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# **Assessment of Fate and Transport of Mixed Contaminants in Soil: An Incubation Experiment**

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## *Authors' contributions*

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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# **ABSTRACT**

A study on fate and transport of mixed contaminants (Cr, Cd and PFOA- Perfluorooctanoic acid) in soil was studied through incubation experiment. The study was carried over in Department of Environmental Sciences, Tamilnadu Agricultural University, Coimbatore between February 2022 and April 2022. The experiment includes 10 treatments with 3 replications and studied for 60 days of incubation. The contaminants (Cr, Cd and PFOA) and amendments (Biochar and humic acid) were spiked in known concentration in the unpolluted soil and analytical methods (physical and chemical properties) carried over to know their degradability, mobility, availability in soil. After 60 days of incubation period, the concentration of chromium, cadmium and PFOA was reduced to 31.5 per cent, 27.5 per cent and 30 per cent respectively. This was due to the addition of organic amendments (Biochar and humic acid). These amendments act as a sorbent in accumulating the contaminants within their pore spaces. Hence the concentration of the contaminants reduced in the soil during the incubation period.

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*Keywords: Incubation; chromium; cadmium; perfluorooctanoic acid.*

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# **1. INTRODUCTION**

Soil is considered as an unconsolidated material on the earth's surface that has been exposed to and impacted by the genetic and environmental elements of parent material, as well as climate, creatures and terrain mall of which have acted through time [1]. The major soil available in India is alluvial soil – 43 per cent of any other soil. The paramount soils of Tamilnadu are red loam, laterite, black, alluvial and saline soils. The red loam soil- 62 per cent occupies major part of Tamilnadu especially inner and coastal districts [2]. Environmental pollution is on-going topic of the day and so air, water and soil are being polluted. Soil can be considered as "universal sink" which hold up largest burden of environmental pollution [3]. In recent years, heavy metal contamination in soil is a major issue due to uncontrolled anthropogenic activities. Heavy metals are a class of metals and metalloids with a high density that are harmful even at low concentrations (ppb). Pb, As, Hg, Cd, Zn, Ag, Cu, Fe, Cr, Ni, Pd, and Pt are among examples [4]. Toxic metals (Ni, Cd, Hg, Zn, Pb, and Cr) & minerals (F and As) that cause pollution are currently a major national concern in India. Gujarat, Maharashtra, and Andhra Pradesh are responsible for 80 percent of India's heavy metal pollution [5]. Along with heavy metals, persistent organic pollutant also plays a major role in contaminating the soil. In 2006, India became a party to the Stockholm Convention, which added PFOS (Perfluorooctane sulfonic acid) to its global list of restrictions in 2009. However, India has refused to accept the modification identifying this compound, and it, like other PFAS (Per and polyfluoro Alkyl Substances), is unregulated. In women from Chidambaram, Kolkata, and Chennai, high PFAS levels for PFOS, PFOA (Perfluoro octanoic acid), PFHxS (Perfluoro hexane sulfonic acid), and PFBS (Perfluoro butane sulfonic acid) were discovered in 2008 research. Overall, PFOS levels in Indian breast milk averaged 46 ppt, more than double the health advice limit of 20 ppt for drinking water [6]. In this article, the fate and transport of mixed contaminants which includes Chromium, Cadmium and Perfluorooctanoic acid in unpolluted soil are discussed. The incubation experiment of soil along with above mentioned contaminants have been studied for 60 days.

## **2. MATERIALS AND METHODS**

## **2.1 Collection of Soil**

The unpolluted soil of 60 kg was collected from Eastern block of Tamil Nadu Agricultural University, Coimbatore – 641003. The soil was of clay loam soil. The soil was then sieved in 2mm sieve and dried at room temperature. The initial parameters of the soil including physical, chemical and biological properties were analyzed (Table 3).

#### **2.2 Spiking Soil with Mixed Contaminants and Amendments**

The experiment follows 10 treatments with 3 replications. Each pot was filled with 2 kg of soil. For incubation experiment optimum moisture was maintained through alternate wetting and drying method were followed to maintain the moisture of the soil. The water holding capacity of soil was found to be 54%. Accordingly, each pot of soil the moisture was maintained. Cr (III), Cd (II), PFOA, biochar and humic acid at fixed concentration were added according to the treatment details (Table 1). The soil was spread on a plastic sheet. The water with the chemicals and amendments were added little by little and mixed thoroughly by flipping the sheets from four corners. This ensures uniform mixing of all chemicals and amendments in the soil [7].

#### **Table 1. Treatment details of incubation experiment**



## **2.3 Incubation Period**

The first three days after spiking was considered to be stabilization stage where the spiked chemicals stabilize in the soil. The incubation period was counted after the stabilization stage completes. During the 30 days of incubation period, the soil was taken for analysis at  $15<sup>th</sup>$ ,  $30<sup>th</sup>$ , 45<sup>th</sup> and 60<sup>th</sup> day for physico- chemical analysis.

#### **2.4 Parameters to be Analyzed**

The fate and transport of the mixed contaminants was studied by analyzing the physical, chemical and biological properties of experimental soil (Table 3). The results of the analysis taken at  $15^{th}$ , 30<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> day of analysis were compared with the initial parameters.

## **2.5 Characterization of Soil**

#### **2.5.1 Physical properties of soil**

The bulk density and particle density were determined by following Cylinder method [8]. It was determined from the apparent and true volumes of the soil measured by adding a known quantity of water to a measuring cylinder containing a weighed quantity of soil.

#### **2.5.2 Chemical properties of soil**

The pH and EC of the soil samples were examined by using digital pH and EC meter respectively [9]. Organic carbon was estimated by following [10] method. Estimation of Calcium and Magnesium is done by following Versenate method.

Total Chromium and total Cadmium was estimated using Microwave Plasma Atomic Emission Spectroscopy (MP- AES). It is an atomic emission technique, once an atom of a specific element is excited, it emits light in a characteristic pattern of wavelengths, as it returns to the ground state. The samples were digested initially in Microwave digestion works by exciting water molecules to tear sample material apart. Adding Aquaregia (3:1 ratio of hydrochloric acid and nitric acid) of about 10 ml added to each sample speeds up the sample homogenization [11].

#### 2.5.2.1 Speciation of heavy metals

Metals can form organic complexes with dissolved organic materials, inorganic complexes

with dissolved anions and free hydrated metal ions in soil solution. Speciation refers to the chemical form in which metals reside in the solid and solution phases of the soil [12].

Determination of chromium and cadmium speciation were carried following the sequential extraction method and determined using MP-AES.

*Step 1 (Water soluble fraction):* In a 50 ml polypropylene centrifuge tube, one gram of airdried soil sample was weighed and 25 ml of double distilled water was added. It was shaken for 2 hours at 25°C in an end-over-end shaker. The tubes were then centrifuged for 10 minutes at 8000 rpm and filtered using Whatmann No. 42 filter paper. The amount of soluble Cr and Cd in the water extract was calculated.

*Step 2 (Exchangeable fraction):* 25 ml of 0.5 M KNO3 was added to the residue from step 1 and agitated for 16 hours. As described in step 1, centrifugation, filtering, and measurement were carried out.

*Step 3 (Organic fractions):* 0.5 M NaOH was added to the residue from step 2 and agitated for 16 hours. As described in step 1, centrifugation, filtering, and measurement were carried out.

*Step 4 (Organic plus iron – oxide bound fraction):* 0.05 M Na2EDTA was added to the step 3 residue and agitated for 6 hours. As described in step 1, centrifugation, filtering, and measurement were carried out.

*Step 5 (Residual fraction):* Using a jet of water, the soil residue from step 4 was placed into a 100 mL conical flask and dried in an oven. The contents were digested at 1100º C after 10 mL of strong nitric acid was added. The contents were diluted and filtered using Whatmann No. 4 filter paper after digestion.

Before and after extraction, the tube and contents were weighed to determine the amount of entrapped solution and heavy metal transfer between extractants. The quantity of Cr and Cd extracted by each extractant was estimated independently using the following equation.

Cr and Cd extracted  $(\mu g g^{-1}) = C \times (E+M)$  -(C' x M) / weight of soil

Where,

C – Concentration of heavy metal in the extraction solution

(m/z)	(m/z)	Compound Precursor Fragment ion	<b>Fragment voltage</b> Collision energy (V)	Dwell time (ms)
<b>PFOA</b>	$369*$	80		30
413	169	80		30

**Table 2. MRM acquisition parameters for the detection of PFOA in LC/MS**.

- M Mass (g) of the entrained solution carried over from previous extraction
- C' Concentration of the heavy metal in the extraction solution proceeding step of the sequence

 $E -$  Mass (g) of the extractant

2.5.2.2 Perfluorooctanoic acid estimation

PFOA (Perfluorooctanoic acid) was determined using LC/MS (Liquid Chromatography – Mass Spectrometer) – SHIMADZU LC/MS/MS 8040. PFOA was purchased from Sigma Aldrich with 95% purity. The standards were prepared at concentrations of 50, 40, 20, 10, 5, 2, 1 ppb (parts per billion) and the linearity curve arrived. The soil sample were prepared as done by Bugsel and Zwiener [13]. In a 50-mL polypropylene (PP) tube, five grams of soil samples were weighed, and 10 mL MeOH was added. The mixture was vortexed for 2 minutes, sonicated for 10 minutes, and then shaken horizontally for 24 hours. The supernatant was transferred to a 20-mL PP jar using a glass pipette after centrifugation (10 min, 4000 rotation per minute (rpm)). As specified, the extraction was repeated with 10 mL MeOH. The supernatants were mixed, heated to 40 °C, and evaporated with a moderate stream of nitrogen to less than 1 mL. To make the volume 1 mL, pure MeOH was utilised. prior to analysis, the concentrate was placed into a PP vial and centrifuged (10 min, 4000 rpm). Table 2 gives details about the MRM acquisition parameters for the detection of PFOA.

## **2.5.3 Biological properties of soil**

Soil enzyme activity: Urease, phosphatase and dehydrogenase of the soil were determined. For determination of urease Tabatabai and Bremner, 1972 method [14] was adaopted. Dehydrogenase was determined using Chendrayan et al. 1980 method [15]. For the determination of phosphatase Tabatabai and Bremner, 1969 method [16] was adopted.

## **3. RESULTS AND DISCUSSION**

The results emanated from the study is discussed here.

## **3.1 Characteristics of Experimental Soil and Amendment**

Before spiking the soil with contaminants (Cr, Cd and PFOA) along with the amendments (Biochar and humic acid), the initial characteristics of soil were analysed (Table 3). From the table, it was evident that the soil and amendments were not contaminated by chromium, cadmium nor PFOA. These contaminants not exceeded the permissible limit and were in below detectable limit. Hence the soil was suitable for incubation experiment.

## **3.2 Effect of Contaminants and Amendments on Soil pH and EC during Incubation Period**

The pH of the soil in the treatments from  $T_1$  to  $T_4$ for 60 days of incubation period was moreover in a stable state. The pH was gradually increased in the treatments  $T_5$  to  $T_{10}$  when compared with  $T_1$ to  $T<sub>4</sub>$ . This was due to addition of amendments to the soil. Since biochar and humic acid homogenized with the soil, it influenced the chemical properties of the soil.

The EC of the soil when compared with the  $15<sup>th</sup>$ and 60<sup>th</sup> showed varied difference. During the initial analysis of the soil, EC of all treatments were similar. After course of time the EC got decreased to 62.5 per cent due to the influence of the contaminants and amendments.

## **3.3 Effect of Contaminants and Amendments on Soil Organic Carbon on 0th and 60th day of Incubation Period**

Comparing the results from  $0<sup>th</sup>$  and  $60<sup>th</sup>$  day of soil organic carbon analysis, the organic carbon reduced tremendously for the treatments  $T_5$  to  $T_{10}$ , comparing with the treatments  $T_1$  to  $T_4$ , when comparing with their initial and final values. The treatments from  $T_1$  to  $T_4$  does not have any of the amendments, hence doesn't showed any variations. The treatments from  $T_5$  to  $T_{10}$  showed huge variation due to the addition of amendments. Collectively, the organic carbon of the soil was reduced to about 66.37 per cent. Hence this result confirms that the contaminants along with the amendments biochar [17] and humic acid have effect on soil organic carbon.

**3.4 Effect of Biochar and Humic acid on Water Soluble Fraction (H2O Fraction) of Chromium and Cadmium in Soil at Different Incubation Period (mg kg-1 )**

The concentration of chromium and cadmium had reduced in every treatment. Comparing the treatments with amendments plus contaminants and treatments with only contaminants, the treatments with amendments plus contaminants have reduced in their chromium and cadmium concentration to 38 per cent and 34.5 per cent respectively. This could be as a result of the surface complexation and/or adsorption just on soil particles converting the soluble form of Cr and Cd into insoluble ones [18].

## **3.5 Effect of Biochar and Humic Acid on Organic Fraction (NaOH Fraction) of chromium and Cadmium in Soil at Different Incubation Period (mg kg-1 )**

The NaOH – Cr and Cd concentration was observed to steadily decrease over the course of 60 days of incubation. The many physicochemical interactions between Cr, Cd and soil, such as complexation, adsorption, and precipitation, may be too responsible for this drop in concentration [19].

## **3.6 Effect of Biochar and Humic Acid on Residual Fraction (HNO3 Fraction) of Chromium and Cadmium in Soil at**  Different Incubation Period (mg kg<sup>-1</sup>)

Comparing with the initial and final values taken at 60 days of incubation period, the residual fraction had increased in both chromium and cadmium. The fact that Cr and Cd is immobile may be the cause of its persistence in soil. The sorption of Cr and Cd may have significantly increased the residual proportion of Cr and Cd respectively. Chromium is mostly concentrated in residual fractions [20].

## **3.7 Effect of Biochar and Humic Acid on Availability of Trivalent Chromium (Cr (III)) and Divalent Cadmium (Cd (II))**

Comparing all the treatments,  $T_7$  and  $T_{10}$  had significantly reduced the availability of cadmium and chromium in soil respectively. The humic acid in  $T<sub>z</sub>$  which contains mixed contaminants, had specifically decreased cadmium [21] to lower concentration than chromium. The biochar in  $T_{10}$ which contains mixed contaminants, had specifically decreased chromium to lower concentration than cadmium. But both the biochar and humic acid had a great influence in reducing the chromium and cadmium in the soil. This may be due to reduced mobility and transport of heavy metals in the soil influenced by organic amendments [22]. The concentration of chromium and cadmium was reduced to 31.5 and 27.5 per cent respectively.



**Fig. 1. Effect of contaminants and amendments on soil pH during incubation period**

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**Fig. 2. Effect of contaminants and amendments on soil EC during incubation period**



**Fig. 3. Effect of contaminants and amendments on soil organic carbon on 0th and 60th day of incubation period**



**Fig. 4. Effect of biochar and humic acid on water soluble fraction (H2O fraction) of chromium in**  soil at different incubation period (mg kg<sup>-1</sup>)

<b>Parameters</b>	Soil	<b>Biochar</b>	<b>Humic acid</b>
Physical properties			
Bulk density (g $cm^{-3}$ )	1.11	0.49	1.75
Particle density (g $cm^{-3}$ )	2.5	0.28	1.26
<b>Chemical properties</b>			
рH	8.06	8.56	6.20
Electrical Conductivity (dS $m^{-1}$ )	0.16	0.87	1.42
Organic carbon (%)	0.54	9.49	21.0
Available nitrogen (kg ha <sup>-1</sup> )	149.7		
Available phosphorus (kg ha <sup>-1</sup> )	12.1		
Available potassium (kg ha <sup>-1</sup> )	226		
Total nitrogen (%)		18	4
Total phosphorous (%)		2.89	1.21
Total potassium (%)		18	10
Exchangeable Ca (cmol $(P^+)$ kg <sup>-1</sup> )	6.23		
Exchangeable Mg (cmol $(P^+)$ kg <sup>-1</sup> )	2.67		
Exchangeable Na (cmol $(P^+)$ kg <sup>-1</sup> )	1.34		
<b>Total Cadmium</b>	BDL*	BDL*	BDL*
<b>Total Chromium</b>	BDL*	BDL*	BDL*
<b>PFOA</b>	BDL*	BDL*	BDL*
<b>Biological properties</b>			
Urease (µg of $NH_4$ -N released g <sup>-1</sup> of soil day <sup>-1</sup> )	13.5		
Phosphatase ( $\mu$ g p-Nitrophenyl Phosphate g <sup>-1</sup> of soil)	11.4		
Dehydrogenase ( $\mu$ g triphenyl formazan g <sup>-1</sup> of soil)	9.3		

**Table 3. Initial characteristics of experimental soil and amendments**

*BDL\* - Below detectable level*



**Fig. 5. Effect of biochar and humic acid on water soluble fraction (H2O fraction) of cadmium in soil at different incubation period (mg kg-1 )**

The exchangeable fractions and organic plus iron – oxide bound fractions of chromium and cadmium was found below detectable limit. This may be caused by the quick conversion of exchangeable Cr and Cd to organic form or by adsorption on highly maintained exchangeable sites in the soil [23,24]. It's also possible that the Cr and Cd ions got into the pore spaces of the biochar and humic acid. The availability of chromium and cadmium thus reduced in the soil during the incubation experiment and the major influence was created by the amendments.



**Fig. 6. Effect of biochar and humic acid on organic fraction (NaOH fraction) of chromium and**  in soil at different incubation period (mg kg<sup>-1</sup>)



**Fig. 7. Effect of biochar and humic acid on organic fraction (NaOH fraction) of cadmium and in soil at different incubation period (mg kg-1 )**



Fig. 8. Effect of biochar and humic acid on residual fraction (HNO<sub>3</sub> fraction) of chromium in soil **at different incubation period (mg kg-1 )**

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**Fig. 9. Effect of biochar and humic acid on residual fraction (HNO3 fraction) of cadmium in soil at different incubation period (mg kg-1 )**



**Fig. 10. Effect of biochar and humic acid on availability of trivalent chromium (Cr (III))**



**Fig. 11. Effect of biochar and humic acid on availability of divalent cadmium (Cd (II))**



**Fig. 12. Effect of biochar and humic acid on availability of Perfluorooctanoic acid (PFOA)**

## **3.8 Effect of Biochar and Humic Acid on Availability of Perfluorooctanoic Acid (PFOA)**

The treatments  $T_3$  and  $T_4$  doesn't show any reduction in the concentration of PFOA. Though PFOA is water soluble, it does not bind well with the soil. Hence it is extremely resistant to environmental degradation [25]. The treatments with biochar ( $T_9$  and  $T_{10}$ ) showed reduction in PFOA concentration. Biochars are considered potential sustainable sorbents to reduce the perand polyfluoroalkyl substances (PFAS) from contaminated soils [26]. The humic acid contributes to contaminant removal individually or in combination with other mineral adsorbents [27]. The PFOA concentration was reduced to 30 per cent at the end of the incubation period. The reduction in concentration was greatly influenced by the addition of amendments.

## **4. CONCLUSION**

The use of amendments biochar and humic acid have significantly reduced the concentration of the mixed contaminants (Cr, Cd and PFOA). The contaminants easily transported into the pore spaces of the amendments during the incubation period and the concentration reduced during incubation period. Rather than the amendment treatments, the contaminants (Cr and Cd) concentration reduced due to their degradability, solubility property in the medium (soil and water).

## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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