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# APPLICATION OF AQUEOUS EXTRACTS OF Alternanthera brasiliana, Chromolaena odorata AND Tridax procumbens PLANTS IN REMEDIATING LEAD CONTAMINATED SOILS

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#### ABSTRACT

Soil washing is an effective method of removing lead from contaminated soils. However, great limitations abound in the choice of washing solution that is ecologically sustainable for agricultural soils. In this study, applications of plant soluble extracts in remediating of Pb contaminated soils for ecological sustainability were carried out. Batch laboratory experiments were done using aqueous extracts of Alternanthera brasiliana, Chromolaena odorata, Tridax procumbens and water as control at varying soil-pulp-densities (SPD) of 3, 6, 9, 12, 15%, and washing time of 1, 3, 6, 12 h. For contaminated soil, percentage removal efficiency followed the order: Alternanthera brasiliana  $(38.1\pm0.28\%) > Chromolaena odorata (21.8\pm0.12\%) > Tridax procumbens (21.3\pm0.18\%)$ . The most appropriate ratio for contaminated soil was 3% SPD at 12 h washing time. Removal efficiency was found to be substantially depended on geochemical phase of Pb (exchangeable-4.04%) in contaminated soil and washing solution pH (5.68). Lead removal efficiency was observed to increased proportionally with increasing washing time, but decreased with increasing SPD. Spike soil with high exchangeable Pb-78.9% recorded significant Pb removal of 63.4±0.24% with Alternanthera brasiliana, 47.8±0.22% with Chromolaena odorata, 38.3±0.38% with *Tridax procumbens* and  $52.0\pm0.26\%$  with water. Analysis of variance at *p*=0.05 indicated a significant difference in percentage Pb removal efficiency across all the four washing solutions. However, the statistical T-test indicated no significant difference at p=0.05 in percentage Pb removal efficiency between both soils. Also, moderately positive correlations were observed between contaminated and spiked soils for Alternanthera brasiliana (0.676), Chromolaena odorata (0.570) and Tridax procumbens (0.517) while negative correlation observed for water (0.485) which served as the control. The three plant extracts exhibited good potential characteristics as washing solutions for the treatment of Pb contaminated soils. Chemical modifications are recommended to enhance and improve their efficiencies when considering the geochemical phase of Pb in soil.

Keywords: Soil contamination, Plant extracts, Soil washing, Lead, Spike soil.

#### **INTRODUCTION**

Lead contamination of soil is considered a major environmental problem due to its persistency, non-biodegradability and toxicity (Muarrugo-Negrete et al., 2017; Jiang et al., 2019). Although lead occurs naturally in the ecosystem at varying concentrations, accumulation of lead in soils resulting from contamination degrades water bodies, food crops and the biosphere through surface runoffs and dust storms (Kankia and Abdulhamid, 2014). Bioaccumulation of lead in food chain poses serious human health risk such as cardiovascular diseases, cancer, cognitive impairment, and renal dysfunction (Jaishankar et al., 2014; Pierart et al., 2015; Xiong et al., 2016; Demey et al., 2017). Globally, there is a significant awareness of environmental impact of lead, hence the need for economically efficient technique for remediating lead contaminated soils.

Soil washing is a promising technique for remediation contaminated soils, based on

desorption of trace metals from contaminated soils using different extractants (Maturi and Reddy, 2008; Kulikowska et al., 2015). The success of this technique depends on various soil properties such as pH, permeability, concentration and speciation of metals. In this regard, conventional reagents such as acids, salts, surfactants, chelating and reducing agent have been applied with varying degree of success (Im et al., 2015; Satyro et al., 2016). For example, strong acids can render the soil sterile, while chelating and reducing agents can result in secondary pollution due to their persistence in soil environment (Kaurin et al., 2018). Low molecular weight organic acids on the other hand usually have low percentage removal efficiency (Etim, 2017; Etim, 2020). Therefore, there is the need to research for new and eco-efficient washing agents for soil lead remediation.

Recently, the use of plant based extractants for washing metal contaminated soils is gaining intense interest globally (Chaing et al., 2016; Cao et al., 2017a; Cao et al., 2017b; Feng et al., 2018; Etim, 2019; Feng et al., 2020). This is because they are strongly biodegradable, cost effective and ecofriendly and can increase essential soil nutrients when used. A wide variety of local plants are found in the tropical climates of Nigeria. For instance, Terminalia mantaly, Panicum maximum and Eleusine indica have previously been applied in washing lead contaminated soils with removal efficiency of 27.2±0.6% and 27.0±0.5% for Terminalia mantaly and Panicum maximum respectively (Etim, 2017). Alternanthera brasiliana, Chromolaena odorata and Tridax procumbens are perennial plants and widely distributed in Southern Nigeria. No report is found to have investigated their applications in removing lead from contaminated soils via washing. Hence, screening these new plant materials may be of great significance in seeking enhanced removal efficiency of lead. The objective of this study was to assess the effectiveness of aqueous extracts of Alternanthera brasiliana, Chromolaena odorata and Tridax procumbens for removing lead from contaminated soils.

# MATERIALS AND METHODS Sampling and Preparation of Soil

Field contaminated soil was collected from the impact berm of a major military shooting range with obvious presence of large quantity of spent bullet fragments. These bullet fragments are known to contain high levels of lead which over time can leached and contaminate the surrounding soils. About 10 kg of the field contaminated soil sample was collected at a depth of 0-30 cm using a stainless steel hand trowel, air dried and sieved using a 2 mm mesh sieve in the laboratory. Similarly, about 10 kg of a noncontaminated soil was also sampled at a depth of 0-30 cm around the University of Ibadan Botanical Garden where there is no obvious sign of pollution. This soil was artificially contaminated with lead by spiking it with 1000 mL of  $Pb(NO_3)$  at a concentration of 10,000 mg/L after which it was thoroughly homogenized by mixing for about 48 h and dried at ambient temperature for 3 days with continuous mixing. The field and spiked contaminated soils were store in a polythene bag for further analysis and batch washing experiment.

**Preparation of Water Soluble Plant Extracts** The plants chosen for this washing experiment are: Alternanthera brasiliana, Chromolaena odorata and Tridax procumbens. These plants are locally available and are of no economic value. Large quantities of these three plants (i.e. leaves and stem, which are likely free from metal contamination) were collected and air dried for a week in the laboratory. The dried plants were grounded into fine particles. A 5 kg portion of each grounded plant material was cold extracted with 20 L of deionized water for 12 h using a mechanical mixer (Griffin and George Limited KQPS/34). The aqueous mixtures were later filtered using a 10 µm mess cloth and stored at 20 °C for the washing experiment. This extraction method is a slight modification of the procedure previously describe by Etim (2019) in terms of weight of plant to volume of water ratio. This modification is used to investigate the effect of higher extract concentration on Pb removal efficiency. The pH value of the extracts was measured by directly dipping a clean pH glass electrode into the solutions (Hanna Instrument). Lead concentrations was determined using Varian SpectrAA 600 Flame Atomic Absorption Spectrophotometer.

#### Soil Washing Experiment

The aqueous extract of the three plant spices *Alternanthera brasiliana, Chromolaena odorata* and *Tridax procumbens* as well as water which serve as the control were used for the batch washing of field and spiked contaminated soils to assess their efficiency in removing Pb. Soil-Pulp-Density and washing time were considered as variables in the experiment as described in previous studies (Etim, 2017; Etim, 2019). However, the washing time was slighted adjusted to 1, 3, 6 and 12 h since the extracts where highly concentrated.

For the washing of contaminated soil, the experiments were carried out by weighing 3, 6, 9, 12 and 15 g of soils into five plastic bottles, into which 100 mL of aqueous *Alternanthera brasiliana* solution was added. This is equivalent to 3%, 6%, 9%, 12% and 15% soil pulp density, calculated as:

$$SPD(\%) = Ms/V_{sol}$$
(1)

where Ms is mass of soil in grams,  $V_{sol}$  is volume of

aqueous washing solution or water added in mL.

The five bottles were then placed on a mechanical shaker (Edmund Buhler SM 25) and agitated for 1 h. The resulting mixtures were filtered using Whatman (Cat No 1001, 110 mm) filter paper and the residue washed with deionized water. The extracts were stored for further analysis. The agitation process was repeated for 3, 6 and 12 h intervals with same weight-to-volume ratio of contaminated soil and Alternanthera brasiliana aqueous washing solutions. This process was also repeated using aqueous extracts of Chromolaena odorata, Tridax procumbens and water at the same weight-to-volume ratio of contaminated soil and washing solutions. Similarly, the entire experiment was also carried out for the spiked soil using the various washing solutions and same variables. The extracts from each of the experiments were digested with 2 mL concentrated nitric acid on a hot plate, filtered and stored for Pb determination using Varian SpectrAA 600 Flame Atomic Absorption Spectrophotometer.

The percentage removal efficiency of lead from the soils was calculated using the equation reported by Reddy and Chinthramreddy (2000) and Wuana *et al.* (2010) given as:

Removal efficiency (%)  $C_1 x V_1 / C_s x Ms \times 100$  (2) where  $C_1$  and  $C_s$  are the concentrations of Pb in the supernatants (mg/L) and soil samples (mg/kg) determined using FAAS,  $V_1$  is the volume of supernatants (L), and  $M_s$  is the weight of the soil (kg) used for the washing experiment.

The batch washing experiments were performed in triplicate and the standard deviation was within the range of 5%.

#### Soil Analysis

Soil pH values were determined using a soil-water ratio of 1:1 (w/v) (McLean, 1982). Particle size analysis was carried out using the hydrometeric method (IITA, 2001). Walkley and Black method (Walkley and Black, 1934) for soil organic carbon content. Available phosphate and nitrate was determined by Bray No. 1 method (Bray and Kurtz, 1945) and by distillation method (Bremner and Mulvaney, 1982) respectively. Cation exchange capacity (CEC) was determined using 1 M ammonium acetate at pH 7 as the exchangeable base (Rhoades, 1982). Total lead concentration in the soils was determined using HCl:HNO<sub>3</sub> aquaregia acid mixtures (Niskavaara *et al.*, 1997) and the Pb analyzed using Varian SpectrAA 600 Flame Atomic Absorption Spectrophotometer. Speciation study of lead fractions was done using Tessier sequential extraction method (Tessier *et al.*, 1979).

## **Quality Control and Assurance**

Analar grade (Sigma-Aldrich) reagents and deionized water were used for the experiment. The washing experiments were performed in triplicates incorporated with split samples for instrument data validation. Statistical T-test showed no significant differences in actual and split results. The Varian SpectrAA 600 Flame Atomic Absorption Spectrophotometer was calibrated using 1, 3, 5, and 10 mg/L REHHGFT working standards.

#### **RESULTS AND DISCUSSION**

# Characterization of Experimental Soils and Washing Solutions

The physicochemical properties of the experimental soils are presented in Table 1. The pH in 1:1 soil to water ratio indicated slight acidic soil conditions for both contaminated - 5.44 and spiked - 4.18 soils. Soil texture showed that both soils were loamy sand with low organic carbon content. Nitrate and phosphate levels were typical of any tropical soil which supports plant growth. However, cation exchange capacity of spiked soil was slightly higher than that of the contaminated soil. High soil CEC allows for easy exchange of soil contaminants if present. Lead levels in contaminated soil (9750 ± 250 mg/kg) was significantly higher than that of the spiked soil  $(7175 \pm 106 \text{ mg/kg})$  at P=0.05. For washing extract solutions, pH values where relatively acidic for Alternanthera brasiliana (5.68), moderately neutral for Chromolaena odorata (6.70) and Tridax procumbens (6.85), but basic for water (8.10). These variations in pH values may impact on the washing solutions ability to extract lead from the experimental soils. Mean concentrations of lead in all the extracting solutions were below detection limit  $\leq 0.002$  mg/L. From the speciation data (Table 2), the bioavailable (non-residual) fractions of Pb in contaminated soil was 69.5% which was

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lower than spiked soil value of 94.3%. The most mobile of the bioavailable fraction was only about 4.04% in contaminated and 78.9% in spiked soil sample. The low levels of mobile phase Pb in contaminated soil would therefore minimize the extraction of Pb as against spiked soil.

Parameter	Contaminated soil	Spiked soil	
pH	5.44	4.18	
Sand content (%)	71.2	74.4	
Silt content (%)	17.2	19.4	
Clay content (%)	11.6	6.20	
Organic carbon (%)	2.39	2.27	
Organic matter (%)	4.13	3.92	
CEC (cmol/kg)	134	228	
Nitrate (mg/kg)	66.4	69,4	
Phosphate (mg/kg)	18.6	15.8	
Lead	9750±250mg/kg	7175±106mg/kg	

**Table 1:** Characteristics of experimental soils and washing solutions (n=3)

n- Number of samples analyzed. CEC-Cation exchange capacity.

Table 2: Lead speciation	(% geochemical )	phases) in ex	perimental so	ils $(n=3)$
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Datamatat	Pb		
rarameter	Contaminated soil	Spiked soil	
Exchangeable metal	4.04	78.9	
Metals bound to carbonates	35.7	4.20	
Metals bound to Fe-Mn oxides	20.4	6.92	
Metals bound to organic matter	9.40	4.30	
Residual metals	30.5	5.69	

# Lead Removal Efficiency from Contaminated Soil

Table 3 shows percentage removal efficiency of lead from contaminated soil using four washing solutions: Alternanthera brasiliana, Chromolaena odorata, Tridax procumbens and water as control. This experiment assessed efficiency of three aqueous plant extracts, as washing solutions in removing Pb from contaminated soil comparatively to water as control. The pH values of the plant extracts shown in Table 1 indicates that the extracting solutions have a complex matrix which could affect Pb removal efficiency. Percentage removal efficiency of lead from contaminated soil ranged between 7.04  $\pm$  0.09 to  $38.1 \pm 0.28\%$  for Alternanthera brasiliana,  $1.45 \pm$ 0.10 to 21.8  $\pm$  0.12% for *Chromolaena odorata*, 7.18  $\pm$  0.17 to 21.3  $\pm$  0.18% for *Tridax procumbens*, and  $0.85 \pm 0.04$  to  $19.8 \pm 0.18\%$  for water extracting solutions. The highest removal efficiency of lead by the three aqueous plant extracts were observed to be obtained for 3% soil pulp density at 12 h extraction time. However, water extraction solution showed rather the lowest removal efficiency of lead at only 15 % soil pulp density for 15 h extraction time. Removal efficiency of Pb from contaminated soil was observed to significantly decrease with increasing soil-pulpdensity for the three plant extract washing solutions irrespective of washing time. And the most efficient soil ratio was observed to be 3 % SPD. This implies that, removal efficiency is only enhanced at lower soil to washing solution ratio [i.e. 3 g in 100 mL], which is also shown in similar studies (Chaiyaraksa and Sriwiriyanuphap, 2004; Demir and Koleli, 2013; Etim, 2017; Etim, 2019; Etim, 2020). Washing time also played a key role in determining removal efficiency, vis-à-vis, the longer the washing time the greater the removal efficiency as shown in Figure 1. For instance,

highest Pb removal efficiency was obtained for 3 SPD at 12 h extraction with *Alternanthera brasiliana* recording 38.1  $\pm$  0.28%; *Chromolaena odorata* 21.8  $\pm$  0.12% and *Tridax procumbens* 21.3  $\pm$  0.18%. Slight acidic pH value of *Alternanthera brasiliana* could explain the relative high removal efficiency of Pb comparatively to *Chromolaena odorata* and *Tridax procumbens* with about neutral pH values. Acidic conditions of washing solutions and soils favours dissolution and solubilization of specific soil components containing metals (Neale *et al.*, 1997). Besides other soil conditions such as organic matter and CEC may also be a main factor as observed in Table 1. Lead removal efficiency did not differ significantly in control water washing solution in relation to washing time and SPD. However, percentage removal efficiency was much lower compared to aqueous plant extract washing solutions. Analysis of variance (p=0.05) showed significant differences in percentage removal efficiency of Pb across the four washing solutions. This further confirms the role the pH values of washing solutions in solubilization of Pb in the contaminated soil. Comparatively, *Alternanthera brasiliana* (38.1 ± 0.28%) seems to be a better washing solution in removing lead from contaminated soil than *Treminalia mantaly* (21.7 ± 0.10%) under the same condition of 3 SPD and 12 h washing time (Etim, 2019).

Soil pulp	Washing time	Alternanthera	Chromolaena	Tridax	Water
density (%)	(h)	brasiliana,	odorata	procumbens	
3	1	16.3±0.05	7.86±0.32	13.0±0.24	0.85±0.04
6		11.8±0.06	8.55±0.22	8.55±0.17	6.84±0.09
9		$10.4 \pm 0.17$	$1.48 \pm 0.15$	8.32±0.21	8.66±0.06
12		8.72±0.18	$1.45 \pm 0.10$	7.61±0.12	9.06±0.11
15		$7.04 \pm 0.09$	3.08±0.18	8.27±0.10	$10.7 \pm 0.10$
3	3	19.2±0.21	16.1±0.14	17.5±0.13	4.27±0.12
6		14.8±0.11	10.3±0.13	12.5±0.22	7.69±0.21
9		12.8±0.15	6.38±0.10	11.5±0.26	8.43±0.14
12		$11.2 \pm 0.14$	8.43±0.21	16.9±0.16	8.03±0.15
15		9.85±0.02	4.24±0.22	7.18±0.17	10.9±0.14
3	6	$23.6 \pm 0.06$	$10.1 \pm 0.21$	15.7±0.10	6.41±0.13
6		$20.8 \pm 0.08$	$9.55 \pm 0.18$	13.6±0.11	9.30±0.11
9		$16.0 \pm 0.10$	8.32±0.07	11.0±0.10	8.89±0.16
12		17.8±0.13	8.72±0.11	10.5±0.10	9.06±0.14
15		$10.4 \pm 0.08$	5.33±0.11	9.85±0.14	11.1±0.11
3	12	38.1±0.28	$21.8 \pm 0.12$	21.3±0.18	9.36±0.09
6		25.7±0.16	21.3±0.16	20.6±0.12	13.6±0.10
9		23.7±0.21	19.5±0.09	17.3±0.14	15.2±0.13
12		20.0±0.19	17.5±0.13	16.6±0.17	16.8±0.12
15		$18.2 \pm 0.16$	$14.4 \pm 0.10$	13.8±0.13	$19.8 \pm 0.18$

Table 3: Percentage removal efficiency of Pb from contaminated soil





Figure 1: Removal efficiency (%) of Pb from contaminated soil by aqueous extracts of dried Alternanthera brasiliana, Chromolaena odorata, Tridax procumbens and water

#### Lead Removal Efficiency from Spiked Soil

Lead spiked soil was similarly used to assess removal efficiency using plant extract washing solutions (Table 4) considering matrix geochemical phase differences between spike and contaminated soils. For the soil spiked with lead, the percentage removal efficiency of lead ranged between  $4.55 \pm 0.15$  to  $63.4 \pm 0.24\%$  for Alternanthera brasiliana, 7.08  $\pm$  0.23 to 47.8  $\pm$ 0.22% for Chromolaena odorata,  $11.4 \pm 0.47$  to 38.3  $\pm$  0.38% for *Tridax procumbens* and 14.4  $\pm$  0.17 to  $52.0 \pm 0.26\%$  for water extracting solutions. Removal efficiency of lead from the spiked soil was relatively higher than that of contaminated soil. Lead removal for Alternanthera brasiliana and Chromolaena odorata were observed more efficient for 3 % soil pulp density at 12 h extraction time, and 3 % soil pulp density at 6 hrs extraction for Tridax procumbent. Water extraction solution reported its highest efficiency at 15% soil pulp density for 15 h extraction time. Basically, it was similarly observed, that removal efficiency of Pb decreased proportionally with increased soil pulp density for the three plant extracts across all washing duration. The differences between removal efficiency of 3-SPD and 15-SDP was relatively very significant (p=0.05) for Alternanthera brasiliana, Chromolaena odorata and Tridax procumbens. The 3-SPD recorded very high Pb removal efficiency especially for Alternanthera brasiliana  $63.4 \pm 0.24\%$  at 12 h; Chromolaena odorata  $47.8 \pm 0.22\%$  at 12 h and *Tridax procumbens*  $38.3 \pm$ 0.38% at 6 h. As shown in Figure 2, positive impact (i.e. increasing removal efficiency) of washing time could only be observed for 3% SPD for Alternanthera brasiliana and Chromolaena odorata while negative impact (i.e. decreasing removal efficiency) was observed for 3 to 15% SPD for Tridax procumbens. On the other hand, Pb removal efficiency of control water washing solution was on the reversed order- increasing with increasing soil pulp density. Larger quantity of mobile phase Pb with increasing SPD explains this trends for spike soil. Analysis of variance showed significant difference (p=0.05) in removal efficiency of Pb for the four plant extract washing solutions. The percentage removal efficiency of Pb from spiked soil (63.3  $\pm$  0.24%) was higher than contaminated soil (38.1  $\pm$  0.28%). The reason could be attributed largely to high exchangeable Pb (78.9%)

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in spike soil as against contaminated soil (4.04%). Metals in artificially polluted soils are more loosely bound and therefore easily leached. Whereas, the contaminated soil had more Pb in the carbonate (35.7%), Fe-Mn oxide (20.4%) and residual (30.5%) forms which should be rather difficult to wash with the plant extract, but requiring stronger acidic medium for washing (Table 4). Besides the CEC also affect soil washing by enhancing greater removal efficiency through exchange of metal cations in spiked soil (Table 1). Statistical T-testing showed no significant difference (p=0.05) in removal efficiency of Pb between contaminated and spiked soils for all four washing solutions. Consequently, a positive correlation was observed between contaminated and spiked soils for *Alternanthera brasiliana* (0.676), *Chromolaena odorata* (0.570) and *Tridax procumbens* (0.517) while negative correlation observed for control water (0.485).

Soil pulp	Washing time	Alternanthera	Chromolaena	Tridax	Water
density	(h)	brasiliana,	odorata	procumbens	
3	1	45.7±0.43	35.8±0.39	27.2±0.22	16.1±0.13
6		32.5±0.31	24.6±0.34	14.4±0.53	19.8±0.15
9		18.9±0.23	19.0±0.11	17.2±0.20	19.8±0.15
12		22.2±0.21	17.5±0.52	11.8±0.05	25.5±0.19
15		4.55±0.15	7.08±0.23	15.3±0.08	39.3±0.32
3	3	46.5±0.44	43.2±0.45	36.9±0.11	24.2±0.14
6		36.0±0.25	23.7±0.26	26.0±0.30	31.4±0.15
9		35.6±0.31	23.4±0.41	24.9±0.31	31.1±0.18
12		27.1±0.22	$21.5 \pm 0.20$	23.8±0.22	40.9±0.27
15		28.2±0.18	19.7±0.15	19.7±0.32	44.7±0.29
3	6	57.3±0.79	46.9±0.19	38.3±0.38	18.7±0.16
6		45.7±0.34	$20.9 \pm 0.28$	26.5±0.21	22.8±0.18
9		47.9±0.52	22.3±0.36	22.9±0.18	24.4±0.31
12		33.0±0.21	$24.5 \pm 0.22$	19.1±0.19	25.5±0.13
15		17.3±0.82	$20.9 \pm 0.10$	14.5±0.24	35.9±0.33
3	12	63.4±0.24	47.8±0.22	33.4±0.28	16.7±0.17
6		36.5±0.22	42.5±0.31	$20.0 \pm 0.18$	14.4±0.17
9		21.5±0.12	24.5±0.35	19.7±0.35	17.8±0.19
12		23.1±0.22	18.4±0.24	14.1±0.33	38.6±0.13
		$20.1 \pm 0.21$	22.8±0.20	$11.4 \pm 0.47$	52.0±0.26

Table 4: Percentage removal efficiency of Pb from spiked soil







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Figure 2: Removal efficiency (%) of Pb from spiked soil by aqueous extracts of dried *Alternanthera brasiliana, Chromolaena odorata, Tridax procumbens* and water

## CONCLUSION

This batch laboratory experiment has demonstrated a promising start-up for using aqueous plant extracts as potential washing solutions for removal of Pb from certain geochemical phase contaminated soils. As much as 38.1 % Pb removal has been achieved using Alternanthera brasiliana and about 21% for Chromolaena odorata and Tridax procumbens from contaminated soils. The most appropriate ratio for soil was 3% SPD at 12 h washing time. Based on this finding, the authors recommend the use of Alternanthera brasiliana aqueous extract for washing of contaminated soils, specifically soils with high percentage exchangeable and carbonate bound Pb. This will promote ecologically sustainable soils in restoring contaminated soils for agricultural purposes. Further studies should be conducted to investigate how to enhance this removal efficiency by application of certain cation salts like potassium chloride which could aid the leaching of Pb from contaminated soils.

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## **CONFLICT OF INTEREST**

The authors declare no conflict of interest for this research work.

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