

# **Vermicomposting for the bioremediation of PCB congeners in SUPERUND site media**

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## **Abstract**

In this study we investigated the potential of using earthworms (*E.foetida*) to effect the biotransformation of polychlorinated biphenyls (PCB's) in sludge and sediment from the Ralston Street Lagoon (RSL) SUPERFUND site in Gary, IN. Vermicomposting bioreactors (VB's) were established with mass fractions of contaminated sludge ranging from 10% to 75%. The VB's comprised a drainage layer overlaid with a mesh screen and then the earthworm bedding mixed with contaminated sludge. The VB's were each inoculated with equal biomass of earthworms. Suitable negative controls to monitor reproduction and biomass increase without contaminated sludge were also established. The VB's were kept moist by water spray and earthworms were periodically fed cornmeal. At set times, VB's were sampled and analysed by GC-ECD for PCB levels. Earthworm biomass and number were also measured. Results demonstrate that earthworms survived and reproduced in the presence of contaminated media; however, biomass increase decreased rapidly with increasing mass fraction of sludge. Biomass increased ranged from 103% in the negative control to biomass reduction of 54% with 75% sludge. Gas chromatography results demonstrated an 80% reduction in PCB level in all VB's, although the time required for this level of reduction increased with increasing sludge mass fraction. Analysis of earthworms showed elevated PCB levels in the worm. These results suggest vermicomposting may be a viable option for on-site *in situ* bioremediation and site clean-up.

**Keywords:** vermicomposting, earthworms, polychlorinated biphenyls, bioremediation, SUPERFUND.



## 1 Introduction

Polychlorinated Biphenyl's (PCB's) are chlorinated aromatic organics that were extensively used in industry until they were banned in 1976 by the United States Congress's Toxic Substances Control Act (TSCA) due to their adverse impact on health and the environment. This limited the distribution and future usage of PCB products, as described by Chawla et al [1]. PCB's were manufactured as Aroclors (USA), Kanechlors (Japan), and Sovols (Soviet Union) [2]. The Aroclors vast range of utility as a key ingredient in various adhesives, transformer dielectric fluids and machine oils due to its capacity for heat resistance led to its global distribution and subsequent contamination of multiple environmental matrices such as sediments, soils, and inland water-bodies [2]. Lax PCB disposal practices have lead to almost ubiquitous PCB environmental distribution. Approved treatment technologies, such as incineration, are expensive and can generate harmful by-products. Biotransformation of the PCB contaminants is a potential alternative method to be developed as a possible process for site cleanup.

## 2 Earthworms and chemical toxicity

Earthworms are the key organisms in the breakdown of plant organic matter; their populations expand in relation to the availability of organic matter. Due to their widespread distribution and importance in soil systems, they are considered as very useful organisms for evaluating contamination of the soil environment. Residues of chemicals can bio-accumulate in the earthworms and may be distributed by them to the tissues of animals in higher trophic levels within the food web, as discussed in Edwards and Thompson [3]. There has also been concern over the fate of long-lived contaminants such as dioxin [4] and PCBs [5]. Earthworms have a role in bio-monitoring because they can bio-accumulate or bio-concentrate xeno-biotic chemicals. They have been used to measure the level of heavy metal and persistent organic contamination of soil media.

## 3 Materials and methods

### 3.1 Earthworms

*E.foetida* earthworms were reared in our laboratory and these worms were used in all our experiments. This is the species most commonly used for degrading organic wastes. The rationale for choosing this species is that it tends to be ubiquitous and many organic wastes become naturally colonized by this species as described by Edwards and Neuhauser [6]. It also has a wide temperature tolerance and can live in wastes with a wide range of moisture content. It is a tough worm that is readily handled and, in mixed cultures, usually becomes dominant so that even when field systems begin with other species they end up with a large proportion of *E. foetida*.



### 3.2 Soil matrix

PCB contaminated sludge was obtained from the SUPERFUND site at the Ralston Street Lagoon in Gary, Indiana. The Gary Sanitary District (GSD) was responsible for the excavation, removal and transport of the sludge to our laboratory. Three five-gallon buckets of sludge were delivered to the Howard University Biochemical Engineering Lab (HUBEL) containing 3 different levels of PCB contamination. The samples were from different locations within the RSL: northwest, mid-west, and southwest, with approximate concentrations of 1000ppm, 780ppm, and 220ppm respectively, as reported to us by GSD. The sludge from the mid-west sample was chosen for our experiments.

### 3.3 Experimental setup

Two sets of VB's were established, each containing different mass fractions of sludge and hence different starting PCB concentrations. Each set of VBs contained five reactors with 0%, 10%, 25%, 50%, and 75% sludge mass fractions, respectively. The VBs were established with a gravel drainage layer on which was overlaid a mesh screen. The rock layer permitted excellent drainage so earthworms did not get flooded during the periodic VB wetting. The mesh screen, placed on the single layer of rock ensured that the rock would not mix with the soil above. All the VBs were covered with mesh on top so earthworms would not escape. One set of the different sludge mass fraction containing VBs was inoculated with 9 gms of live earthworms. The VBs were also wrapped in aluminium foil so that external light would not keep the earthworms away from the reactor walls.

## 4 Experimental protocol

All VB's were periodically sprayed with water to maintain the moisture content at around 50%. At given time intervals, each set of VBs was emptied, the earthworms were separated from the sludge using light and the sludge-earthworm bedding mixture was mixed and sampled for analysis by GC-ECD.

### 4.1 PCB measurement

The VB samples were placed in a laboratory fume hood to air dry. After drying, samples were extracted with acetonitrile. One gram of the dried sample was extracted in 15ml acetonitrile. The solvent was then filtered through a 0.4- $\mu$ m pore-size nylon filter. 1 ml of this filtered sample was injected into the gas chromatograph (GC) and run using an electron capture detector (ECD). The instrument used was a HP 5890 Series II GC utilizing a 0.32 mm internal diameter, 30 m fused silica column with a 0.5 $\mu$ m film and ECD. Calibration curves were run prior to each set of samples; Aroclor 1248 was used as the calibration standard, since the initial report from the GSD RSL demonstrated that the main PCB congener grouping in the RSL sludge was Aroclor 1248.



## 4.2 Total PCB mass balance

After 180 days, the experiments were terminated for 10% and 25% sludge mass fractions, and the total biomass of the earthworms in the different VB's was measured. The final sludge in the VB's was weighed and dried for extraction and analysis. The complete sludge-bedding mixture for each VB was dried and extracted which provided the information to complete the mass balance on the fate and distribution of the PCBs. The earthworms were also crushed and dried until a constant weight was recorded. The dried earthworm samples were extracted with methylene chloride in a Soxhlet extraction apparatus for 18 hrs with a reflux rate of 3-4 cycles per hour. The extract was then concentrated to 2 ml and the solvent exchanged with hexane [7] before being brought to a final volume of 10 ml. The 10 ml sample was washed with equivalent volume of concentrated sulfuric acid until the sample became clear; the washed and cleaned samples were then run on the GC with ECD.

## 5 Results and discussions

### 5.1 PCB concentration

The total final reduction of PCB's in the different sludge concentrations was fairly similar in all VBs although the time required to achieve this level of reduction increased significantly with increase in the contaminated sludge mass fraction in the VBs. All sludge concentrations demonstrated around an 80% reduction in total PCB concentration by the time of termination of the experiment. Table 1 show the reduction rate of PCB's in different sludge concentrations.

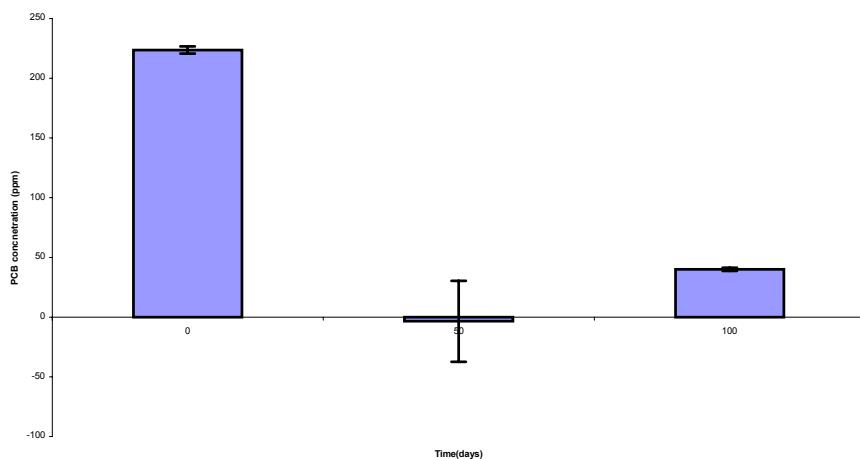


Figure 1: PCB concentration in 10% sludge from T= 0 days to T=150 days.



Figures 1, 2, 3, and 4 show the PCB reduction in the VBs with RSL sludge mass fractions of 10%, 25%, 50% and 75%, respectively. The increase in PCB concentration that appears after particular periods in some of the VBs suggests de-chlorination of higher chlorinated PCB congeners and generation of greater amounts of lower chlorinated compounds, hence resulting in a net increase in integrated area from the GC chromatograms. The x-axis in each figure shows the end-time for each VB experiments and this was different for the different sludge mass fractions. Hence, the rates of biotransformation decreased as the concentration of PCB contaminants increased.

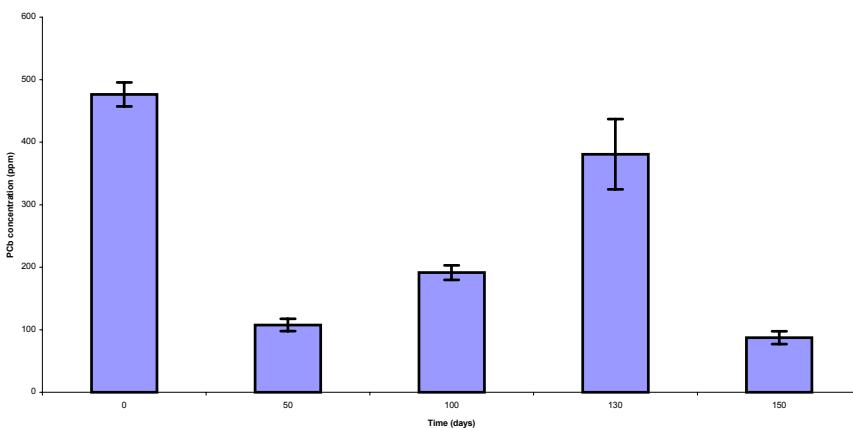


Figure 2: PCB concentration in 25% sludge from T= 0 days to T=150 days.

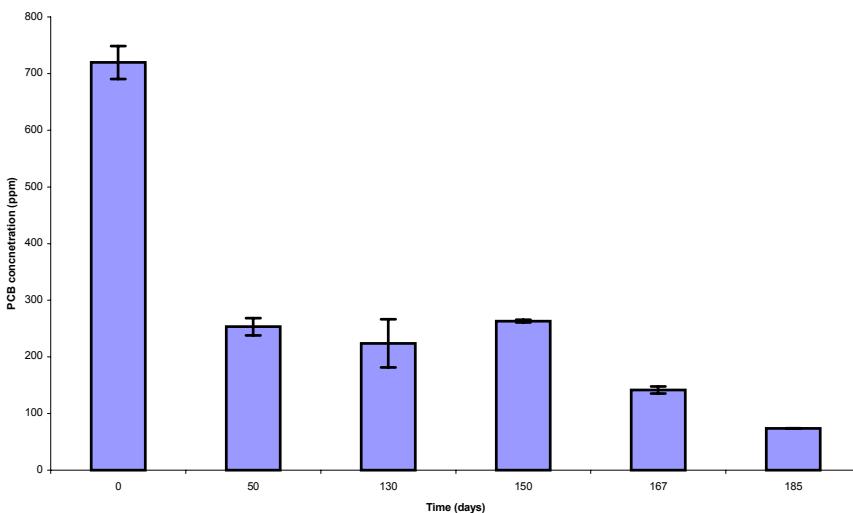


Figure 3: PCB concentration in 50% sludge from T= 0 days to T=185 days.



Table 1: PCB concentrations in different sludge concentrations.

Sludge Concentration (%)	PCB concentration at $t_{\text{initial}}$ (ppm)	PCB concentration at $t_{\text{final}}$ (ppm)[days]	Net % reduction in PCB concentration
10	223.65	40.07 [150]	82.08
25	476.28	87.46 [150]	81.63
50	719.57	73.8 [185]	89.67
75	942.17	160.83 [200]	82.93

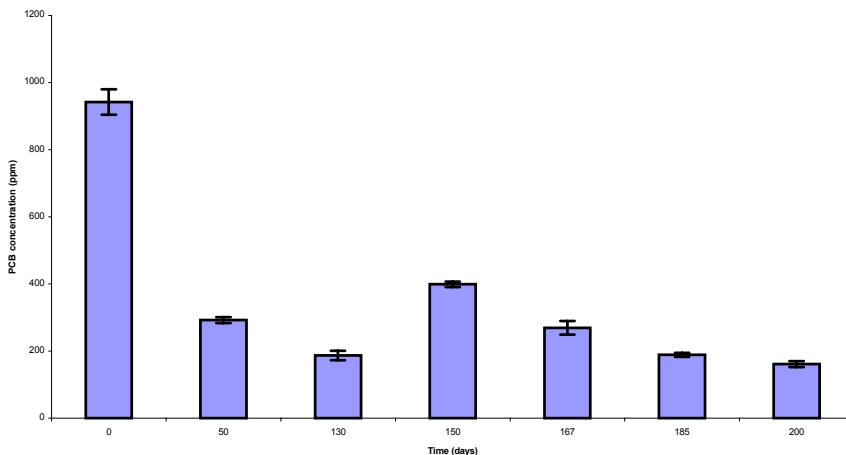


Figure 4: PCB concentration in 75% sludge from T= 0 days to T=200 days.

## 5.2 Earthworms

The total biomass of earthworms in all the VBs was also monitored. Table 2 shows the earthworm biomass from inoculation to time of termination. The negative control, containing earthworms with no sludge, demonstrated an earthworm biomass increase of 103%.

Table 2: Earthworm biomass in different sludge concentrations.

Sludge Concentration (%)	Initial worm weight (grams)	Final worm weight (grams)	Net % gain/loss in weight
10	9.026	12.2	35.16
25	9.057	5.563	-38.58
50	9.09	7.173	-21.089
75	8.997	4.11	-54.318

Table 3 shows the PCB concentration in the earthworms from each of the VBs. Analysis of dried and extracted earthworms from the control VB with no PCB contaminated sludge demonstrated no peaks and hence no PCBs. By the end of the experiment, the earthworms in the different sludge mass fraction VBs had bio-accumulated PCBs in their bodies, and the data shows that the higher the surrounding PCB levels, more PCBs were accumulated in the worms.

Table 3: PCB concentrations in the earthworm present in different sludge concentrations.

Sludge Concentration (%)	PCB concentration in earth worm (ppm)
10	148.02
25	212.9
50	188.35
75	313.08

## 6 Conclusion

This study shows that the earthworms (*E.foetida*) were able to bio-accumulate the PCBs from the sludge and reduce the amount of PCB congeners left in the sludge. Some biotransformation of PCBs is suggested by the data, especially a close examination of the various congener peaks. However, the bulk of the removal of PCBs from the VB matrices appeared to be by transport into the earthworm biomass. There is a decrease in the PCB concentrations in the sludge after exposure to, and working over by, the earthworms. Approximately 20% of the PCBs remain in the sludge-earthworm bedding mixture at the time of termination of each of the experimental studies. The fate of this remaining 20% and the reasons for its recalcitrance to further biotransformation requires more study and investigation in order to elucidate what might be occurring. Several possibilities make themselves apparent. Of course, it is entirely possible that a certain percentage of PCB contamination is hard to access and hence recalcitrant to any transformative activity. Bioavailability of the PCB in the sludge may be a large hindering factor, especially given the nature of the sludge which was extremely dense and clay-like. Another possibility is that the earthworms did not have sufficient time and sufficient additional feeding; hence it may be that with sufficient additional resources and longer experiments, more complete biotransformation may occur. Much of the PCBs are not biotransformed but simply bioaccumulated into the earthworms. The higher the surrounding PCB concentrations, the larger the amount of PCBs found in the earthworm biomass, as shown in Table 3. A high degree of bioaccumulation is occurring, and it is likely that bioaccumulation and biotransformation may take place together.



Nevertheless, these results do suggest that vermicomposting may be a potentially viable alternative for removal of PCB congeners from contaminated sludge or soils. Some further processing, however, may be required for complete elimination of the PCB.

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