.62±0.33b	2.81±0.46a	<0.01
Geoaccumulation index (I_{geo}) (Pb)	2.88±0.44b	2.70±0.25a	2.44±0.31a	<0.01

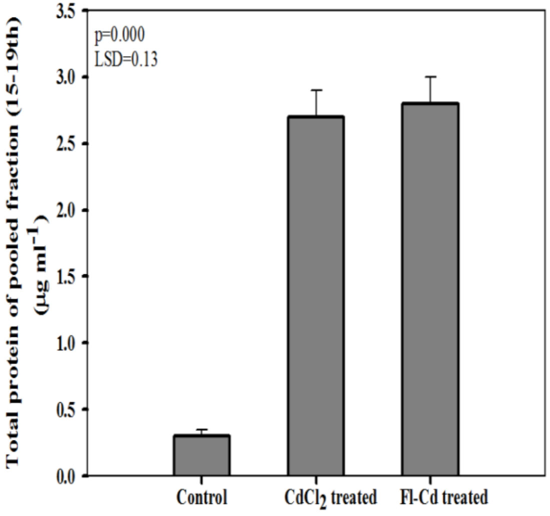
SPES= Silk processing effluents and sludge; CTS= Cotton textile sludge; MSW=Municipal solid waste. Rows with same letter are not statistically different as per Duncan's Multiple Range Test at p<0.05

Ecotoxicological indices	Vermicomposted SPES	Vermicomposted CTS	Vermicomposted MSW	Composted SPES	Composted CTS	Composted MSW	*p*
Pollution risk index (PI)	0.5±0.02c	0.41±0.05c	0.2±0.06d	0.83±0.03a	0.85±0.05a	0.65±0.05b	<0.05
Contamination index (CI)	22.1±0.11c	24.2±2.18c	24.2±2.18c	57.4±2.21a	58.5±1.76a	48.5±0.98b	<0.01
Ecological risk index (ERI)	93.9±0.41d	102.4±0.53c	91.8±0.33d	127.5±3.43b	145.1±3.17a	145.2±2.77a	<0.03
I_{geo} (Cr)	-4.87±0.02e	2.41±0.01b	-0.64±0.17d	0.25±0.07c	2.86±0.03a	0.18±0.02b	<0.05
I_{geo} (Cd)	-5.06±0.11e	-4.35±0.12d	-0.87±0.07c	-0.16±0.01a	-0.22±0.02a	-0.72±0.01b	<0.05
I_{geo} (Pb)	2.45±0.25b	0.78±0.27d	3.39±1.00a	2.99±0.32b	1.38±0.11c	3.89±0.03a	<0.05

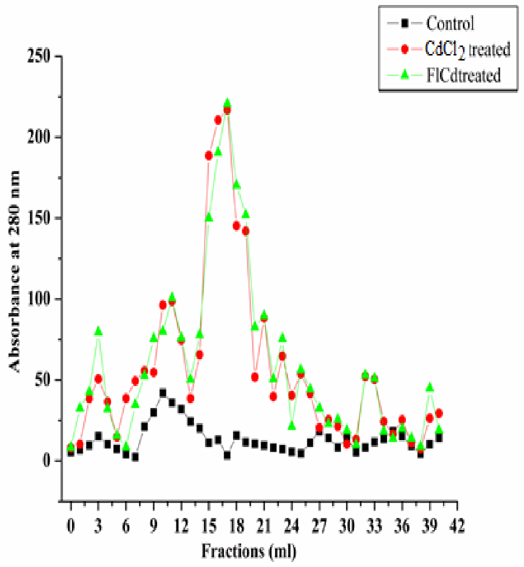
SPES= Silk processing effluents and sludge; CTS= Cotton textile sludge; MSW=Municipal solid waste. Rows with same letter are not statistically different as per Duncan's Multiple Range Test at p<0.05

Supple item 11

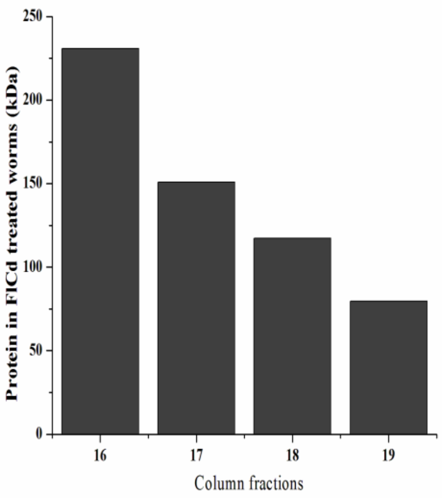
a



b



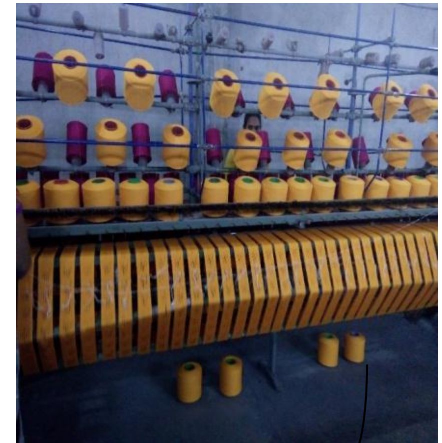
c



Supple item 12
Pictures

Sample Collection

ERI Industry



Cotton Industry

Vermicomposting

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GFR 12 – A [(See Rule 238 (1))]
UTILIZATION CERTIFICATE (UC) FOR THE YEAR 2019-20...
in respect of *NON-RECURRING*
as on 29-03-2020 to be submitted to SERB
Is the UC Provisional (Provisional) *(Provisional/Audited)*
(To be given separately for each financial year ending on 31st March)

1. Name of the grant receiving Organization: ... SERB ....
2. Name of Principal Investigator (PI) .... Dr. Satya Sundar Bhattacharya... ....
3. SERB Sanction order no. & date ...... EMR/2016/002609 dated 27/03/2017...
4. Title of the Project "Utilization of Textile Industry Sludge Through Application of Vermitechnology: An In – Sight on Metal Accumulation Potential of Earthworms."
5. Name of the SERB Scheme : ...... EMR (Presently CRG)... .... (CRG/NPDF/ECR etc.)
6. Whether recurring or non-recurring grants : ***NON-RECURRING***
7. Grants position at the beginning of the Financial year

(i) Carry forward from previous financial year : Rs.2,39,189/-..

(ii) Others, If any : ...Nil........................

(iii) Total : Rs. 2,39,189/-...........................

8. Details of grants received, expenditure incurred and closing balances: (Actuals)

Unspent Balance of Grants received previous years [figure as at Sl. No. 7(iii)]	Interest Earned there on	Interest deposited back to the SERB	Grants received during the year			Total Available funds (1+2-3+4)	Expenditure incurred	Closing Balances (5-6)
1	2	3	4			5	6	7
			Sanction No. (i)	Date (ii)	Amount (iii)			
Rs. 2,39,189/-	Nil	N/A	Nil	Nil	Nil	Rs. 2,39,189/-	Nil	Nil (Unspent balance refunded to SERB vide D.D. No. 434777 dt.07.11.2019)

Component wise utilization of grants:

Grants-in-aid- General	Grant-in-aid-creation for capital assets	Total
		Rs. 2,39,189/-

Details of grants position at the end of the year

(i) Balance available at end of financial year : Nil..

(ii) Unspent balance refunded to SERB (If any) : Rs. 2,39,189/- vide D.D. No. 434777 dt.07.11.2019

(iii) Balance (Carried forward to next financial year) if applicable : Nil...

GFR 12 – A [(See Rule 238 (1))]
UTILIZATION CERTIFICATE (UC) FOR THE YEAR 2019-20
in respect of *NON-RECURRING*
as on 29-03-2020 ....to be submitted to SERB
Is the UC *Provision* .... *(Provisional/Audited)*
(To be given separately for each financial year ending on 31st March)

Certified that I have satisfied that the conditions on which grants were sanctioned have been duly fulfilled/are being fulfilled and that I have exercised following checks to see that the money has been actually utilized for the purpose for which it was sanctioned:

(i) The main accounts and other subsidiary accounts and registers (including assets registers) are maintained as prescribed in the relevant Act/Rules/Standing instructions (mention the Act/Rules) and have been duly audited by designated auditors. The figures depicted above tally with the audited figures mentioned in financial statements/accounts.

(ii) There exist internal controls for safeguarding public funds/assets, watching outcomes and achievements of physical targets against the financial inputs, ensuring quality in asset creation etc. & the periodic evaluation of internal controls is exercised to ensure their effectiveness.

(iii) To the best of our knowledge and belief, no transactions have been entered that are in violation of relevant Act/Rules/standing instructions and scheme guidelines.

(iv) The responsibilities among the key functionaries for execution of the scheme have been assigned in clear terms and are not general in nature.

~~(v) The benefits were extended to the intended beneficiaries and only such areas/districts were covered where the scheme was intended to operate.~~

(vi) The expenditure on various components of the scheme was in the proportions authorized as per the scheme guidelines and terms and conditions of the grants-in-aid.

(vii) It has been ensured that the physical and financial performance under ... EMR (Presently CRG)... .... **(CRG/NPDF/ECR.....etc.)** (Name of the scheme has been according to the requirements, as prescribed in the guidelines issued by Govt. of India and the performance/targets achieved statement for the year to which the utilization of the fund resulted in outcomes given at Annexure – I duly enclosed.

(viii) The utilization of the fund resulted in outcomes given at Annexure – II duly enclosed (to be formulated by the Ministry/Department concerned as per their requirements/specifications.)

(ix) Details of various schemes executed by the agency through grants-in-aid received from the same Ministry or from other Ministries is enclosed at Annexure –II (to be formulated by the Ministry/Department concerned as per their requirements/specifications).

Date: 08-09-2020

Place: Tezpur University

8/9/2020 P. I. & Assistant Professor Dept. of Environmental Science Tezpur University **Signature of PI**	**Signature with Seal** .... **Name:** .... **Chief Finance Officer** **(Head of Finance)** Finance Officer Tezpur University	14/9 **Signature with Seal**...................... **Name:** .... Registrar **Head of Organisation** Tezpur University

(Strike out inapplicable terms)

GFR 12 – A [(See Rule 238 (1))]
UTILIZATION CERTIFICATE (UC) FOR THE YEAR ...2019-20
in respect of *RECURRING*
as on 29-03-2020 to be submitted to SERB
Is the UC Provisional (*Provisional/Audited)*
(To be given separately for each financial year ending on 31st March)

1. Name of the grant receiving Organization : ... SERB..
2. Name of Principal Investigator(PI) .... Dr. Satya Sundar Bhattacharya ....
3. SERB Sanction order no. & date .... EMR/2016/002609 dated 27/03/2017..
4. Title of the Project...........“Utilization of Textile Industry Sludge Through Application of Vermitechnology: An In – Sight on Metal Accumulation Potential of Earthworms.”
5. Name of the SERB Scheme : .... EMR (Presently CRG)... .... (CRG/NPDF/ECR etc.)
6. Whether recurring or non-recurring grants : ***RECURRING***
7. Grants position at the beginning of the Financial year

    (i) Carry forward from previous financial year : Rs. 19479/-

    (ii) Others, If any :...Nil........................

    (iii) Total : Rs.19,479/-

8. Details of grants received, expenditure incurred and closing balances: (Actuals)

Unspent Balance of Grants received previous years [figure as at Sl. No. 7(iii)]	Interest Earned thereon	Interest deposited back to the SERB	Grants received during the year			Total Available funds (1+2-3+4)	Expenditure incurred	Closing Balances (5-6)
1	2	3	4			5	6	7
			Sanction No. (i)	Date (ii)	Amount (iii)			
Rs.19479/-	Nil	N/A	EMR/2016/002609	01-11-2019	Rs. 840000.00	Rs. 8,59,479	Rs. 8,59,479	NIL

Component wise utilization of grants:

Grants-in-aid- General	Grant-in-aid-creation for capital assets	Total
		NIL

Details of grants position at the end of the year

(i) Balance available at end of financial year : Nil
(ii) Unspent balance refunded to SERB (If any) : Nil

(iii) Balance (Carried forward to next financial year) if applicable: N/A

GFR 12 – A [(See Rule 238 (1))]

UTILIZATION CERTIFICATE (UC) FOR THE YEAR 2019-20.........

in respect of *RECURRING*

as on 29-03-2020......... to be submitted to SERB

Is the UC Provisional .... *(Provisional/Audited)*

(To be given separately for each financial year ending on 31st March)

Certified that I have satisfied that the conditions on which grants were sanctioned have been duly fulfilled/are being fulfilled and that I have exercised following checks to see that the money has been actually utilized for the purpose for which it was sanctioned:

(i) The main accounts and other subsidiary accounts and registers (including assets registers) are maintained as prescribed in the relevant Act/Rules/Standing instructions (mention the Act/Rules) and have been duly audited by designated auditors. The figures depicted above tally with the audited figures mentioned in financial statements/accounts.

(ii) There exist internal controls for safeguarding public funds/assets, watching outcomes and achievements of physical targets against the financial inputs, ensuring quality in asset creation etc. & the periodic evaluation of internal controls is exercised to ensure their effectiveness.

(iii) To the best of our knowledge and belief, no transactions have been entered that are in violation of relevant Act/Rules/standing instructions and scheme guidelines.

(iv) The responsibilities among the key functionaries for execution of the scheme have been assigned in clear terms and are not general in nature.

(v) ~~The benefits were extended to the intended beneficiaries and only such areas/districts were covered where the scheme was intended to operate.~~

(vi) The expenditure on various components of the scheme was in the proportions authorized as per the scheme guidelines and terms and conditions of the grants-in-aid.

(vii) It has been ensured that the physical and financial performance under ...EMR(Presently CRG).... .... **(CRG/NPDF/ECR.....etc.)** (Name of the scheme has been according to the requirements, as prescribed in the guidelines issued by Govt. of India and the performance/targets achieved statement for the year to which the utilization of the fund resulted in outcomes given at Annexure – I duly enclosed.

(viii) The utilization of the fund resulted in outcomes given at Annexure – II duly enclosed (to be formulated by the Ministry/Department concerned as per their requirements/specifications.)

(ix) Details of various schemes executed by the agency through grants-in-aid received from the same Ministry or from other Ministries is enclosed at Annexure –II (to be formulated by the Ministry/Department concerned as per their requirements/specifications).

Date: 08-09-2020

Place: Tezpur University

[signature] 08/09/2020 P. I. & Assistant Professor Dept. of Environmental Science Tezpur University **Signature of PI:..........**	[signature] 11/9/20 **Signature with Seal..............** **Name: ....** **Chief Finance Officer** **(Head of Finance)** Finance Officer Tezpur University	[signature] 14/9/20 **Signature with Seal........................** **Name: ....** Registrar **Head of Organisation** Tezpur University

(Strike out inapplicable terms)

Annexure-II

REQUEST FOR ANNUAL INSTALMENT WITH UP-TO-DATE STATEMENT OF EXPENDITURE

1. SERB Sanction Order No and date :EMR/2016/002609 dated 27/03/2017
2. Name of the PI :Dr. Satya Sundar Bhattacharya
3. Total Project Cost :Rs. 57,60,600/-
4. Revised Project Cost (if applicable) : Not Applicable
5. Date of Commencement : 30.03.2017
6. Statement of Expenditure :
   (Month wise expenditure incurred during current financial year)

Month & year	Expenditure incurred/ committed
January 2020	Rs. 49,991/-(Contingencies expenditure incurred)
January 2020	Rs. 1,90,734/-Overhead expenditure incurred)
February 2020	Rs.2,38,046/-(Consumable expenditure incurred)
April 2020	Rs.47,574/-(Travel expenditure incurred)
April 2020	Rs.3,33,134/-(Manpower costs incurred)
Total	Rs. 8,59,479/-

1. Grant received in each year:
   a. 1stYear :Rs 400,08,000/-(Rs 31,08,000 was received during 2016-17 and Rs 9,00,000/- was received during 2017-18)
   b. 2nd Year :Rs. 7,00,000/-(Received during 2018-19)
   c. 3rd Year :Rs. 8,40,000/-
   d. Interest, if any : NIL
   e. Total (a + b + c + d): Rs 55,48,000

Statement of Expenditure

(to be submitted financial year wise i.e. DOS* to 31st March of that financial year say 2019, 01-04-2019 till 29.03.2020)

Sr No (I)	Sanctioned Heads (II)	Total Funds Allocated (indicate sanctioned or revised (III)	Expenditure Incurred 1st Year (30.03.2017 (DOS) to 31.03.2018) (IV)	2nd Year (01.04.2018 to 31.03.2019) (V)	3rd Year & so on (01.04.2019 to 29.03.2020) (VI)	Total Expenditure till 29.03.2020 (VII = IV + V +VI)	Balance as on (date) (VIII = III–VII)	Requirement of Funds upto 31st March next year	Remarks (if any)
1.	Manpower costs	Rs 10,29,600	Rs. 2,46,266	Rs.3,00,000	Rs.3,33,134	Rs. 8,79,400	Rs. 1,50,200	NA	
2.	Consumables	Rs.9,00,000	Rs.3,61,718	Rs.3,00,000	Rs. 2,38,046	Rs. 8,99,764	Rs. 236	NA	
3.	Travel	Rs.1,50,000	Rs.48,555	Rs.44,201	Rs.47,574	Rs 1,40,330	Rs. 9,670	NA	
4.	Contingencies	Rs.1,50,000	Rs.50,000	Rs.50,000	Rs.49,991	Rs.1,49,991	Rs. 9	NA	
5.	Others, if any	Nil	NA	NA	NA	NA	NA	NA	
6.	Equipment	Rs.30,08,000	Rs.21,19,419	Rs. 6,49,392	NIL	Rs. 27,68,811	Rs. 2,39,189	NA	UNSPENT BALANCE OF 2,39,189 HAS BEEN REFUNDED TO SERB FAVOURING "FUND FO SCIENCE & ENGINEER RESEARCH" N DELHI V DD NO. 43477 DT.07.11.2
7.	Overhead expenses	Rs. 5,23,000	Rs. 1,70,718	Rs.1,09,063	Rs. 1,90,734	Rs.4,70,515	Rs. 52,485		
8.	Total	Rs.57,60,600	Rs. 29,96,676	Rs. 14,52,656	Rs. 8,59,479	Rs.53,08,811	Rs. 4,51,789		

S Bhattacharya

DR. SATYA SUNDAR BHATTACHARYA

Name and Signature of Principal Investigator:

Date: 08/09/2020

Assistant Professor
Dept. of Environmental Science
Tezpur University

Signature of Competent financial authority: ____________

(with seal)

Finance Officer
Tezpur University

Date: ____________

* DOS – Date of Start of project

Note:

1. Expenditure under the sanctioned heads, at any point of time, should not exceed funds allocated under that head, without prior approval of SERBi.e.FiguresinColumn(VIII)shouldnotexceedcorrespondingfiguresinColumn(III)
2. Utilization Certificate (Annexure III) for each financial year ending 31st March has to be enclosed along with request forcarry-forward permission to the next financial year.