Bioremediation for Sustainable Soil Health: Harnessing Phytochemicals for Remediation of Agrochemicals and Heavy Metal Contamination in Soil

^{1*}Damilola Tope Ogundele, ¹Abdulfatai Temitope Ajiboye, ¹Damilola Jumoke Ogunleye, ²Yusuf Ayodeji Iyanda, ¹Olanrewaju Samsudeen Azeez

¹Department of Chemistry and Industrial Chemistry, Kwara State University, Malete, Nigeria ²Department of Environmental Management & Toxicology, Kwara State University, Malete, Nigeria

Sent for review: 2 September 2024

Accepted: 30 September 2024

ABSTRACT

Background: Soil functions as a major sink for environmental pollutants, particularly heavy metals and agrochemicals released through agricultural and industrial activities. Recent research highlights the potential of plants, through their phytochemical constituents, to remediate contaminated soils. This study evaluated the remediation efficacy of crude methanolic extracts from *Sida acuta, Senna alata, and Sacosephallous latifolius* on soils contaminated with heavy metals and agrochemicals.

Methods: Soil samples were artificially spiked with arsenic (As), cadmium (Cd), lead (Pb), zinc (Zn), and five agrochemicals (Cypermethrin, Terminator, DD-Force, Furan, and Rido). Crude extracts were prepared via maceration and methanolic fractionalization and applied to the contaminated soils. Heavy metal concentrations were determined using atomic absorption spectrometry (AAS), while agrochemical residues were analyzed using gas chromatography-mass spectrometry (GC-MS).

Results: Heavy metal remediation (% reduction) followed distinct trends: Cd > As > Zn > Pb (*S. acuta*), Zn > Pb > Cd > As (*S. alata*), and Pb > Zn > As > Cd (*S. latifolius*). A total of 21 agrochemical compounds were detected, with 1,1'-biphenyl, 2,2',4 showing the highest concentration (38.06 mg/mL) from Terminator agrochemicals. All plants achieved bioaccumulation factor (BAF) values <1 for most metals, except for As (at 40 ppm) and Zn (at 10 ppm) in *S. latifolius*, where BAF >1. Additionally, three key agrochemical compounds showed notable concentration reductions following treatment with *S. acuta* and *S. alata* extracts.

Conclusion: The findings demonstrated that the phytochemical-rich extracts of *Sida acuta*, *Senna alata*, and *Sacosephallous latifolius* hold strong potential for phytoremediation of heavy metals and persistent organic pollutants (POPs) in contaminated soils, supporting their use in sustainable soil recovery and environmental management strategies.

Keywords: Agrochemicals, bio-accumulation factors, contaminated soil, heavy metal, phytochemical, remediation potential

Corresponding author: Email: damilola.ogundele@kwasu.edu.ng: Phone: +2348034396529

This is an open-access article distributed under the Creative Commons Attribution License, (<u>http://creativecommons.org/licenses/by/4.0/</u>) which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

1. INTRODUCTION

The rapid growth of industrialization, urbanization, and modern agricultural practices worldwide has led to a sharp rise in anthropogenic activities that release various contaminants into the atmosphere, soil, and water systems [1]. Among these concerns, soil contamination from industrial discharges and agricultural wastes has become particularly pressing [2]. Pollutants such as pesticides, fertilizers, textile industry effluents, electroplating byproducts, and petroleum refining wastes contribute significantly to soil and water pollution, alongside natural sources like wind, soil erosion, urban runoff, and volcanic activity [3]. While inorganic contaminants, especially heavy metals, often require immobilization or physical removal [4, 5], organic contaminants-including persistent organic pollutants (POPs)—are largely resistant to microbial degradation in soils. Heavy metal contamination has emerged as a global environmental challenge due to the metals' non-biodegradability and long-term persistence in soil ecosystems [6]. These metals, characterized by atomic weights between 63.54 and 200.5 g and specific gravities above 4, pose significant health hazards [7, 8]. Toxic heavy metals such as cobalt (Co), cadmium (Cd), iron (Fe), zinc (Zn), lead (Pb), and chromium (Cr) are frequently found in wastewater and have increasingly contaminated soils [9]. Human exposure occurs via direct soil ingestion, dust inhalation, consumption of crops grown on contaminated soils, or through the food chain, posing both ecological and public health risks [10–16]. Although various physicochemical and biological methods have been employed to mitigate soil and water contamination, traditional remediation approaches are often costly, disruptive, and insufficiently effective [17, 18]. Conventional methods frequently destroy beneficial soil components, including nitrogen-fixing bacteria, essential nutrients, mycorrhizal fungi, and fauna, leaving the soil unsuitable for plant growth [19]. In contrast, plant-based remediation-known as phytoremediation-has emerged as an effective, affordable, and eco-friendly alternative for removing heavy metals and organic contaminants from polluted environments [20-23]. Derived from the Greek prefix phyto- (plant) and the Latin root remedium (to correct or remove an evil), phytoremediation refers to the use of certain plant species to absorb, extract, immobilize, or detoxify pollutants, reducing their bioavailability in soil [24, 25]. This technology can address both organic and inorganic contaminants across soil, water, and atmospheric matrices [26]. Plants owe much of their protective and adaptive capacity to phytochemicals—naturally occurring, biochemically active secondary metabolites with significant nutritional and medicinal value [27]. These compounds, including alkaloids, carotenoids, polyphenols, polysaccharides, isoprenoids, phytosterols, saponins, and dietary fibers, are distributed across various plant parts such as leaves, bark, stems, and roots [28]. Phytochemicals not only contribute to a plant's color, aroma, and flavor but also serve as defensive agents against environmental stressors like pollution, drought, UV radiation, and pathogens. This study aimed to: investigate the mechanisms of phytoremediation of heavy metals and selected organic pollutants, particularly agrochemicals, in contaminated or spiked soils using plant phytochemicals; conduct quantitative phytochemical screening of methanolic extracts from selected plant materials (Sida acuta, Senna alata, and Sacosephallous latifolius); and simulate contaminated soil by spiking with heavy metals (Pb, Cd, As, Zn) and agrochemicals, followed by remediation using crude plant extracts.

2. MATERIALS AND METHODS

2.1 Materials

Ethanol, Chloroform, Glacial Acetic, n-Hexane, Ethyl acetate, Feric Chloride, NaOH, Sida Acuta, sienna Acuta, sacosephallus latifolius,

2.2 Methods

2.2.1. Study Area

In this study, two (2) different study locations were chosen from North Central region of the Federal Republic of Nigeria. The soil samples were collected from Flower Garden, G. R. A., Ilorin, and the plant samples were collected from Irewolede, Ilorin, both within Kwara State, Nigeria. The coordinates for these locations were obtained (Table.1) and employed in generating the geographical information using GPS (geographical positioning



system) as presented in Figure 1.

Table 1: Coordinates of Study Locations

Samples	Longitude	Latitude
Soil	4° 34' 56''	8° 29' 14''
Plants	4° 32' 56''	8° 28' 19''



Figure 1: Map of Kwara State Showing Soil Sample Location

2.2.2 Collection and Identification of Plant Samples

The leaves of *S. acuta, S. alata,* and stembarks of *S. latifolius* were sampled from Irewolede, Ilorin, Nigeria and were taxonomically identified and authenticated at the Department of Plant and Environmental Biology, Kwara State University Malete, Nigeria.

2.2.3 Preparation of Plant Samples

The leaves of *S. acuta, S. alata,* and stembarks of *S. latifolius* were cut into smaller pieces, washed under running tap water and dehydrated at ambient temperature $(25 \pm 2^{\circ}C)$ for 14 days to ensure crispy texture. Subsequently, the dehydrated plant materials were separately pulverized into powdery form and kept in a polythene bag until further use.







Figure: 2 A. S. acuta, B. S. alata C. S. latifolius

2.2.4 Extraction of Plant Material

A 500 g of the pulverized plant materials (*S. acuta, S. alata* and *S. latifolius*) were separately macerated in 95 % methanol (1000 mL) for 72 hours at room temperature with occasional shaken. The resultant crude extracts were separately decanted, filtered and concentrated at a lowered pressure with rotary evaporator set at 40 $^{\circ}$ C to get a solvent-free methanol extracts and stored in an air-tight bottle for later use.

2.2.5 Preliminary Phytochemical Screening of Crude Methanol Leaf Extracts

The procedure described by Ajiboye *et al.* [29] was followed in the determination of phytochemical constituents of the methanolic extracts and fraction the plants.

2.2.6 Collection and Preparation of Soil Sample

The soil sample was collected from GRA, Ilorin, Nigeria, by random sampling method with the aid of a hand trowel that was pre-cleaned with 20 % nitric acid into a well labelled polythene bag. The soil samples were air dried for 48 hours, blended, and sieved with 2 mm stainless sieve. Each sample were labelled and stored in a dry plastic bag for further use.

2.2.7 Soil Contamination (Spiking) Procedure

The method of Ugwu *et al.* [30] with slight modification was adopted for this study. The soil spiking with agrochemicals (cypermethrin, terminator, D-D force, furan and rido), heavy metals (Cd, Pb, Cu and Zn) and PCBs was done to establish the presence of these environmental pollutants. About 20 g of dry soil samples was artificially contaminated with 2 mL solutions of 1000 ppm of the agrochemicals by serial dilution. The soil samples were thoroughly mixed with solutions of the pollutants to form a slurry body, this was thereafter left to age and cure for weeks. During the period of aging and curing the slurry soils were frequently mixed. This was subsequently air dried to a constant mass.

2.2.8 Remediation of the Heavy Metals Spiked Soil

2.2.8.1 Preparation of Washing Solutions

A 0.5 mg of crude methanol extract was dissolved in 95 mL distilled water and stirred thoroughly to ensure total dissolution. The pH of solution was taken and recorded accordingly. The resulting solution was kept in a refrigerator at 4°C until further use. The method of Ugwu *et al.* [30] was adopted with slight modification. Briefly, the extract solution (100 mL) was added into contaminated soil (20 g). The mixture was shaken at room temperature over a rotary shaker at 230 rpm for 15 minutes. The aliquot was collected and centrifuged at 1000 rpm for 15 minutes. The supernatant was filtered, and the residue (remediated soil) was collected and kept for digestion.

2.2.8.2 Procedure for Soil Washing Study

The 100 mL crude extract (*S. acuta, S. alata,* and *S. latifolius*) were added to Agrochemical contaminated soil sample and constantly shaken over rotary shaker at 230 rpm for 15 minutes at 25°C. The aliquot was centrifuged at 1000 rpm for 15 minutes and the supernatant was filtered, concentrated and kept in a refrigerator at 4° C further analyses [31].



2.2.8.3 Digestion of the spiked and remediated soil samples

Both the spiked and remediated samples were prepared for elemental composition analysis by Atomic Absorption Spectroscopy following the sample preparation procedure. Digestion method was adapted from Anton Pear's multiwave 3000 microwave digestion system. The dissolution of the spike and remediated samples were done by weighing accurately 0.2 g of each sample into digestion flasks and 10 mL of conc. HNO₃, HCl and HClO₄ was added to almost dryness and 10 mL conc. HCl was finally added. The digested sample were filtered and transferred into pre-cleaned 250 mL polypropylene vials and filled to 250 mL with distilled water for AAS analysis. The digestate was analyzed for Pb, Cd, As and Zn.

2.2.9 Remediation of Agrochemical Spiked Soil

2.2.9.1 Remediation potential and Bio-accumulation factor

The ability of plants to remediate heavy metal spiked soil (*remediation potential*) was calculated using the formula stated in [31].

Remediation potential (% reduction) = $\frac{H_{P,E}}{H_{S,S}} \times 100$ 1

 $H_{P,E}$ = amount of heavy metal remove by plants extracts

 $H_{S.S}$ = amount of heavy metal in spiked soil

Amount of heavy metal lost by plant extract

Bioaccumulation factor (BAF) is the ratio of the concentration of remediated heavy metal by plant and contaminated soils [32], it indicates the plant's ability to accumulate metal.

 $BAF = \frac{C_P}{C_S} \dots 3$

 C_P = concentration of heavy metal remediated with plants (mg/kg)

 C_8 = concentration of heavy metal in spiked soil (mg/kg)

BAF values > 1 indicate accumulation is greater than the medium such as soil.

2.2.10 The GC-MS Determination of Agrochemical Spiked and Remediated Soil

A 5 g of each sample for both (spiked and remediated) and 5 g of anhydrous sodium sulphate were weighed and homogenized to a complete mixture. The mixtures were transferred to pre-cleaned extraction tubes, and 40 mL of dichloromethane was added. The tubes were tightly capped, allowed to stand for 30 minutes, and then shaken vigorously for 30 minutes. The solids were allowed to settle, and solvent layers were filtered. The procedure was repeated with 25 mL dichloromethane. The two extracts were combined, concentrated on a rotary evaporator (Buchi Rotavapor R-114), exchanged with 10 mL of n-hexane and re-concentrated to 1 mL for clean-up for both (spiked and remediated sample). The extracts were then eluted with 40 mL dichloromethane/hexane on a silica gel column. The extracts were evaporated and re-dissolved in 1 mL n-hexane. The cleaned extracts were analyzed for the representative agrochemicals using a Shimadzu GC/MS QP 2010 model.

2.2.11 Procedure for AAS analysis



Digestion method was adapted from Anton Pear's multiwave 3000 microwave digestion system. The dissolution of the spike and remediated samples were done by weighing accurately 0.2 g of each sample into digestion flasks and 10 mL of conc. HNO₃, HCl and HClO₄ was added to almost dryness and 10 mL conc. HCl was finally added. The digested sample were filtered and transferred into pre-cleaned 250 mL polypropylene vials and filled to 250 mL with distilled water for AAS analysis. The digestate was analyzed for Pb, Cd, As and Zn.

2.3 Statistical Analysis

The mean, standard deviation, t-test, and correlation matrix coefficient were used to determine the relationships in contaminated and remediated concentrations of heavy metals study, as well as also their significant difference, at 95% confidence level ($p \le 0.05$). Similarly, remediation potential (% reduction) and bio-accumulation (BAF value) and graphs were used to illustrate the concentration of the heavy metals in the spiked soil and remediated by plants extracts.

3. RESULT

Table 2 shows the plant samples of *Sida acuta, Senna alata,* and *Sacosaphallous latifolius* (each weighing 500 g) extracted using methanol, along with their respective pH levels, weight percentage yields, and colours.

3.1 Result for Phytochemical Screening of Plant Extracts

Table 3 shows the result of phytochemical screening of the plant materials. Phenol was found in S. *acuta*, and S. *alata* in trace amount but presence in large quantity in S. *latifolius* crude extract. The crude extracts significantly demonstrated the presence of alkaloids, terpenoids, saponins and glycosides. These suggest that the crude extracts from these S. *acuta*, S. *alata and S. latifolius* are good sources of phytochemicals that could be used in remediation of soil contaminants through their metal chelating properties.

S/N	Name	pН	Weight of	Weight of	Colour	Percentage
		•	Pulverized	Crude		Yield (%)
			Sample (g)	Extract (g)		
1	S. acuta	5.6	500	10.2	Dark green	2.04
2	S. alata	5.0	500	9.4	Dark green	1.88
3	S. latifolius			7.8	Dark green	1.56

Table 2: Extraction and percentage yield of the methanol extracts of leaves of S. acuta, S. alata and S.latifolius

Table 3: The results showing comparism of phytochemical screening in each of the samples

	8 1 1 7	8	1	
Phytochemical	Specific test.	S.acuta	S.alata	S.latifolius
Phenols	FeCl ₃ solution	+	+	+
Flavonoids	Conc. H ₂ SO ₄	+	-	+
	NaOH solution	+	-	+
Alkanoids	Dragendorff's	+	+	+
	Meyer's	+	-	+
	Wagner's	+	+	+
Terpenoids	Salkowski's test	+	+	+
Steriods	Libermann Burchard's test	+	+	+
Saponins	Frothing test	+	+	+
Glycosides	Keller-killani's test	+	+	+
- absent, + sma	ll quantity, ++ larger quantit	ies		



3.2 Results of Heavy Metals Analysis of Contaminated Soil

Table 4 and Figure 2 show the results of heavy metal concentrations (As, Cd, Pb and Zn) at different spiked concentrations from 10 ppm to 50 ppm. The As was found to be 7.3 ± 0.001 , 18.40 ± 0.002 , 28.30 ± 0.001 , 36.60 ± 0.001 and 46.20 ± 0.001 at 10ppm, 20ppm, 30ppm, 40ppm and 50 ppm respectively. The concentration of cadmium varied from (8.55 ± 0.004 , 16.9 ± 0.002 , 27.75 ± 0.002 , 31.35 ± 0.001 and 45.02 ± 0.004) at 10 to 50 ppm respectively. Also, the Table revealed concentration of Lead (Pb) in spike soil sample of (7.70 ± 0.002 , 17.90 ± 0.006 , 25.30 ± 0.005 , 30.51 ± 0.003 and 43.30 ± 0.002 ,) at 10 to 50 ppm. While the concentration of Zinc (Zn) 9.10 ± 0.002 , 15.80 ± 0.003 , 23.90 ± 0.001 , 35.20 ± 0.001 and 41.4 ± 0.003 was within (WHO) permissible limit (50mg/kg) at all concentration (ppm).

Spiked Sample	Metal Concentration (mg/kg±SD)				
Ppm	As	Cd	Pb	Zn	
10	7.30± 0.001	8.55±0.004	$7.70{\pm}0.002$	9.10±0.002	
20	18.40±0.002	16.90 ± 0.002	17.90 ± 0.006	15.80 ± 0.003	
30	$28.30{\pm}\ 0.001$	27.75 ± 0.002	$25.30{\pm}0.005$	23.90±0.001	
40	36.60 ± 0.001	31.35±0.001	30.51±0.003	35.20±0.001	
50	$46.20\pm\!0.001$	45.02±0.004	43.30±0.002	41.4±0.003	
*WHO PL	5	0.8	85	50	

 Table 4: Concentrations of heavy metal in contaminated soil WHO [33]

World Health Organization Permissible Limit (WHO PL)





3.3 Effect of plants extract in remediating heavy metal concentrations in spiked soil

The table 5 - 7, shows the comparison of heavy metals concentrations in the spiked before and after remediating soil samples with *S. acuta, S. alata* and *S. latifolius* and their remediation potential (% reduction). The results revealed a significant reduction in the heavy metals concentration accordingly.







Figure 3: Graphical representations of remediation potential of heavy metal with a). S. acuta,b

Figure 4: Graphical representations of remediation potential of heavy metal with b) S. alata

It was observed that, Arsenic (As) was found to have percentage reduction (36, 75 and 90 %). Cadmium (Cd) was found to have metal reduction with (64, 91 and 93 %), (Zn) had remediation potentials of (43 %, 55 % and 64 %) while (Pb) showed percentage reduction of (34, 60, 50 %) at 10, 30 and 40 ppm respectively by *S. acuta* as presented in Table 5 and Figure 3. Cd with percentage concentration of (36, 30, and 42 %), As (62, 30, and 42 %), Pb, (42, 26, 50 %), and Zn, (41, 43, and 53 %), were found in all the samples remediated with S. alata. Pb at 30 ppm was found to have least remediation potential (% reduction). Cadmium, Lead, and Zinc showed the highest reduction at 40 ppm while Arsenic has positive reduction of (62 %) at 10 ppm in *S. alata* as presented in Table 6 and Figure 4. In *S. latifolius*, all the remediated metals showed highest reduction at 30 ppm with Pb & Zn found to have the highest reduction (55 %) among all ppm studied as presented in Table 7 and Figure 5.





Figure 5: Graphical representations of remediation potential of heavy metal with c)S. latifolius.

The trend of remediation potential (% reduction) were in order of (Cd> As > Zn > Pb), (Zn> Pb Cd > As) and (Pb> Zn> As> Cd) for *S. acuta, S alata* and *S. latifolius* respectively

Metals ppm	In ppm	Spiked soil	S. acuta remediated	% reduction
		mg/kg	soil	
As	10	7.3	4.7	36
	30	28.30	7	75
	40	36.60	3.5	90
Cd	10	8.55	3.05	64
	30	27.75	2.6	91
	40	31.35	2.1	93
Pb	10	7.70	5.07	34
	30	25.30	10.21	60
	40	30.51	15.20	50
Zn	10	9.10	5.20	43
	30	23.90	10.8	55
	40	35.20	12.7	64

Table 5: Comparison of heavy metal concentrations in spiked and remediated soil by S. acuta

p-value = 0.377 p > 0.05

3.4 Effect of Bio-accumulation of heavy metal by the plants

The table 8 and figure 6-8, show the bio-accumulation factor of As, Cd, Pb and Zn by plant extracts, the result showed that Zn and As metals by *S.latifolius* were found to have highest accumulation BAF value of 1.36 and 2.09 respectively, also high BAF value was observed in *S. acuta* plant around (0.84) for both metals. This was follow by lead Pb in *S.alata* of BAF value of (0.98). Cadmium Cd was found to has least bioaccumulation factor by all the



plants with BAF value around (0.051 - 0.097, 0.19 - 0.51 and 0.13 - 0.55) for S. acuta, S. alata and S. latifolius respectively

Metals	ppm	Spiked soil	S. alata Remediated	Percentage (%) reduction
As	10	7.30	2.80	<u>62</u>
	30	28.30	19.90	30
	40	36.60	21.15	42
Cd	10	8.55	5.50	36
	30	27.75	19.30	30
	40	31.35	18.10	42
Pb	10	7.70	4.50	42
	30	25.30	18.60	26
	40	30.51	15.20	50
Zn	10	9.10	5.35	41
	30	23.90	13.55	43
	40	35.20	16.50	53

Table 6: Comparison of heavy metal concentrations in spiked and remediated soil with S. alata





www.nijophasr.net



Figure 6-8: Graphical representations of bio-accumulation (BAF) of heavy metal with a) *S. acuta*, b) *S. alata* and c) *S. latifolius*

Table 7: Comparison of heavy metal concentrations in spiked and remediated soil by *S. latifolius*

Metals	Conc. (ppm)	Spiked soil (mg/kg)	S. alata Remediated soil	Percentage (%) reduction
As	10	7.3	4.35	40
	30	28.30	13.05	54
	40	36.60	19.20	48
Cd	10	8.55	4.45	48
	30	27.75	13.45	52
	40	31.35	15.75	50
Pb	10	7.70	3.65	53
	30	25.30	11.35	55
	40	30.51	15.70	49
Zn	10	9.10	5.90	35
	30	23.90	10.75	55
	40	35.20	17.35	51

Table 8: Bio-accumulation factor of heavy metal by plant extract

S. acuta	S. alata	S. latifolius
0.53 - 0.84	0.23 - 0.38	0.36 - 1.36
0.051 - 0.097	0.19 - 0.51	0.13 - 0.55
0.21 - 0.29	0.71 - 0.98	0.075 - 0.40
0.32 - 0.84	0.11 - 0.181	0.28 - 2.09
	S. acuta 0.53 - 0.84 0.051 - 0.097 0.21 - 0.29 0.32 - 0.84	S. acuta S. alata 0.53 - 0.84 0.23 - 0.38 0.051 - 0.097 0.19 - 0.51 0.21 - 0.29 0.71 - 0.98 0.32 - 0.84 0.11 - 0.181



3.5 Correlation matrices of Heavy metal and Plants extract

Table 9 – 11, showed the correlative potential of H metal in contaminated soil and remediate plants, as seen, in the table, a positive correlation PC is represented blue color and negative correlation NC with red color, the PC revealed a presence of strong ($\geq 0.70 \text{ PC} \leq 1.00$), fair ($\geq 0.30 \text{ PC} \leq 0.69$) and weak ($\geq 0.00 \text{ PC} \leq 0.29$) correlations [34]. There was a strong positive correlation between As and As. p (0.9979), and between Pb and Pb. p (0.8800) remediated by *S. acuta* as showed in table 9. For *S. alata* there a strong positive correlation between Zn & Zn. p (0.711), fairly correlation between Pb & Pb. p (0.5175) and weak correlation between Cd and Cd. p (0.1202) as presented in the table 10. While for *S. latifolius*, there's fairly positive correlation between Pb & Pb. p and also between Zn & Zn. p of (0.6217) and (0.4273) respectively as presented in the table 11. The correlation matrices conducted produced positive regression that implies heavy metals from contaminated soil component were from similar remediated by plants extracts source [35].



Table 9: Correlation matrices of heavy metals in spiked soil and remediated by S. acuta

AS. p, Cd. p, Pb. p, & Zn. p means metals remediated by plants extracts.

Table 10: Correlation matrices of heavy metals in spiked soil and remediated by S. alata







Table 11: Correlation matrices of heavy metals in spiked soil and remediated by S. latifolius

AS. p, Cd. p, Pb. p, & Zn. p means metals remediated by plants extracts.

3.6 FTIR Analysis of Plant Extracts and Remediated Effluent

The results of FTIR spectra and analysis of *S. acuta, S. alata and S. latifolious* crude extracts and the **remediated effluents** of different heavy metals are presented in (Figures 9-11) with their characterization data.

3.6.1 FTIR results of S. acuta

Crude extract – (KBr, cm⁻¹): 3339 (O-H), 2925 (C-H), 1735 (C=O), 1559 & 1508 (C=C), 1464 & 1383 (C-H) and 1071 (C-O). **As** – (KBr, cm⁻¹): 3620 (O-H), 2853 (C-H), 1744 (C=O), 1629 (C=C), 1464 (C-H) and 1076 (C-O). **Pb** – (KBr, cm⁻¹): 3442 (O-H), 2900 (C-H), 1825 (C=O), 1632 (C=C), 1400 (C-H) and 1209 (C-O). **Cd** – (KBr, cm⁻¹): 3439 (O-H), 2903 (C-H), 1801 (C=O), 1633 (C=C), 1400 (C-H) and 1036 (C-O). **Zn** – (KBr, cm⁻¹): 3445 (O-H), 2901 (C-H), 1719 (C=O), 1633 (C=C), 1401 (C-H) and 1200 (C-O).

3.6.2 FTIR results of S. alata

Crude extract – (KBr, cm⁻¹): 3397 (O-H), 2935 (C-H), 1625 (C=C), 1400 (C-H) and 1073 (C-O). **As** – (KBr, cm⁻¹): 3440 (O-H), 2918 (C-H), 1634 (C=C), 1450 (C-H) and 1032 (C-O). **Pb** – (KBr, cm⁻¹): 3438 (O-H), 2853 (C-H), 1633 (C=C), 1428 (C-H) and 1032 (C-O). **Cd** – (KBr, cm⁻¹): 3428 (O-H), 2850 (C-H), 1629 (C=C), 1421 (C-H) and 1036 (C-O). **Zn** – (KBr, cm⁻¹): 3428 (O-H), 2892 (C-H), 1636 (C=C), 1409 (C-H) and 1029 (C-O).

3.6.3 FTIR results of S. latifolius

Crude extract– (KBr, cm⁻¹): 3379 (O-H), 2932 (C-H), 1609 (C=C), 1400 (C-H) and 1110 (C-O). **As** – (KBr, cm⁻¹): 3434 (O-H), 1871 (C-H), and 1622 (C=C). **Pb** – (KBr, cm⁻¹): 3438 (O-H), 1871 (C-H), and 1625 (C=C). **Cd** – (KBr, cm⁻¹): 3619 (O-H), 1872 (C-H), and 1624 (C=C). **Zn** – (KBr, cm⁻¹): 3442 (O-H), 1871 (C-H), 1629 (C=C) and 1040 (C=O).

The FTIR spectra were used to identify the chemical composition based on the functional group present in the samples relative to the peak value in the region of infrared radiation. It is seen that the crude extracts showed



absorption peaks between 3339-1071cm⁻¹. Although the prominent peaks were observed at 3339, 2925, 1735, 1559, 1464, and 1071, corresponding to the O-H of hydroxyl group, C-H of aliphatic alkenes, C=O of carbonyl group, C=C of aromatic alkenes, respectively. The peak at 1071 cm⁻¹ confirms the presence of C- O in the carbonyl functional group. Furthermore, comparing the absorption peaks of (*S. acuta, S. alata and S. latifolius*) crude extracts and the remediated effluents from various heavy metals, it is evident that there was shifting and disappearance of some absorption peaks, indicating the disappearance of some functional groups. Hence, the absence of these functional groups in the remediated effluents probably confirms their involvement in the remediation process.



Figure 9: Graphical representation of four heavy metals plotted against the *Sida acuta* extract (SAE) crude extract



Figure 10: Graphical representation of four heavy metals plotted against the Sida alata crude (SAC) extract



Figure 11: Graphical representation of four heavy metals plotted against the S. latifolius extract (S.LA) crude extract.



3.7 GC-MS Analysis of Agrochemical Spiked Soil

Table 12 shows, the concentrations, retention time and compounds present in the different soil samples spiked with various agrochemicals such as Cypermethrin, Terminator, Furan, DD-force and Rido. It was observed that among the four (4) compounds present in CYPERMETHRIN spiked sample, 1,1'-Biphenyl, 2,3,4'- was found to be the most abundant with concentration of 0.38 mg/mL. For terminator, the result revealed 7 compounds found present in the agrochemical with 1,1'-Biphenyl, 2,2',4, was found to be the most abundant with concentration of 38.06mg/ml. For Rido, the result shows the present of 4 compounds in the agrochemical with 1,1'-Biphenyl, 2,2',4, has the highest concentration of 0.79mg/ml. also for DD-force, the result shows the present of compounds in the agrochemical with 1,1'-Biphenyl, 2,3,4'-, has the highest concentration with 2.10mg/ml for furan, the most abundant compound in the sample is Biphenyl, 2,2',4, with 0.53mg/ml.

S/N	Name	Retention	Molecular	Molecular	Conc
А	CYPERMETHRIN				· · · ·
1	1,1'-Biphenyl, 4-chloro-	5.593	Cl ₂ H ₉ Cl	188.65	0.11
2	1,1'-Biphenyl, 2,3-dichloro	7.030	$C1_2H_8Cl_2$	223.10	0.11
3	1,1'-Biphenyl, 2,3,4'-	8.649	C1 ₂ H ₇ Cl ₃	257.54	0.38
4	1,1'-Biphenyl, 2,2',4,	8.895	C12H7Cl3	257.54	0.01
В	TERMINATOR				
1	1,1'-Biphenyl, 4-chloro-	5.525	C1 ₂ H ₉ Cl	188.65	0.11
2	1,1'-Biphenyl, 2,3-dichloro	7.047	$C1_2H_8Cl_2$	223.10	0.23
3	1,1'-Biphenyl, 2,3,4'-	8.214	$C1_2H_7Cl_3$	257.54	0.34
4	1,1'-Biphenyl, 2,2',4,	8.998	$C1_2H_7Cl_3$	257.54	38.06
5	2,3',4,5-Tetrachloro-1	9.553	$C1_4H_{13}C_{15}$	358.51	2.99
6	1,1'-Biphenyl, 2,2',4,	9.559	$C_{12}H_7C_{13}$	257.54	0.65
7	1,1'-Biphenyl, 2,2',3,	13.599	$C_{12}H_7C_{13}$	257.54	0.33
С	RIDO				
1	1,1'-Biphenyl, 4-chloro-	5.536	C ₁₂ H ₉ Cl	188.65	0.20
2	1,1'-Biphenyl, 2,3-dichloro	7.087	$C_{12}H_8C_{12}$	223.10	0.12
3	1,1'-Biphenyl, 2,3,4'	8.065	$C_{12}H_7C_{13}$	257.54	0.12
4 D	1,1'-Biphenyl, 2,2',4, DD-FORCE	8.901	$C_{12}H_7C_{13}$	257.54	0.79

Table 12: GCMS Chromatograph of Agrochemical Spiked Soil



1 2	1,1'-Biphenyl, 4-chloro- 1,1'-Biphenyl, 2,3-dichloro	5.359 7.281	C ₁₂ H ₉ Cl C ₁₂ H ₈ C ₁₂	188.65 223.10	0.05 0.65
3	1,1'-Biphenyl, 2,3,4'	8.660	$C_{12}H_7C_{13}$	257.54	2.10
4	1,1'-Biphenyl, 2,2',4,	8.895	$C_{12}H_7C_{13}$	257.54	0.14
Е	FURAN				
1	1,1'-Biphenyl, 2,3,4'	256	$C_{12}H_7C_{13}$	257.54	0.12
2	1,1'-Biphenyl, 2,2',4,	292	$C_{12}H_7C_{13}$	257.54	0.53

3.8 Effect of Crude Extract of S. acuta and S. alata on Agrochemical Spiked Contaminated Soil

The result of the GC-MS analysis (Table 13) revealed total number of three (3) compounds Identified from the soil sample remediated by *S. acuta* and *S. alata*. The table xx shows the concentrations, molecular formular and weight, retention time and compounds. From the table, three compounds were detected in the Terminator agrochemical which are (2,2',4'-trichloro-[1,1'-biphenyl]-4-amine, 1,1'- Biphenyl, 2,3',4,4',5'-pentachloro-3-methoxy-and 1,1'- Biphenyl, 2,2',4,4'-tetrachloro-5- (methylthio)-) with 1,1'-Biphenyl, 2,3',4,4',5'-pentachloro-3-methoxy-was found to have 9.13 mg/mL concentration for *S. acuta* plant. While the result of the GCMS analysis (Table 14) revealed total number of two (2) compounds Identified from the remediated sample by *S. alata*, the two compounds were detected in the Terminator agrochemical which are (1,1'-Biphenyl, 2,3',4,4',5'-pentachloro-3-methoxy- and 1,1'-Biphenyl, 2,2',4,4'- tetrachloro-5-(methylthio) while 1,1'-Biphenyl, 2,3',4,4',5'-pentachloro-3-methoxy- and 1,1'-Biphenyl, 2,2',4,4'- tetrachloro-5-(methylthio) while 1,1'-Biphenyl, 2,2',4,4'-tetrachloro-5-(methylthio) while 1,1'-Biphenyl, 2,2',4,4'-tetrachloro-5-(methylthio)- was found to have 0.45 mg/L concentration below calibration limit and (1,1'-Biphenyl, 2,3',4,4',5'- pentachloro-3-methoxy- is 0.45 mg/L, whereas other compounds have concentration below calibration limit.

Table 13: GC-MS analysis of agrochemical remediated by Sida acuta

S/N	Name	Retention Time	Molecular Formula	Molecular Weight	Conc. (mg/L)
	Terminator				
1	2,2',4-trichloro- 1,1,	7.596	$C_{12}H_8C_{13}N$	272.55	Below cal
2	1,1'-Biphenyl,	6.980	$C_{13}H_7C_{15}O$	356.45	9.13
3	1,1'-Biphenyl,	6.489	$C_{13}H_8C_{14}S$	356.45	Below cal



S/N	Name	Retention	Molecular	Molecular	Conc.
		Time	Formula	Weight	(mg/L)
	Terminator				
1	1,1'-Biphenyl,	8.603	C13H7C15O	356.45	0.45
2	1,1'-Biphenyl,	8.489	$C_{13}H_8C_{l4}S$	338.07	Below cal

-1 (11/1C) $1 = 1$. X IX / 1 VIV / (11)(11) X (11) (12) (12) (12) (12) (12) (12) (12)	Table	14:	GC-MS	analysis	of agroc	hemical	remediated	with Senna	alata
--	-------	-----	-------	----------	----------	---------	------------	------------	-------

4. DISCUSSION

The choice of a plant species for accumulation of heavy metal contaminated soil medium depends on several factors like the level of tolerance of the plant and the capability of the plant to absorb and accumulate the heavy metal [36]. In this study, three plant were used (Sida acuta, Sienna alata and Sacosaphallous latifolius) to remediate spiked soil samples, their concentration was determined and compared with remediated soil sample of heavy metal, also remediation potential (% reduction), BAF and reduction in concentration(mg/kg) was examined. The results obtained in this study indicate that remediation using Sida acuta, Senna alata and S. latifolius have positive remediation potential (% reduction) and effect with lead (Pb), Cadmium (Cd), Zinc (Zn) and Arsenic (As), except Arsenic (As) was found to have no reduction at 40 ppm and Zn at 10 ppm for S. latifolius. Significant reduction of these metals is due to the presence of organic contents (phytochemicals) in the plants, which point to the fact that the plants can be used to reclaim soil contamination of heavy metals especially those with positive remediation potential. The BAF value for all metals by plants were all < 1, except for Arsenic (As) and zinc (Zn) which were found to have BAF value > 1 at 40ppm and Zn at 10ppm for S. latifolius. The t-test and p-value were used to compare the heavy metal concentration of both contaminated and remediated by plants, the result showed that there were significant differences (t = 0.330; p-value = 0.0203 p < 0.05) in the concentration of heavy metals contaminated and remediated by S.alata plant. while for both S. acuta and S. latifolius have little significance difference differences (t = 0.015; pvalue = 0.377 p < 0.05) and (t = 0.039; p-value = 0.22 p < 0.05) respectively in the concentration of heavy metals in spiked and remediated indicating that soil-plant heavy metal bio-accumulation was efficient and effective similar to that of Fakhri et al.[37]. It is also observed in the agrochemical remediation, 3 compounds were detected by S. acuta with reduction in concentration and 3 compounds are detected by S. alata respectively, while the rest 19 agrochemicals were clean-up by the plants extract. This bio-accumulation and uptake of heavy metals and agrochemicals in the plants depend on the plant species, the levels of the metals in the, the element bioavailiability, pH, cation exchange capacity, climacteric condition, vegetation period and multiple other factors [38]. The results obtained can be compared with already published results in the sense that the intensity of heavy metal and POPs contamination, accumulation as well as remediation potential in the plant. The obtained results prove that the plant extract can successfully be used as a phytoremediation for POPs and Heavy, especially Pb and Zn metals by S. acuta, S. alata showed the ability to absorb metal and the concentration of metal in the spiked soil showed a good agreement with the concentration of metals in the soil [39]. The availability of these POPs and heavy metal is a major factor that affect the uptake and remediation by plants, the organic contents (phytochemicals) in the plant is one of the factors that may reduce the ability of metal and POPs to be phytotoxic in the soil due to metal-organic. Such results might be attributed due to phytochemical organic content of the plants. Thus, this shows they have good environment value apart from well reported medicinal value of these plants [40, 41].

5. CONCLUSION

The results of this study revealed that (Sida acuta, Sienna alata and Sacosephallous latifolius) plants has the capacity to fascinate the removal and remediation of different heavy metals and clean up POPs from the



contaminated soil. This study suggests that the phytochemical (organic contents) of the plants positively influence the extents by which plants enhance the remediation of heavy metal and POPs from soil and bioaccumulation by the plants extract. The results show that Lead (Pb) have highest concentration in the spiked soil, The t-test and p-value result showed that there were significant differences (t = 0.330; p-value = 0.0203 p < 0.05) in the concentration of heavy metals contaminated and remediated by *S.alata* plant, while for both *S. acuta* and *S. latifolius* have little significance differences (t = 0.015; p-value = 0.377 p < 0.05) and (t = 0.039; p-value = 0.22 p < 0.05) respectively. The results for POPs detected the presence of 21 agrochemical compounds, with 1,1'-Biphenyl, 2,2',4, having an abundant concentration (38.06 mg/L) of terminator agrochemical. The extent of chemical contaminants removal varied depending on the plant used in this study, thereby suggesting an eco-friendlier chemical contaminant removal pathway.

Acknowledgements

The authors would like to acknowledge the contributions of all researchers and staff involved in the laboratory work that supported this study. Their dedication to scientific inquiry, attention to detail, and commitment to maintaining rigorous experimental standards played a vital role in the successful completion of this research. The collaborative environment and shared pursuit of knowledge within the lab greatly enriched the quality and depth of the study.

Conflict of Interest

The authors declared that there is no conflict of interest. **Funding:** No funding was received for conducting this study.

Authors' Contributions

DTO conceptualization, investigation, methodology, YAI and ATA data curator, draft preparation, and editing. DJO experimental, writing review. OSA review, proofread, editing, and resources. All authors approved the manuscript in the present state and approved it for submission.

Availability of Data and Materials

The datasets/information used for this study are available within the manuscript.

6. REFERENCES

- Mojiri, A., Zhou, J. L., Ohashi, A., Ozaki, N., & Kindaichi, T. Comprehensive review of polycyclic aromatic hydrocarbons in water sources, their effects and treatments. *Science of the total environment*, 2019: 696, 133971. doi: 10.1016/j. scitotenv.2019.133971.
- [2] Ghosh, M., & Singh, S. P. A review on phytoremediation of heavy metals and utilization of it's by products. *Asian J Energy Environ*, 2005: 6(4), 18.
- [3] Akpor, O. B., Ohiobor, G. O., & Olaolu, D. T. Heavy metal pollutants in wastewater effluents: sources, effects and remediation. *Advances in Bioscience and Bioengineering*, 2014:2(4), 37-43.
- [4] Adriano, D. C., & Adriano, D. C. Other trace elements. *Trace elements in the terrestrial environment*, 1986: 470-501.
- [5] Alloway, B. J.. Sources of heavy metals and metalloids in soils. *Heavy metals in soils: trace metals and metalloids in soils and their bioavailability*, 2013:11-50.
- [6] Jothimani, P., Ponmani, S., & Sangeetha, R. Phytoremediation of heavy metals-A review. *International Journal of Research: Studies in Bioscience*, 2013:1, 17-23.
- [7] Kennish, M. J. Ecology of estuaries: anthropogenic effects. CRC press. 2019
- [8] Bibi, S., Jabeen, R., & Hayee, A. A. A review: Phytoremediation: Using aquatic plants to clean up polluted water. *International Journal of Current Research*, 2016:8(8), 35561-35564.



- [9] Singh, B. K., Walker, A., Morgan, J. A. W., & Wright, D. J. Biodegradation of chlorpyrifos by Enterobacter strain B-14 and its use in bioremediation of contaminated soils. *Applied and environmental microbiology*, 2004:70(8), 4855-4863.
- [10] Wuana, R. A., & Okieimen, F. E. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecology 2011: 1–20. *Korean J. Soil Sci. Fert*, 2011:34(1), 33-41. https://doi.org/10.5402/2011/402647.
- [11] Aremu, M. O., Atolaiye, B. O., & Labaran, L. Environmental implication of metal concentrations in soil, plant foods and pond in area around the derelict Udege mines of Nasarawa State, Nigeria. *Bulletin of the Chemical Society of Ethiopia*, 2010: 24(3).
- [12] Roozbahami, M.M., Sobhanardakam, S., Karimi, H. and Sorooshnia, R. Natural and Anthopogenic sources of heavy metal pollution in the soil samples of an industrial complex. A case study. *Iranian Journal of Toxicology*, 2015:9, (29)
- [13] McLaughlin, M. J., Zarcinas, B. A., Stevens, D. P., & Cook, N. Soil testing for heavy metals. *Communications in Soil Science and Plant Analysis*, 2000: *31*(11-14), 1661-1700.
- [14] Chambers, C. D. In Situ Treatment of Hazardous Waste-Contaminated Soils. Soil Science, 1993: 155(1), 71.
- [15] Baker, A. J. M., McGrath, S. P., Sidoli, C. M. D., & Reeves, R. D. The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. *Resources, Conservation and Recycling*, 1994:11(1-4), 41-49.
- [16] Watts R. J. Hazardous Wastes: Sources, Pathways and Receptors. John Wiley, New York. 1997
- [17] Mench, M.J., Didier, V.L., Loffler, M., Gomez, A. and Massio, P. J. Environ. Qual., 1994:23: 785-792.
- [18] Alloway, B. J., & Jackson, A. P. The behaviour of heavy metals in sewage sludge-amended soils. *Science of the Total Environment*, 1991:100, 151-176.
- [19] Burns, R. G., Rogers, S., & McGhee, I. Remediation of inorganics and organics in industrial and urban contaminated soils. In *Contaminants and the Soil Environment in the Australasia-Pacific Region: Proceedings* of the First Australasia-Pacific Conference on Contaminants and Soil Environment in the Australasia-Pacific Region, held in Adelaide, Australia, 18–23 February 1996 (pp. 411-449). Dordrecht: Springer Netherlands.
- [20] Agunbiade, F. O., Olu-Owolabi, B. I., & Adebowale, K. O. Phytoremediation potential of Eichornia crassipes in metal-contaminated coastal water. *Bioresource Technology*, 2009:100(19), 4521-4526.
- [21] Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International journal of chemical engineering*, 2011.
- [22] Otaru, A. J., Ameh, C. U., Okafor, J. O., Odigure, J. O., Abdulkareem, A. S., & Ibrahim, S. Study on the effectiveness of phytoremediation in the removal of heavy metals from soil using corn. *International Journal of Computational Engineering Research*, 2013:3(4), 87-93.
- [23] Kardievel, K., & Jegadeesan, M. Mercury and cadmium accumulation in selected weed plants: implication for phytoremediation. *Asian Journal of Plant Science and Research*, 2014:5, 1-4.
- [24] Cunningham, S. D., & Ow, D. W. Promises and prospects of phytoremediation. *Plant physiology*, 1996:110(3), 715.
- [25] Sadowsky, M. J. Phytoremediation: past promises and future practices. In *Proceedings of the 8th international symposium on microbial ecology. Halifax, Canada* 1999:1-7.
- [26] Salt, D. E., Smith, R. D., & Raskin, I. Phytoremediation. Annual review of plant biology, 1998: 49(1), 643-668.
- [27] Sharma, B. R., Kumar, V., Gat, Y., Kumar, N., Parashar, A., & Pinakin, D. J. Microbial maceration: a sustainable approach for phytochemical extraction. *3 Biotech*, 2018:8, 1-13. doi: 10.1007/s13205-018-1423-8.
- [28] Jaeger, R., & Cuny, E. Terpenoids with special pharmacological significance: A
review. Naturalproductcommunications, 2016:11(9),1934578X1601100946.doi: 10.1177/1934578X1601100946.



- [29] Ajiboye, A. T., Asekun, O. T., Ajiboye, O. F. A., Asekun, O. T., & Familoni, O. B. HPLC profile of phenolic contents, antioxidant and antidiabetic activities of Methanolic extract of the leaves of Sarcocephalus latifolius (Bruce, smith) grown in north central geopolitical zone, Nigeria. *Jordan Journal of Chemistry (JJC)*, 2020:15(3), 103-110.
- [30] Ugwu, E., Gupta, B. S., Adeloye, A. J., & Martínez-Villegas, N. Column experiment for the removal of cadmium, copper, lead and zinc from artificially contaminated soil using EDTA, rhamnolipids, and soapnut. European Journal of Environment and Earth Sciences, 2021:2(2). https://doi.org/10.24018/ejgeo.2021.2.2.63
- [31]Njoku, K. L., & Jolaoso, A. O. Growth and ability of Senna alata in phytoremediation of soil contaminated with heavy metals. *Notulae Scientia Biologicae*, 2020:12(2), 420-432. <u>https://doi.org/10.15835/NSB12210523</u>
- [32]Olowoyo, J. O., Van Heerden, E., Fischer, J. L., & Baker, C. Trace metals in soil and leaves of Jacaranda mimosifolia in Tshwane area, South Africa. *Atmospheric Environment*, 2010:44(14), 1826-1830.
- [33] World Health Organization (WHO). Permissible limits of heavy metals in soil and plants. Geneva, Switzerland, 1996.
- [34] Ojaniyi, O. F., Okoye, P. A., & Omokpariola, D. O. Heavy metals analysis and health risk assessment of three fish species, surface water and sediment samples in Ogbaru Axis of River Niger, Anambra State, Nigeria. Asian Journal of Applied Chemistry Research, 2021:9(1), 64-81. https://doi.org/10.9734/AJACR/2021/v9i130205.
- [35] Onwukeme, V. I., & Okechukwu, V. U. Leaching matrix of selected heavy metals from soil to ground water sources in active dumpsites: A case study of Southern Nigeria. *IOSR J Environ Sci, Toxicol Food Technol*, 2021:15(4), 1-18.
- [36] Sarma, H. Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. *Journal of Environmental Science and Technology*, 2011:4(2), 118-138.
- [37]Fakhri, Y., Mousavi Khaneghah, A., Conti, G. O., Ferrante, M., Khezri, A., Darvishi, A., & Amanidaz, N. (2018). Probabilistic risk assessment (Monte Carlo simulation method) of Pb and Cd in the onion bulb (Allium cepa) and soil of Iran. *Environmental Science and Pollution Research*, 25, 30894-30906.
- [38] Filipovic-Trajkovic, R., Ilic, Z. S., Sunic, L., & Andjelković, S. The potential of different plant species for heavy metals accumulation and distribution. *J Food Agric Environ*, 2012:10(1), 959-964.
- [39]Lu, S., Kong, L., Li, S., & Chen, B. Z. Y. and Pan, Q. Accumulation of heavy metals associated with trees planted in Beijing. *China. Journal of Food Agriculture and Environment*, 2014:12, 508-512.
- [40] Villaseñor, I. M., Canlas, A. P., Pascua, M. P. I., Sabando, M. N., & Soliven, L. A. P. Bioactivity studies on Cassia alata Linn. leaf extracts. *Phytotherapy Research*, 2002:16(S1), 93-96.
- [41] Lewis, A., & Levy, A. Anti-inflammatory activities of Cassia alata leaf extract in complete Freund's adjuvant arthritis in rats. *West Indian medical journal*, 2011:6

