INTERNATIONAL JOURNAL OF PHYTOFUELS AND ALLIED SCIENCES
(A Journal of the Society for the Conservation of Phytofuels and Sciences)
(http://www.phytofuelsciences.com) (ISSN 2354 1784)

Morphological Responses of *Anacardium occidentale* L. (Anacardiaceae) to Lead (PB)

Abdulrahaman, A. A.[1*], Sagaya, A.[1], Sholarin, A. A.[1], Adeniran, S. A.[1], Al sahli, A. A.[2] and Alaraidh, I. A.[2]

[1]Applied Plant Anatomy and Wood Technology Laboratory, Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria

[2]Department of Botany and Microbiology,
College of Science, King Saud University, Riyadh, Saudi Arabia

***CORRESPONDING AUTHOR:** abdulrahamanaa@unilorin.edu.ng; +2348033897870

ABSTRACT

Cashew plant (*Anacardium occidentale* L.) seeds were pretreated with concentrated tetraoxosulphate (IV) acid (H_2SO_4) in three concentrations: 5%, 10% and 20% in 5, 15 and 30 minutes each and were grown in lead (Pb) (Lead (II) acetate) $Pb(C_2H_3O_2)_2$ contaminated soil (7 kg) in three concentrations 1000 ppm, 3000 ppm and 5000 ppm. Seed emergence was appreciated in 5% acid concentration and depressed in 10% and 20%. However, the effect of Pb was significantly observed as it delayed seed emergence in the control plants. Plant height, number of leaves and stem girth increased significantly across the months while the leaf area was depressed. The total Pb content was determined using Atomic Adsorption Spectrophotometry (AAS). The present result showed higher root Pb compared to shoot Pb, indicating limited mobility, once absorbed by the roots and that its accumulation and translocation is concentration dependent in relation to the pretreatment time interval. The result showed that the plant demonstrated a physiological response to Pb pollution, which suggests that cashew plants are indicators of heavy metal accumulation and the effect on the environment.

Keyword: Cashew, Lead acetate, accumulation,

INTRODUCTION

Heavy metals are essential environmental pollutants and their toxicity is a problem of growing importance for ecological, evolutionary, dietary and environmental reasons (Agnihotri *et al.*, 2014). They are included in the main category of environmental pollutants as they can linger in the environment for long periods; their accumulation is potentially hazardous to humans, animals and plants (Webber, 1981; Baker *et al*., 1994; Deram *et al*., 2000; Jayakumara *et al*., 2008a; Abdul Jaleel *et al*., 2009 and Thayer *et al*., 2012). Heavy metals make a significant input to environmental pollution as a result of human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and military operations (Nedelkoska and Deram, 2000). They present a risk for primary and secondary consumers and ultimately humans (Zeller and Feller, 1999). Additionally, these metals can enter the plant system, accumulate and later may enter the food chain and cause harm to humans and animals (Deram *et al*., 2000; Jayakumara *et al*., 2008a).

Lead (Pb), with atomic number 82, atomic weight 207.19, and a specific gravity of 11.34, is a bluish or silvery-grey metal with a melting point of 327.5^{o}C and a boiling point at atmospheric pressure of 1749^{o}C. It has four naturally occurring isotopes with atomic weights 208, 207, 206 and 204 (in decreasing order of abundance) (Laide, 2004). Lead (Pb) exists in many forms in the natural sources throughout the world and is now one of the most widely and evenly distributed trace metals. Lead (Pb) is a major pollutant in aquatic and terrestrial ecosystems. Anthropogenic Pb pollution occurs through a variety of activities, including mining, metal processing, battery manufacture and disposal, the burning of leaded fuels, the release of Pb from the wearing of tires, and application of sludge to agricultural land (Kabata-Pendias and Pendias, 2001). Soil and plants can be contaminated by lead from car exhaust, dust, and gases from various industrial sources (Tangahu *et al*., 2011). Lead (Pb) is one of the non-essential toxic heavy metal for plants (Gopal and Rizvi, 2008; Choudhary *et al*., 2012) and pollutants of the environment with no known biological function with its concentration rapidly increasing in the soil (Choudhary *et al*., 2012). Elevated Pb in soils may adversely affect soil productivity and even a very low concentration can inhibit some vital plant processes, such as photosynthesis, mitosis and water absorption showing toxic symptoms of dark leaves, wilting of older leaves, stunted foliage and brown short roots (Patra *et al*., 2004). Besides physiological changes (germination, growth etc.), Pb also causes ultrastructural (anatomy) variations in the cells of different plant parts (Islam *et al*., 2007; Kaur *et al*., 2012). The extent to which Pb enters plants via the leaves depends on the ability of the leaves to absorb Pb from aerial sources, which in turn depends on the specific leaf morphology as downy leaves are able to absorb heavy metals from the atmosphere (Godzik, 1993). However, it is agreed that the bulk of the Pb taken up by plants remains in the roots (Kumar *et al*., 1995). Leaves differ in their abilities to accumulate Pb depending on age, maximum Pb content found in senescing leaves and least in young leaves (Godzik, 1993). So, better understanding of the effect of Pb in plants requires ultrastructural observations in addition to physiological studies.

Cashew (*Anacardium occidentale* L.) is a tropical tree nut crop that belongs to the family Anacardiaceae. It is native to tropical South American countries around Bolivia, Brazil, Peru and West Indies (Nakasone and Paull, 1998; Samal *et al*., 2003), with the Amazonia forest of Brazil being the centre of origin (Mitchell and Mori, 1987) and consists of about 75 genera and 700 species (Nakasone and Paull, 1998). Other members of the family Anacardiaceae include mango and pistachio. Out of the nine species identified in the genus *Anacardium*, only cashew (*occidentale*) is of economic importance because of its edible hypocarp (apple) and nutritious kernel of the drupaceous nut (Aliyu, 2012; Adeigbe, *et al.*, 2015). It is believed, that cashew nut was brought into Africa from the northern part of South America by Portuguese missionaries in 1400 (Mitchell and Mori, 1987). In western Nigeria, the first planting of cashew started in the 16th century at Agege in Lagos (Ventakaramah, 1976). The commercial cultivation actually started in 1950s at Iwo, Eruwa and upper Ogun in the defunct Western Nigeria Development Corporation (WNDC) (Togun, 1977; Sanwo *et al.*, 1972). Remarkably, cashew trees are planted not only for the production of nuts, but also for afforestation and erosion prevention programmes in escarpment areas (Togun, 1977; Sanwo *et al.*, 1972). Today, cashew cultivation has spread to almost all the states of Nigeria from the South to the North with increased processing, shipping and exporting activities (Ezeagu, 2002; Chemonics International Inc., 2002). It is noteworthy that the majority of export quality nuts comes from the Western and Eastern parts of the country (Adeigbe *et al.,* 2015).

Cashew is well adapted to seasonally wet and dry tropical climates and has the capability to grow and yield adequately on well-drained, light textured soils with at least minimum inputs (Hammed *et al*., 2008). This shows that, cashew can suitably adapt to wide ecological changes. Different environmental factors like drought, temperature, salinity, and heavy metal contamination affect germination and ultimate growth in plants (Sidari *et al*., 2008; Curguz *et al*., 2012).

Therefore, the current study elucidated the effects of lead-polluted soil on the morphology of *A. occidentale*.

MATERIALS AND METHODS

EXPERIMENTAL SITE

The research was carried out at the Botanical Garden of the University of Ilorin, Ilorin, Kwara State, Nigeria. University of Ilorin Botanical Garden lies between latitude 4^0 38.920^1 E and 4^0 39.971^1 E and latitude 8^0 27.810^1 N and 8^0 28.230^1 N. It is located in the transitional zone between the deciduous woodland of the south and dry savannah of North of Nigeria (Jimoh, 2003). The certified viable nuts of *A. occidentale* were procured from the Ministry of Agriculture, Ilorin, Kwara State, Nigeria and authenticated at University of Ilorin, Ilorin Kwara state with voucher number UIL/002/631/2020.

SOIL SAMPLING AND ANALYSIS

Soil samples from 0 to 15cm depth were collected using hand trowel. Soil sample analysis was conducted to determine the soil texture (Gee and Bauder, 1986), organic carbon (Walkley and Black, 1934), organic matter (Walkley and Black, 1934), total nitrogen (Bremmer, 1996), pH, exchangeable acidity (Al+H) (IITA, 1979), exchangeable cations (Anderson and Ingram, 1998), and phosphorus (Bray and Kurtz, 1945).

EXPERIMENTAL DESIGN AND TREATMENT DETAILS

The experimental plots were divided into four sections, each of the sections was further divided into three subsections which had three treatments with three replications per treatment. Preliminary test was carried out on soil to determine the main soil parameters, heavy metals present and its concentration ranging the actual amount of Lead (Pb) used for the experiment. The pretreated nuts with different concentrations were sown at 2 cm depth differently in different pots filled with fertile loamy soil of 7 kg for determining the germination percentage of the seeds and arranged in completely randomized block design after the potted soil has been treated with Lead salt (Lead (II) acetate) $Pb(C_2H_3O_2)_2$ dissolved in equal ml of distilled water at three different concentrations; 1000 ppm, 3000 ppm and 5000 ppm with three replications each. There were three control plants for each concentration; pre-treated acid nuts without Pb treated soil, ordinary sterilized nuts (alcohol) with Pb treated soil and plant without pretreated acid nuts and Pb treated soil.

Table 1: Experiment design and treatment details

Lead (Pb) treatment of soil (ppm)	Acid (H_2SO_4) pre-treatment of nuts									Control experiments				
	5% concentration			**10% concentration**			**20% concentration**			**–Pb+Pr**			**+Pb–Pr**	**–Pb–Pr**
	5 mins	**15 mins**	**30 mins**	**5 mins**	**15 mins**	**30 mins**	**5 mins**	**15 mins**	**30 mins**	**5 mins**	**15 mins**	**30 mins**		
1000	R1	R1	R1	R1	R1	R1	R1	R1	R1	R1	R1	R1	R1	
	R2	R2	R2	R2	R2	R2	R2	R2	R2	R2	R2	R2	R2	
	R3	R3	R3	R3	R3	R3	R3	R3	R3	R3	R3	R3	R3	
3000	R1	R1	R1	R1	R1	R1	R1	R1	R1	R1	R1	R1	R1	
	R2	R2	R2	R2	R2	R2	R2	R2	R2	R2	R2	R2	R2	
	R3	R3	R3	R3	R3	R3	R3	R3	R3	R3	R3	R3	R3	
5000	R1	R1	R1	R1	R1	R1	R1	R1	R1	R1	R1	R1	R1	
	R2	R2	R2	R2	R2	R2	R2	R2	R2	R2	R2	R2	R2	
	R3	R3	R3	R3	R3	R3	R3	R3	R3	R3	R3	R3	R3	
Control														R1R2R3

N.B: ppm: parts per million; R1, R2, R3: Replication 1, 2, 3; (–): without or minus; (+): with or plus; Pr: acid pre-treatment; Pb: Lead

SEED GERMINATION PERCENTAGE

The seeds were considered as germinated when the radicle surfaced to the top soil reaching about a length of 1mm (Kabir *et al.*, 2008) and the germination percentage was calculated using this formula:

$$\text{Seed germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \; x\ 100$$

(Gairola *et al*., 2011).

GROWTH PARAMETERS

Growth parameters were taken on a monthly basis for four months to determine: the plant height which was measured from the soil level to the tip of the plant appropriately using the meter scale. The number of leaves present on the plant was counted and readings were taken. The stem girth of the plant was measured with the aid of a Vernier caliper. Leaves were measured randomly from the top, middle and lower parts of the plants. A sample size of three of leaves was used. The leaf area was determined using the formula:

$$\text{Leaf Area} = L\ x\ B\ x\ K$$

Where L = length, B = breadth, K = Franco constant (0.79) (Franco, 1939)

LEAD ACCUMULATION IN PLANTS

Aqua regia (EPA 3050B-1) method was adopted for the digestion of the plant samples. Half gram (0.5 g) of each plant sample (the root and shoot) was weighed into digesting tube. Fifteen millimeter (15 ml) of concentrated HNO_3 and five (5 ml) of concentrated HCl, ratio 3:1 were added to the samples. The content was heated on a hot plate in a fume cupboard till brownish fumes were expelled. The solution was cooled for 5 minutes. After cooling, 10ml of distilled water was added and then filtered through Whatman No. 42 filter papers. The filtrate was made up separately with distilled water to 100ml of the standard flask. The digested plant samples were transferred separately into separate plastic sample bottles for further analyses. The blanks were also ran.

HEAVY METAL ANALYSIS

The concentration of heavy metal Pb was analyzed with Atomic Absorption Spectrophotometric (Buck Scientific 210 VGP) at FATLAB Nigeria Company, Ibadan, Nigeria.

DETERMINATION OF TRANSLOCATION FACTOR

Translocation Factor (TF) was described as the ratio of heavy metals in plant shoot to that in plant root given in equation (Nouri *et al*., 2009).

$$\text{Translocation Factor} = \frac{\text{Shoot metals}}{\text{Root metals}}$$

QUALITY ASSURANCE AND CONTROL

Appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results. The method used by Bai *et al.*, (2011) in quality assurance and control in heavy metal studies was followed and Standard Reference Materials (SRM) obtained from the National Research Centre for CRMs (IAEA SL-1 Lake sediment for soil samples and IAEA 359-cabbage for plant samples) were used for validation of analytical procedures.

STATISTICAL ANALYSIS

The means of all the parameters were subjected to Analysis of Variance (ANOVA) and means were separated by Duncan Multiple Range Test at $p<0.05$. All statistics were carried out with the use of Statistical Package for Social Sciences (SPSS) version 17.0 and Origin 7 software package. A probability value of 0.05 was used as a yardstick for significance difference between parameters.

RESULTS

Physico-chemical properties of experimental soil

The soil used in the experiment was sandy loam in nature comprising 10% silt, 6.48% clay, 83.52% sand. The chemical analysis of the soil showed that the pH of the soil was slightly alkaline (8.5) and it contained other chemical properties with varying percentage (Table 2).

Table 2: Physico-chemical properties of the experimental soil

Soil parameter determined	Values
Physical properties	**Particle size distribution**
Silt (%)	10.00

Clay (%)	6.48
Sand (%)	83.52
Gravel (%)	–
Chemical properties	
pH	8.5
KCl	7.5
Nitrogen (%)	0.4
Organic carbon (%)	1.226
Organic matter (%)	2.113
Cation exchange capacity	2.95
Base saturation (%)	27.45
Ca (cmolkg^{-1})	0.07
Mg^{2+} (cmolkg^{-1})	0.68
K^{+} (cmolkg^{-1})	0.04
Na^{+} (cmolkg^{-1})	0.20
P (mg/kg)	1.52
Pb (mg/kg)	0.10
Cr (mg/kg)	0.11
Ni (mg/kg)	0.04
Cu (mg/kg)	0.12
Mn (mg/kg)	0.31
Cd (mg/kg)	0.13

Seed germination and growth

Germination of seeds of the *Anacardium occidentale* occurred within 13 – 30 days of sowing. The order of seedling emergence to acid pretreatment was 5%<10%<20% but in the control experiments (–Pb–Pr), recorded (14 – 20 days) while (+Pb–Pr) recorded the longest days of emergence (25 – 30 days). It was however observed that the influence of Pb variations with respect to pretreatment time interval had no severe effect in the duration of days of seedling emergence except in +Pb–Pr and that only the concentration of acid used was influential (Table 3).

Table 3: Duration of days of seedling emergence

Treatment	Time	Lead (Pb) variation	Days to seedlings emergence
5%Acid	5 minutes	1000 ppm	13 – 18
		3000 ppm	
		5000 ppm	

	15 minutes	1000 ppm	
		3000 ppm	
		5000 ppm	
	30 minutes	1000 ppm	
		3000 ppm	
		5000 ppm	
	5 minutes	– Pb (without lead treated soil)	
	15 minutes	– Pb (without lead treated soil)	
	30 minutes	– Pb (without lead treated soil)	
10% Acid	5 minutes	1000 ppm	16 – 23
		3000 ppm	
		5000 ppm	
	15 minutes	1000 ppm	
		3000 ppm	
		5000 ppm	
	30 minutes	1000 ppm	
		3000 ppm	
		5000 ppm	
	5 minutes	– Pb (without lead treated soil)	
	15 minutes	– Pb (without lead treated soil)	
	30 minutes	– Pb (without lead treated soil)	
20% Acid	5 minutes	1000 ppm	20 – 30
		3000 ppm	
		5000 ppm	
	15 minutes	1000 ppm	
		3000 ppm	
		5000 ppm	
	30 minutes	1000 ppm	
		3000 ppm	
		5000 ppm	
	5 minutes	– Pb (without lead treated soil)	
	15 minutes	– Pb (without lead treated soil)	
	30 minutes	– Pb (without lead treated soil)	

Control	– Pr – Pb	(without acid pretreatment and lead treated soil)	14 – 20
	+ Pb – Pr	1000 ppm	25 – 30
		3000 ppm	
		5000 ppm	

Note: – = minus or without; + = plus or with; Pb = lead; Pr = pretreatment

It was recorded that the percentage of seeds germinated in both the pretreated seeds and control seeds differs (Table 4). The table illustrates that the germination percentage was calculated individually, per number of seed sown in the treated soil for each acid concentration, i.e. 5mins (1000ppm, 3000ppm, 5000ppm), then combined to show the germination percentage for the time intervals, i.e. 5mins, 15mins and 30mins and lastly combined to show the total germination percentage for the acid pretreatment i.e. 5% acid, 10% acid and 20% acid. The seeds pretreated with 20% acid recorded the lowest (38.52%) overall germination percentage followed by the pretreated seeds with 5% acid (45.19%) and 10% acid (52.59%) while the control seeds recorded the highest (61.54%) germination percentage.

Table 4: Germination percentage of the seeds sown in different pretreatment

Acid pre-treatment	Time	Pb Treatment variations	Total number of seed sown	Number of germinated seeds	Germination percentage (%)	Total number of seed sown	Number of germinated seeds	Germination percentage (%)	Total number of seed sown	Number of germinated seeds	Germination percentage (%)
5%Acid	5 mins	1000 ppm	15	8	53.33	45	19	42.22	135	61	45.19
		3000 ppm	15	6	40.00						
		5000 ppm	15	5	33.33						
	15 mins	1000 ppm	15	11	73.33	45	24	53.33			
		3000 ppm	15	7	46.67						
		5000 ppm	15	6	40.00						
	30 mins	1000 ppm	15	7	46.67	45	18	40.00			
		3000 ppm	15	6	40.00						
		5000 ppm	15	5	33.33						
10%Acid	5 mins	1000 ppm	15	10	66.67	45	22	48.89	135	71	52.59
		3000 ppm	15	6	40.00						
		5000 ppm	15	6	40.00						
	15mins	1000 ppm	15	12	80.00	45	26	7.78			
		3000 ppm	15	8	53.33						
		5000 ppm	15	6	40.00						

	30 mins	1000 ppm	15	8	53.33	45	23	51.11			
		3000 ppm	15	7	46.67						
		5000 ppm	15	8	53.33						
20%Acid	5 mins	1000 ppm	15	7	46.67	45	21	46.67	135	52	38.52
		3000 ppm	15	6	40.00						
		5000 ppm	15	8	53.33						
	15 mins	1000 ppm	15	7	46.67	45	14	31.11			
		3000 ppm	15	4	26.67						
		5000 ppm	15	3	20.00						
	30 mins	1000 ppm	15	5	33.33	45	17	37.78			
		3000 ppm	15	7	46.67						
		5000 ppm	15	5	33.33						
Control	–Pr–Pb		15	11	73.33	15	11	73.33	195	120	61.54
	+Pb–Pr	1000 ppm	15	7	46.67	45	18	40.00			
		3000 ppm	15	6	40.00						
		5000 ppm	15	5	33.33						
	–Pb +Pr (5% Acid)	5 minutes	15	14	93.33	45	32	71.11			
		15 minutes	15	9	60.00						
		30 minutes	15	9	60.00						
	–Pb +Pr (10% Acid)	5 minutes	15	12	80.00	45	31	68.89			
		15 minutes	15	10	66.67						
		30 minutes	15	9	60.00						
	–Pb +Pr (20% Acid)	5 minutes	15	8	53.33	45	28	62.22			
		15 minutes	15	12	80.00						
		30 minutes	15	8	53.33						

Note: – = minus or without; + = plus or with; Pb = lead; Pr = pretreatment

Mean values on plant height is presented in Table 5. It showed that there was significant difference in the plant height of all treated seedlings compared to the control plants (–Pb–Pr) ($p = 0.0000 < \alpha = 0.05$). It further revealed that there was increase in the height across the months and that the growth trend was reduced from 2 MAP to 3 MAP compared to 1 MAP to 2 MAP and 3 MAP to 4 MAP. The shortest plant height (13.57 cm) was produced by 20% acid pretreatment for 30 minutes at 1000 ppm of Pb treated soil (20%A+Pb 1000 ppm at 30 mins) while 20% acid pretreatment for 30 minutes minus Pb treated soil (20%A – Pb at 30 mins) produced the tallest plant height (29.47 cm) at the first month after planting (1 MAP). At the fourth month after planting (4 MAP), +Pb – Pr had the shortest plant height (34.07 cm) while 20%A – Pb at 15 mins produced the tallest plant height (55.17 cm) (Table 5).

Table 5: Plant height of the various treatments of cashew seedlings

Treatment	Time	1MAP	2MAP	3MAP	4MAP
5%A+Pb 1000 ppm	Control	10.47±2.17[b]	17.53±1.87[b]	28.50±1.97[a]	39.74±1.56[ab]
	5 minutes	20.13±2.86[a]	28.97±4.42[a]	30.17±4.15[a]	38.00±1.15[ab]
	15 minutes	26.37±1.79[a]	35.97±0.61[a]	39.57±1.90[a]	47.00±4.16[a]
	30 minutes	20.77±2.46[a]	28.17±4.48[b]	32.00±4.04[a]	34.23±5.19[b]
5%A+Pb 3000 ppm	Control	10.47±2.17[a]	17.53±1.87[b]	28.50±1.97[a]	39.74±1.56[a]
	5 minutes	21.87±0.98[a]	33.93±2.36[a]	35.00±2.08[a]	41.90±4.97[a]
	15 minutes	19.70±2.60[a]	33.90±3.61[a]	37.40±0.95[a]	44.00±2.08[a]
	30 minutes	18.90±6.00[a]	29.00±6.43[ab]	32.33±4.83[a]	40.33±3.33[a]
5%A+Pb 5000 ppm	Control	10.47±2.17[b]	17.53±1.87[b]	28.50±1.97[a]	39.74±1.56[a]
	5 minutes	21.33±0.88[a]	30.23±1.95[a]	33.37±1.56[a]	40.83±4.09[a]
	15 minutes	15.70±2.22[ab]	24.00±3.61[ab]	27.43±2.69[a]	34.17±3.44[a]
	30 minutes	18.90±1.55[a]	29.33±1.30[a]	31.67±1.30[a]	39.17±1.30[a]
5%A – Pb	Control	10.47±2.17[b]	17.53±1.87[b]	28.50±1.97[b]	39.74±1.56[a]
	5 minutes	27.77±1.67[a]	38.47±1.92[a]	39.33±2.14[a]	41.73±1.97[a]
	15 minutes	20.07±3.78[a]	31.07±3.28[a]	33.23±1.62[ab]	37.40±0.59[a]
	30 minutes	23.73±2.61[a]	37.10±1.05[a]	38.63±1.60[a]	41.33±1.92[a]
+Pb – Pr	Control	10.47±2.17[b]	17.53±1.87[c]	28.50±1.97[b]	39.74±1.56[b]
	1000ppm	20.13±0.32[a]	29.27±1.80[b]	30.10±1.42[b]	34.07±0.58[b]
	3000ppm	20.70±0.42[a]	35.90±0.97[a]	42.50±2.02[a]	51.00±2.65[a]
	5000ppm	23.73±1.16[a]	37.17±2.05[a]	39.23±1.39[a]	48.67±2.05[a]
10%A+Pb 1000 ppm	Control	10.47±2.17[c]	17.53±1.87[c]	28.50±1.97[b]	39.74±1.56[b]
	5 minutes	26.43±2.00[a]	35.87±4.66[a]	39.63±4.31[a]	50.67±3.72[a]
	15 minutes	23.83±2.85[ab]	33.67±2.89[ab]	36.50±1.04[ab]	39.90±1.14[b]
	30	19.00±1.50[b]	25.57±0.32[bc]	30.37±1.67[b]	37.97±3.27[b]

	minutes				
10%A+Pb 3000 ppm	Control	10.47±2.17[b]	17.53±1.87[b]	28.50±1.97[b]	39.74±1.56[b]
	5 minutes	21.53±1.83[a]	33.10±3.51[a]	37.67±1.83[a]	43.13±2.93[ab]
	15 minutes	25.23±0.65[a]	35.20±1.72[a]	37.63±1.03[a]	48.90±3.14[a]
	30 minutes	20.00±1.50[a]	26.60±3.45[a]	29.53±2.57[b]	36.03±2.44[b]
10%A+Pb 5000 ppm	Control	10.47±2.17[b]	17.53±1.87[b]	28.50±1.97[a]	39.74±1.56[a]
	5 minutes	20.40±3.45[a]	33.47±2.23[a]	34.63±2.94[a]	42.50±2.18[a]
	15 minutes	23.20±2.01[a]	36.30±1.01[a]	38.63±1.66[a]	49.40±2.47[a]
	30 minutes	20.50±3.50[a]	28.70±5.00[a]	30.37±5.49[a]	37.90±5.76[a]
10%A–Pb	Control	10.47±2.17[b]	17.53±1.87[b]	28.50±1.97[b]	39.74±1.56[b]
	5 minutes	26.33±1.30[a]	38.87±2.43[a]	40.00±2.31[a]	44.67±0.93[ab]
	15 minutes	28.00±1.04[a]	41.20±1.76[a]	42.00±1.73[a]	47.07±2.94[a]
	30 minutes	28.30±0.46[a]	38.50±0.76[a]	39.53±0.80[a]	46.83±2.17[a]
20%A+Pb 1000 ppm	Control	10.47±2.17[b]	17.53±1.87[c]	28.50±1.97[bc]	39.74±1.56[b]
	5 minutes	24.83±0.60[a]	34.33±1.17[a]	36.27±0.62[a]	46.00±2.08[a]
	15 minutes	20.67±1.74[a]	27.07±0.54[b]	30.10±0.86[b]	37.83±1.09[b]
	30 minutes	13.57±1.87[b]	17.67±3.06[c]	24.50±2.18[c]	34.17±2.24[b]
20%A+Pb 3000 ppm	Control	10.47±2.17[b]	17.53±1.87[b]	28.50±1.97[ab]	39.74±1.56[a]
	5 minutes	28.20±2.00[a]	37.53±3.21[a]	38.53±3.07[a]	47.43±3.12[a]
	15 minutes	14.17±2.68[b]	23.07±4.20[b]	24.17±4.49[b]	35.43±3.92[a]
	30 minutes	27.00±1.32[a]	36.83±4.60[a]	37.83±4.32[a]	45.00±4.62[a]
20%A+Pb 5000 ppm	Control	10.47±2.17[b]	17.53±1.87[b]	28.50±1.97[a]	39.74±1.56[a]
	5 minutes	21.60±2.01[a]	34.83±6.36[a]	36.10±5.83[a]	46.37±6.06[a]
	15	20.20±2.08[a]	30.17±1.92[a]	32.03±1.61[a]	41.50±0.87[a]

	minutes				
	30 minutes	22.83±1.10^{a}	32.33±0.88^{a}	34.50±1.15^{a}	39.67±1.20^{a}
20%A–Pb	Control	10.47±2.17^{b}	17.53±1.87^{b}	28.50±1.97^{b}	39.74±1.56^{a}
	5 minutes	26.63±3.64^{a}	34.77±5.27^{a}	36.87±3.81^{ab}	46.07±4.83^{a}
	15 minutes	26.50±0.87^{a}	42.90±2.55^{a}	44.63±2.60^{a}	55.17±7.80^{a}
	30 minutes	29.47±3.49^{a}	44.00±5.51^{a}	46.37±6.06^{a}	52.33±9.35^{a}

Note: MAP = Month after planting; (–) = minus or without; (+) = plus or with; Pb = lead; Pr = pretreatment. Means in a column not followed by the same letter are significantly different at $p < 0.05$.

Mean data as regards number of leaves is presented in Table 6, which showed that there was a significant difference in the number of leaves among treated seedlings with respect to the control plants (–Pb–Pr) ($p = 0.0000 < \alpha = 0.05$). The table also indicated that, there was an increase in the leaf number across the months and the control had the minimum number of leaves at 1 MAP. The least number of leaves occurred at treatments 5%A+Pb 5000 ppm at 5mins, +Pb – Pr at 1000 ppm and 20%A+Pb 3000 ppm at 15 mins (5.67) while the highest number of leaves occurred at treatments 5%A+Pb 1000ppm at 15mins and 10%A–Pb at 30 mins (7.67) at 1 MAP. At 4 MAP, 10%A+Pb 1000 ppm for 15 mins had the lowest number of leaves (9.33) while 20%A+Pb 1000ppm at 15mins had the highest number of leaves (20.33) (Table 6).

Table 6: Number of leaves of the various treatments of cashew seedlings

Treatment	Time	1 MAP	2 MAP	3 MAP	4 MAE
5%A+Pb 1000 ppm	Control	4.00±0.00^{c}	8.33±0.33^{a}	14.00±0.58^{a}	17.67±0.33^{a}
	5 minutes	6.00±0.00^{b}	8.33±1.20^{a}	10.67±0.88^{a}	14.67±1.20^{a}
	15 minutes	7.67±0.33^{a}	11.00±0.00^{a}	13.33±1.45^{a}	14.00±1.53^{a}
	30 minutes	6.67±0.88^{ab}	9.00±1.53^{a}	11.33±1.45^{a}	13.67±1.86^{a}
5%A+Pb 3000 ppm	Control	4.00±0.00^{a}	8.33±0.33^{a}	14.00±0.58^{a}	17.67±0.33^{a}
	5 minutes	6.00±0.58^{a}	8.67±1.20^{a}	10.33±1.33^{a}	14.00±1.00^{b}
	15 minutes	6.00±0.58^{a}	9.33±0.88^{a}	11.67±1.45^{a}	12.67±0.67^{b}
	30 minutes	6.00±0.58^{a}	9.33±1.45^{a}	12.67±1.33^{a}	13.00±1.73^{b}
5%A+Pb 5000 ppm	Control	4.00±0.00^{b}	8.33±0.33^{a}	14.00±0.58^{a}	17.67±0.33^{a}
	5 minutes	5.67±0.33^{a}	9.33±0.88^{a}	10.33±0.88^{b}	12.67±1.45^{b}
	15	6.33±0.33^{a}	9.33±0.33^{a}	13.00±1.15^{ab}	18.33±2.19^{a}

	minutes				
	30 minutes	6.33±0.33[a]	10.00±0.58[a]	10.67±0.67[b]	18.67±0.88[a]
5%A–Pb	Control	4.00±0.00[a]	8.33±0.33[b]	14.00±0.58[a]	17.67±0.33[a]
	5 minutes	6.67±0.33[b]	10.00±0.58[a]	10.67±0.88[ab]	12.67±1.45[b]
	15 minutes	7.00±1.00[b]	10.33±0.33[a]	12.33±1.20[ab]	13.67±1.20[b]
	30 minutes	7.00±0.00[b]	10.00±0.58[a]	10.00±1.15[b]	13.67±1.20[b]
+Pb – Pr	Control	4.00±0.00[c]	8.33±0.33[c]	14.00±0.58[a]	17.67±1.67[a]
	1000ppm	5.67±0.33[b]	9.33±0.33[bc]	10.33±0.33[b]	13.67±1.20[b]
	3000ppm	7.00±0.58[a]	12.00±0.58[a]	15.67±1.76[a]	17.67±0.33[a]
	5000ppm	6.33±0.33[ab]	10.33±0.33[b]	13.00±0.58[ab]	15.33±0.68[ab]
10%A+Pb 1000 ppm	Control	4.00±0.00[c]	8.33±0.33[b]	14.00±0.58[a]	17.67±0.33[a]
	5 minutes	7.33±0.67[a]	11.00±1.15[a]	13.67±1.67[a]	14.67±0.88[a]
	15 minutes	6.00±0.00[b]	8.67±0.33[b]	9.33±0.33[b]	9.33±2.33
	30 minutes	6.67±0.33[ab]	9.33±0.33[ab]	12.33±0.33[a]	13.33±1.45[b]
10%A+Pb 3000 ppm	Control	4.00±0.00[b]	8.33±0.33[b]	14.00±0.58[ab]	17.67±0.33[a]
	5 minutes	6.33±0.33[a]	11.00±1.00[ab]	13.67±1.20[ab]	14.67±2.19[a]
	15 minutes	7.00±0.58[a]	11.67±0.88[a]	15.00±1.00[a]	18.67±1.20[a]
	30 minutes	7.00±0.58[a]	10.67±1.20[ab]	10.67±1.20[b]	15.00±1.00[a]
10%A+Pb 5000 ppm	Control	4.00±0.00[b]	8.33±0.33[a]	14.00±0.58[a]	17.67±0.33[a]
	5 minutes	6.33±0.67[a]	9.00±0.58[a]	12.33±0.88[a]	14.00±2.65[a]
	15 minutes	6.33±0.33[a]	11.67±0.67[a]	13.33±1.20[a]	17.00±1.15[a]
	30 minutes	7.33±0.33[a]	11.00±2.00[a]	11.67±1.45[a]	15.67±1.45[a]
10%A–Pb	Control	4.00±0.00[b]	8.33±0.33[b]	14.00±0.58[a]	17.67±0.33[a]
	5 minutes	6.67±0.33[a]	10.33±0.67[ab]	10.67±0.33[b]	12.33±0.33[c]
	15 minutes	6.33±0.33[a]	10.00±0.58[ab]	11.33±0.33[b]	12.33±0.67[c]

	30 minutes	7.67±0.67[a]	11.33±0.88[a]	13.00±0.00[a]	14.67±0.33[b]
20%A+Pb 1000 ppm	Control	4.00±0.00[b]	8.33±0.33[b]	14.00±0.58[a]	17.67±0.33[a]
	5 minutes	6.67±0.33[a]	12.00±2.08[ab]	14.00±3.21[a]	17.33±2.40[a]
	15 minutes	7.00±0.00[a]	13.67±1.76[a]	16.00±0.58[a]	20.33±2.40[a]
	30 minutes	6.33±0.88[a]	9.33±1.20[ab]	14.00±1.15[a]	18.67±1.20[a]
20%A+Pb 3000 ppm	Control	4.00±0.00[b]	8.33±0.33[b]	14.00±0.58[a]	17.67±0.33[a]
	5 minutes	7.33±0.33[a]	12.00±1.15[a]	13.00±1.15[ab]	13.67±0.88[bc]
	15 minutes	5.67±0.88[a]	9.33±0.88[ab]	10.33±0.88[b]	16.67±0.88[ab]
	30 minutes	6.33±0.33[a]	9.33±1.45[ab]	11.00±1.00[ab]	12.00±1.73[c]
20%A+Pb 5000 ppm	Control	4.00±0.00[b]	8.33±0.33[a]	14.00±0.58[a]	17.67±0.33[a]
	5 minutes	6.67±0.88[a]	10.67±1.45[a]	11.67±1.33[a]	14.67±1.86[ab]
	15 minutes	6.33±0.33[a]	11.00±0.58[a]	12.33±0.33[a]	15.67±0.33[ab]
	30 minutes	7.00±0.58[a]	10.00±1.15[a]	12.33±1.76[a]	12.67±0.88[b]
20%A–Pb	Control	4.00±0.00[b]	8.33±0.33[b]	14.00±0.58[a]	17.67±0.33[a]
	5 minutes	6.33±0.88[a]	10.33±0.67[ab]	12.67±1.45[a]	16.67±1.76[a]
	15 minutes	6.67±0.33[a]	12.33±1.33[a]	13.33±1.45[a]	15.33±1.76[a]
	30 minutes	6.67±0.33[a]	12.00±1.00[a]	13.33±2.33[a]	15.00±1.73[a]

Note: MAP = Month after planting; (–) = minus or without; (+) = plus or with; Pb = lead; Pr = pretreatment. Means in a column not followed by the same letter are significantly different at $p < 0.05$.

Stem girth

The stem girth of the cashew seedlings indicated that a significant difference exists in the stem girth between the seedlings ($p = 0.0000 < \alpha = 0.05$). A treatment +Pr–Pr at 1000 ppm produced the thinnest stem (4.52) while treatment 10%A–Pb at 5 mins produced the fattest stem (5.98) at 1 MAP. At 4 MAP, treatment +Pb–Pr at 1000 ppm produced thinnest stem (7.59) while 10%A+Pb 5000 ppm at 5 mins produced the fattest stem (10.04) (Table 7).

Table 7: Stem girth of the various treatments of cashew seedlings

Treatment	Time	1MAP	2MAP	3MAP	4MAE
5%A+Pb 1000 ppm	Control	4.66±0.19[b]	5.74±0.19[c]	6.18±0.07[b]	7.6067±0.21[c]
	5 minutes	4.95±0.04[ab]	7.87±0.09[a]	8.01±0.03[a]	8.70±0.17[b]
	15 minutes	5.34±0.26[ab]	8.05±0.05[a]	8.53±0.22[a]	9.81±0.17[a]
	30 minutes	5.72±0.45[a]	6.97±0.14[b]	8.24±0.25[a]	9.17±0.44[ab]
5%A+Pb 3000 ppm	Control	4.66±0.19[b]	5.74±0.19[b]	6.18±0.07[c]	7.61±0.21[c]
	5 minutes	5.03±0.05[ab]	6.81±0.20[a]	8.48±0.28[a]	9.34±0.28[ab]
	15 minutes	5.04±0.32[ab]	6.93±0.08[a]	7.95±0.06[b]	8.69±0.17[b]
	30 minutes	5.45±0.24[a]	7.02±0.01[a]	7.91±0.06[b]	9.65±0.21[a]
5%A+Pb 5000 ppm	Control	4.66±0.19[a]	5.74±0.19[b]	6.18±0.07[a]	7.61±0.21[b]
	5 minutes	4.64±0.23[a]	6.74±0.21[a]	8.16±0.37[a]	8.79±0.56[a]
	15 minutes	5.39±0.26[a]	7.00±0.02[a]	7.77±0.18[a]	9.11±0.09[a]
	30 minutes	5.18±0.39[a]	6.97±0.07[a]	7.94±0.05[a]	9.46±0.27[a]
5%A – Pb	Control	4.66±0.19[b]	5.74±0.19[b]	6.18±0.07[b]	7.61±0.21[b]
	5 minutes	5.41±0.26[a]	6.63±0.19[a]	8.00±0.01[a]	9.31±0.38[a]
	15 minutes	5.10±0.15[ab]	6.57±0.23[a]	7.86±0.14[a]	8.82±0.18[a]
	30 minutes	5.32±0.04[a]	7.12±0.19[a]	8.47±0.57[a]	9.36±0.38[a]
+Pb – Pr	Control	4.66±0.19[b]	5.74±0.19[b]	6.18±0.07[c]	7.60±0.21[b]
	1000ppm	4.52±0.25[b]	5.61±0.21[b]	6.81±0.15[b]	7.59±0.21[b]
	3000ppm	5.02±0.02[ab]	6.97±0.03[a]	8.81±0.10[a]	9.96±0.14[a]
	5000ppm	5.19±0.08[a]	6.65±0.18[a]	8.93±0.08[a]	9.99±0.04[a]
10%A+Pb 1000 ppm	Control	4.66±0.19[b]	5.74±0.19[b]	6.18±0.07[d]	7.61±0.21[b]
	5 minutes	5.40±0.23[a]	7.33±0.33[a]	8.55±0.22[a]	9.12±0.05[a]
	15 minutes	4.65±0.18[b]	6.09±0.06[b]	6.89±0.05[c]	7.97±0.18[b]
	30	4.83±0.22[ab]	6.26±0.27[b]	7.69±0.19[b]	8.11±0.44[b]

	minutes				
10%A+Pb 3000 ppm	Control	4.66±0.19a	5.74±0.19b	6.18±0.07c	7.61±0.21b
	5 minutes	4.58±0.14a	6.77±0.18a	8.09±0.10a	8.70±0.55ab
	15 minutes	5.10±0.10a	6.78±0.27a	8.03±0.10a	9.03±0.05a
	30 minutes	5.29±0.36a	6.78±0.14a	7.37±0.28b	8.38±0.26ab
10%A+Pb 5000 ppm	Control	4.66±0.19b	5.74±0.19b	6.18±0.07c	7.61.21c
	5 minutes	5.07±0.20b	7.36±0.30a	8.61±0.32a	10.04±0.03a
	15 minutes	5.75±0.15a	6.99±0.04a	7.98±0.02b	9.22±0.42b
	30 minutes	4.77±0.14b	6.93±0.26a	7.86±0.16b	8.93±0.04b
10%A–Pb	Control	4.66±0.19b	5.74±0.19c	6.18±0.07c	7.61±0.21b
	5 minutes	5.98±0.39a	7.09±0.32ab	7.62±0.19b	8.96±0.28a
	15 minutes	5.40±0.30a	7.54±0.27a	8.47±0.26a	8.99±0.44a
	30 minutes	5.02±0.01a	6.66±0.21b	8.83±0.12a	9.34±0.33a
20%A+Pb 1000 ppm	Control	4.66±0.19a	5.74±0.19b	6.18±0.07c	7.61±0.21b
	5 minutes	5.54±0.30a	6.78±0.18a	8.30±0.36a	8.76±0.35a
	15 minutes	5.29±0.28a	6.49±0.21ab	8.66±0.16a	9.00±0.00a
	30 minutes	5.15±0.25a	5.74±0.31b	7.34±0.19a	8.49±0.15a
20%A+Pb 3000 ppm	Control	4.66±0.19b	5.74±0.19b	6.18±0.07c	7.61±0.21c
	5 minutes	5.73±0.23a	6.99±0.04a	7.98±0.01a	9.02±0.04a
	15 minutes	5.03±0.04b	6.47±0.26a	7.34±0.32b	8.18±0.15bc
	30 minutes	5.00±0.00b	6.78±0.16a	7.91±0.11ab	8.73±0.26ab
20%A+Pb 5000 ppm	Control	4.66±0.19b	5.74±0.19b	6.18±0.07b	7.61±0.21b
	5 minutes	5.00±0.00b	6.41±0.22a	7.85±0.16a	9.24±0.40a

	15 minutes	4.94±0.07^{b}	6.54±0.14^{a}	7.80±0.14^{a}	9.17±0.12^{a}
	30 minutes	5.85±0.12^{a}	6.94±0.06^{a}	8.05±0.03^{a}	9.01±0.05^{a}
20%A–Pb	Control	4.66±0.19^{b}	5.74±0.19^{c}	6.18±0.07^{c}	7.61±0.21^{c}
	5 minutes	4.66±0.33^{b}	6.13±0.09^{b}	7.52±0.27^{b}	8.40±0.30^{bc}
	15 minutes	5.14±0.13^{b}	6.05±0.03^{bc}	7.66±0.30^{b}	8.70±0.28^{b}
	30 minutes	5.86±0.19^{a}	6.98±0.04^{a}	9.00±0.50^{a}	9.82±0.23^{a}

Note: MAP = Month after planting; (–) = minus or without; (+) = plus or with; Pb = lead; Pr = pretreatment. Means in a column not followed by the same letter are significantly different at $p < 0.05$.

Leaf area

It was observed that the tagged leaf dried up which could be as resultant effect of the Pb treatment or the trend of growth in cashew plant. However, there was a significance difference in the leaf area among the treated seedlings ($p = 0.0000 < \alpha = 0.05$). The thinnest leave was observed in treatment 10%A+Pb 5000 ppm at 5 mins (6.41) while the widest leave was observed in 5%A+Pb 3000 ppm at 5 mins (33.23) at 1 MAP. At 4 MAP, the thinnest leave was observed in 5%A–Pb at 5 mins (8.56) while the widest leave was observed in +Pb–Pr 5000 ppm (59.91) (Table 8).

Table 8: Leaf area of the various treatments of cashew seedlings

Treatment	Time	1MAP	2MAP	3MAP	4MAE
5%A+Pb 1000 ppm	Control	7.3865±3.65519^{b}	6.5535±1.14^{a}	4.65±0.58^{b}	12.11±0.71^{a}
+					
	5 minutes	22.57±3.61^{a}	22.96±9.64^{a}	18.12±10.02^{ab}	30.08±4.37^{a}
	15 minutes	28.16±4.95^{a}	22.96±6.50^{a}	34.04±9.09^{a}	40.05±22.79^{a}
	30 minutes	18.05±4.10^{ab}	20.68±8.24^{a}	23.76±4.90^{ab}	14.85±5.59^{a}
5%A+Pb 3000 ppm	Control	7.39±3.66^{b}	6.55±1.14^{b}	4.65±0.58^{a}	12.11±0.71^{b}
	5 minutes	33.27±1.86^{a}	38.37±16.24^{a}	21.98±11.79^{a}	42.11±10.17^{a}
	15 minutes	12.36±1.64^{b}	16.33±5.60^{b}	19.24±13.69^{a}	48.60±3.33^{a}
	30 minutes	20.07±8.87^{ab}	22.90±1.14^{ab}	19.86±12.47^{a}	25.64±10.31^{ab}
5%A+Pb 5000 ppm	Control	7.39±3.66^{a}	6.55±1.14^{b}	4.65±0.58^{b}	12.11±0.71^{b}
	5 minutes	16.86±5.07^{a}	15.84±6.86^{ab}	22.91±3.71^{a}	42.62±6.00^{a}

	15 minutes	7.64±1.28[a]	32.84±9.07[a]	29.71±1.57[a]	31.83±11.12[ab]
	30 minutes	15.70±3.59[a]	15.37±5.00[ab]	23.54±5.08[a]	24.94±4.56[ab]
5%A – Pb	Control	7.39±3.66[a]	6.55±1.14[b]	4.65±0.58[c]	12.11±0.71[b]
	5 minutes	20.94±3.80[a]	37.74±7.34[a]	30.59±7.15[ab]	8.56±3.27[b]
	15 minutes	14.06±2.66[a]	33.91±8.84[ab]	18.23±13.13[bc]	23.14±4.34[b]
	30 minutes	22.13±9.12[a]	41.10±14.03[a]	54.51±2.72[a]	45.27±9.66[a]
+Pb – Pr	Control	7.39±3.66[b]	6.55±1.14[a]	4.65±0.58[b]	12.11±0.71[b]
	1000ppm	18.64±2.48[a]	17.41±6.38[a]	42.88±17.31[a]	34.98±14.39[ab]
	3000ppm	11.04±3.04[ab]	9.45±2.81[a]	16.38±6.28[ab]	34.27±4.83[ab]
	5000ppm	17.09±1.55[a]	17.37±4.27[a]	14.03±5.13[ab]	59.91±5.32[a]
10%A+Pb 1000ppm	Control	7.39±3.65[b]	6.55±1.14[b]	4.65±0.58[a]	12.11±0.71[b]
	5 minutes	17.04±2.71[a]	12.94±2.29[ab]	28.36±20.87[a]	52.21±12.48[a]
	15 minutes	17.40±2.98[a]	33.31±12.42[a]	36.34±6.33[a]	22.00±8.14[ab]
	30 minutes	6.90±1.51[b]	18.13±7.19[ab]	8.17±4.02[a]	26.39±11.05[ab]
10%A+Pb 3000ppm	Control	7.39±3.66[a]	6.55±1.14[a]	4.6543±0.58[a]	12.11±0.71[b]
	5 minutes	8.09±2.64[a]	21.82±14.12[a]	19.57±10.05[a]	15.98±5.56[ab]
	15 minutes	17.07±3.16[a]	24.77±15.40[a]	16.67±6.42[a]	12.82±6.24[b]
	30 minutes	15.97±5.80[a]	9.10±2.42[a]	31.24±12.08[a]	34.11±8.65[a]
10%A+Pb 5000 ppm	Control	7.39±3.66[b]	6.55±1.14[b]	4.65±0.58[a]	12.11±0.71[a]
	5 minutes	6.41±1.33[b]	49.38±12.06[a]	8.23±0.64[a]	40.96±9.85[a]
	15 minutes	23.31±7.99[a]	30.56±7.52[ab]	23.71±10.40[a]	36.05±16.55[a]
	30 minutes	9.44±1.88[ab]	19.30±7.30[b]	21.89±3.38[a]	36.06±5.57[a]
10%A–Pb	Control	7.39±3.66[a]	6.55±1.14[b]	4.65±0.58[a]	12.11±0.71[b]
	5 minutes	22.32±8.76[a]	8.42±1.98[b]	20.95±13.20[a]	46.63±11.28[a]
	15	18.11±1.93[a]	25.70±13.33[ab]	3.23±0.57[a]	46.47±4.07[a]

	minutes				
	30 minutes	10.43±0.27[a]	37.63±5.21[a]	17.38±14.37[a]	48.57±6.11[a]
20%A+Pb 1000 ppm	Control	7.39±3.66[b]	6.55±1.14[a]	4.65±0.58[a]	12.11±0.71[b]
	5 minutes	29.31±3.54[a]	25.19±20.56[a]	20.82±15.21[a]	35.85±13.37[ab]
	15 minutes	17.03±5.06[b]	5.25±0.49[a]	28.72±12.19[a]	56.37±7.59[a]
	30 minutes	11.93±1.84[b]	4.94±1.87[a]	26.91±8.77[a]	47.97±7.35[a]
20%A+Pb 3000 ppm	Control	7.39±3.66[b]	6.55±1.14[a]	4.65±0.58[a]	12.11±0.71[a]
	5 minutes	19.96±7.84[ab]	19.14±5.24[a]	31.04±12.88[a]	41.66±12.70[a]
	15 minutes	13.06±2.76[ab]	11.57±2.58[a]	20.04±9.48[a]	20.56±9.78[a]
	30 minutes	26.52±5.24[a]	25.21±9.68[a]	17.37±6.41[a]	42.70±10.64[a]
20%A+Pb 5000 ppm	Control	7.39±3.66[a]	6.55±1.14[b]	4.65±0.58[a]	12.11±0.71[c]
	5 minutes	11.79±3.38[a]	20.63±10.72[ab]	19.24±12.99[a]	57.99±9.78[a]
	15 minutes	17.49±3.66[a]	18.10±4.72[ab]	22.39±14.37[a]	40.25±1.84[ab]
	30 minutes	14.92±3.68[a]	38.3747±4.32[a]	8.56±4.00[a]	34.28±6.65[b]
20%A–Pb	Control	7.39±3.66[a]	6.5535±1.14[a]	4.65±0.58[a]	12.11±0.71[a]
	5 minutes	17.41±8.58[a]	16.4812±9.38[a]	12.32±7.92[a]	21.31±10.14[a]
	15 minutes	18.67±4.42[a]	11.5261±4.14[a]	9.90±5.39[a]	44.81±20.59[a]
	30 minutes	16.86±4.60[a]	4.1638±0.31[a]	17.43±9.80[a]	14.99±4.99[a]

Note: MAP = Month after planting; (–) = minus or without; (+) = plus or with; Pb = lead; Pr = pretreatment. Means in a column not followed by the same letter are significantly different at $p < 0.05$.

LEAD ACCUMULATION IN CASHEW

Accumulation of Pb content in cashew seedlings was reliant on the concentration of Pb existing in the Pb treated soil (Table 9). The Pb accumulation level was found to be higher in roots compared to the shoots. The level of Pb accumulation in roots and shoots showed positive linear relationships with the Pb concentration in the Pb treated soil. The maximum accumulation of Pb content found in roots and shoots were 6.04 mg/L and 0.62 mg/L respectively. The translocation factor value was found to be less than 1. Though, Pb was largely stored in plant roots exposed to 5000 ppm Pb treatment, only lesser amounts of Pb were translocated to aerial parts of the plants. However, the influence of pretreatment with acid was observed and it trends in term of time or duration that is, the Pb contents found in the roots reduces with the duration of pretreatment (30minutes < 15minutes < 5minutes). Also in the shoots, the trend observed was based on the amount of Pb content in the root. This result clearly indicate that the large amount of metal content was accumulated in roots, but only lower levels of Pb content was translocated into the shoots of cashew seedlings.

The variations observed in the physiological parameters (seed germination, plant height, number of leaves, stem girth, leaf area) may be linked to the level of Pb present in the root and shoot as there was delayed in seed germination in the plants grown in Pb treated soil compared to the control plants. Furthermore, it was observed that though the delay in germination, the plant height, number of leaves and stem girth of the plants grown in Pb treated soil is higher than the control which could be as a result of the Pb content in the soil. However, there was a decline in the leaf area of the plants grown in Pb treated compared to the control as the leaf gets dry, which could be as a result of the Pb concentration in the soil.

Table 9: Lead accumulation in the cashew seedlings

Treatment	Time	Root (mg/L)	Shoot (mg/L)
5%A+Pb 1000 ppm	5 minutes	0.82	0.15
	15 minutes	0.71	0.12
	30 minutes	0.53	0.10
5%A+Pb 3000 ppm	5 minutes	0.75	0.11
	15 minutes	0.51	0.10
	30 minutes	0.42	0.09
5%A+Pb 5000 ppm	5 minutes	6.04	0.44
	15 minutes	3.01	0.35
	30 minutes	1.43	0.20
5%A – Pb	5 minutes	0.10	0.01
	15 minutes	0.10	0.02
	30 minutes	0.10	0.01
+Pb – Pr	1000 ppm	0.20	0.10
	3000 ppm	0.21	0.10
	5000 ppm	0.20	0.10
10%A+Pb 1000 ppm	5 minutes	0.92	0.13
	15 minutes	0.75	0.11
	30 minutes	0.31	0.10
10%A+Pb 3000 ppm	5 minutes	1.62	0.31
	15 minutes	0.92	0.21
	30 minutes	0.20	0.10
10%A+Pb 5000 ppm	5 minutes	3.10	0.40

	15 minutes	1.10	0.32
	30 minutes	0.51	0.14
10%A–Pb	5 minutes	0.10	0.03
	15 minutes	0.10	0.02
	30 minutes	0.11	0.02
20%A+Pb 1000 ppm	5 minutes	0.30	0.10
	15 minutes	0.21	0.08
	30 minutes	0.13	0.07
20%A+Pb 3000 ppm	5 minutes	1.74	0.21
	15 minutes	1.31	0.17
	30 minutes	1.10	0.10
20%A+Pb 5000 ppm	5 minutes	1.22	0.62
	15 minutes	1.16	0.44
	30 minutes	1.10	0.35
20%A–Pb	5 minutes	0.10	0.02
	15 minutes	0.11	0.01
	30 minutes	0.10	0.01
– Pb – Pr (Control)		0.10	0.08

DISCUSSION

The percentage of germination may reflect the reaction rate of seeds to their living environment. In this experiment, it was observed that seedlings pretreated with 5%Acid concentration emerged earlier (13 – 18 days) than other treatments and even the control (–Pr–Pb) (14 – 20 days) which shows a positive influence on the seeds. This assertion cohort the works of Durrant and Mash (1991); Bockarie and Duryea (1993); Khazaei (2001); Duan *et al*. (2004). However, it was also observed that with the increase in concentration of acid i.e. 10% and 20%, there was an increment in the emergence days (16 – 23 days and 20 – 30 days respectively), this may be as a result of Pb treatment or the concentration of the acid has detrimental effects on the seeds to reduce its emergence. Inhibition of germination may result from the interference of lead with important enzymes like β-amylase and protease which results in decrease in seed germination (Verma and Dubey, 2003). Mukherji and Maitra (1976) observed 60 μM lead acetate inhibited protease and amylase by about 50% in rice endosperm. Further, decrease in the seed germination under heavy metal stress could be attributed to the accelerated breakdown of stored nutrients in seeds and alteration of selection permeability properties of cell membrane (Shafiq *et al*., 2008).

The effect of Pb was significantly observed as it delayed seed emergence (25 – 30 days) of cashew and has a depreciating effect on the germination percentage (40.00%). This research conforms with the works of Tantrey and Agnihotri (2010); Heidari and Sarani (2011); Gubrelay *et al*. (2013). Lead is known to inhibit seed germination of *Spartiana alterniflora* (Morzck and Funicclli, 1982), *Pinus helipensis* (Nakos, 1979). Early seedling growth was also inhibited by lead in soya bean (Huang *et al*., 1974), rice (Mukherji and Maitra, 1976), maize (Miller *et al*., 1975), barley (Stiborova *et al*., 1987), tomato, eggplant (Khan and Khan, 1983) and certain legumes (Sudhakar *et al*., 1992).

Also in this experiment, the increment of the plant height, number of leaves and stem girth was observed in all treated seedlings across the months. This assertion however, is contrary to reports by Tomar *et al*. (2000); Munzuroglu and Geckil (2002); Muranyi and Kodobocz (2008); and Rezvani and Zaefarian (2011) in which the reduction in plant growth by increasing Pb doses were observed. Ardakani *et al*. (2009) also found a reduction in barley (*Hordeum volgare* L.) growth, when grown in Pb contaminated soil. Sinha *et al*. (2006) have proposed that Pb was interfering with a factor directly involved in cell elongation suggesting the specific involvement of enzymes in the wall and ATPase associated with the plasmalemma. This could be that cashew been a woody plant has resistance to Pb at these levels or has a means of translocating Pb without its effect on the physiological parameters such as plant height and number of leaves. In contrary, the work of Kabir *et al*. (2009) reported reduction in number of leaves; reduced plant height; decrease in plant biomass of Portia tree (*Thespesia populnea*). The leaf area was although reducing as the plant height increase, this probably may be due to the fact that cashew translocated the heavy metals to the leaves which causes the dryness of leaves. This observation was in accordance to Gopal and Rizvi (2008) and Kabir *et al*. (2009) in the reduction of leaf area of radish and Portia tree respectively treated with different concentrations lead (Pb).

Lead (Pb) was taken up by *A. anacardium* seedlings grown in the experiment. Pb was detected in all the tissues examined (root and shoots). Pichtel *et. al*. (2000) reported that soil Pb was taken up in substantial quantities by herbaceous and woody species grown in contaminated sites. In this experiment, all seedlings contained higher root Pb compared to shoot Pb, indicating limited mobility once absorbed by the roots. Similar results were reported by Patra *et al*. (2004); Sinha *et al*. (2006) and Ghani (2010). In plants exposed to lead contaminated rooting media, the roots always contained lead concentrations considerably greater than other above ground tissues and that lead is bound to the roots where such binding serves to protect the remaining plant parts from injury (Koeppe, 1981). The movement or translocation of lead from absorbing roots or root hairs is apparently impeded by a number of biochemical and or physical processes involving lead binding, inactivation and or precipitation (Koeppe, 1981). Miller and Koeppe (1971) demonstrated that *Zea mays* L. plants translocated and accumulated significant quantities of Pb in the leaves in a concentrated dependent manner. Jarvis and Leung (2002) stated that the combination of chelating agents (such as H-EDTA or EDTA) and low pH prevented root cell wall retention of lead (Pb) making Pb available for translocation to shoots. Pb moves predominantly into the root apoplast and thereby in a radial manner across the cortex and accumulates near the endodermis. The endodermis acts as a partial barrier to the movement of Pb between the root and shoot. This may in part account for the reports of higher accumulation of Pb in roots compared to shoots (Jones *et al*., 1973; Verma and Dubey, 2003; Dubey and Sharma, 2005).

CONCLUSION

In this experiment, the results revealed that pretreating cashew seeds with sulphoric acid enhances its germination and aids absorption of Pb in relation to its concentration and pretreatment time interval as it showed higher root Pb compared to shoot Pb, indicating limited mobility once absorbed by the roots. In addition, the result also showed that cashew plants demonstrated a physiological response to lead (Pb) pollution, which suggests that cashew plants are indicators of heavy metals accumulation.

REFERENCES

Abdul Jaleel, C., Jayakumar, K., Chang-Xing, Z. and Iqbal, M. (2009). Low concentration of cobalt increases growth, biochemical constituents, mineral status and yield in *Zea mays*. *Journal of Scientific Research*, 1: 128-137.

Adeigbe, O. O., Olasupo, F. O., Adewale, B. D. and Muyiwa, A. A. (2015). A review on cashew research and production in

Nigeria in the last four decades. *Scientific Research and Essays*, 10(5):196-209.

Agnihotri, R. K., Imtiyaz, S., Ahmad, S. and Sharma, R. (2014). Effect of cobalt and lead induced heavy metal stress on some physiological parameters in *Glycine max*. *International Journal of Agriculture and Crop Sciences*, 7(1):26-34.

Aliyu, O. M. (2012). Genetic Diversity of Nigerian Cashew Germplasm. *Genetic Diversity in Plants*, pp. 163-184.

Baker, A. J. M., McGrath, S. P., Sidoli, C. M. D. and Reeves, R. D. (1994). The possibility of *in-situ* heavy metal decontamination of polluted soils using crops of metal-accumulating plants. *Resource Conservation and Recycling*, 11: 41-49.

Choudhary, S., Ansari, M., Khan, Z. and Gupta, H. (2012). Cytotoxic action of lead nitrate on cytomorphology of *Trigonella foenum-graecum* L. *Turkish Journal of Botany*, 36:267.

Curguz, V. G., Raicevic, V., Veselinovic, M., Tabakovic-Tosic, M. and Vilotic, D. (2012). Influence of heavy metals on seed germination and growth of *Picea abies* L. Karst. *Polish Journal of Environmental Studies*, 21(2): 355.

Deram, A., Petit, D., Robinson, B., Brooks, R. R., Gregg, P., and Van-Halluvyn, C. (2000). Natural and induced heavy-metal accumulation by *Arrhenatherum elatius*: implications for phytoremediation. *Communication in Soil Science and Plant Analysis*, 31: 413-421.

Ezeagu, W. (2002). Assessment of the situation and development prospects for the cashew nut sector. A report of the International Trade Center UNCTAD/WTO(ITC). pp. 6 – 28.

Godzik, B. (1993). Heavy metal contents in plants from zinc dumps and reference area. *Pol. Bot. Stud.*, 5: 113-132.

Gopal, R. and Rizvi, A. (2008). Excess lead alters growth, metabolism and translocation of certain nutrients in radish. *Chemosphere*, 70:1539.

Hammed, L. A., Anikwe, J. C. and Adedeji, A. R. (2008). Cashew nuts and production development in Nigeria. *American-Eurasian Journal of Scientific Research*, 3(1): 54-61.

Islam, E., Yang, X., Li, T., Liu, D., Jin, X. and Meng, F. (2007). Effects of Pb on root morphology, physiology and ultrastructure in the two ecotypes of *Elsholtzia argyi*. *Journal of Hazardous Materials*, 147:806.

Jayakumar, K., Vijayarengan, P., Chang-Xing, Z. and Abdul Jaleel, C. (2008a). Soil applied cobalt alters the nodulation, leg-haemoglobin content and antioxidant status of *Glycine max* (L.) Merr. *Colloids and Surfaces B: Biointerfaces*, 67(2): 272-275.

Kabata-Pendias, A., and Pendias, H. (2001). Trace Elements in Soils and Plants (3rd edition). *CRC Press*, Boca Raton, FL.

Kaur, G., Singh, H., Batish, D. and Kohli, R. (2012). Lead (Pb)-induced biochemical and ultrastructural changes in wheat (*Triticum aestivum*) roots. *Protoplasma*, 21:255.

Kumar, N., Dushenkov, V., Motto, H. and Raskin, I. (1995). Phytoextraction: the use of plants to remove heavy metals from soils. *International Journal of Environmental Science and Technology*, 29:1232-1238.

Laide, D. R. (2004). *CRC Handbook of Chemistry and Physics* (84th ed.). CRC Press, Boca Raton. pp. 12-220.

Mitchell, J. D. and Mori, S. A. (1987). The cashew and its relatives (*Anacardium*, Anacardiaceae). *Memoirs of New York Botanical Gardens English, New York*, 42: 1–76.

Nakasone, H. Y. and Paull, R. E. (1998). Tropical Fruits. CAB International, Oxford, UK. 445p.

Nedelkoska, T. V. and Doran, P. M. (2000). Characteristics of heavy metal uptake by plants species with potential for phytoremediation and phytomining. *Minerals Engineering*, 13:549–561.

Ohler, J. G. (1979). *Cashew*. Royal Tropical Institute, Tropen, Amsterdam. p.260.

Patra, M., Bhowmik, N., Bandopadhyay, B. and Sharma, A. (2004). Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. *Environmental and Experimental Botany*, 52:199 – 223.

Samal, S., Rout, G. R. and Lenka, P. C. (2003). Analysis of genetic relationships between populations of cashew by using morphological characterization and RAPD markers. *Plant, Soil and Environment*, 49(4): 176-182.

Sanwo, J.O.; Kuti, B. O. and Osundolire, M. (1972). Cashew Germplasm collections. *The Annual Report of Cocoa Research Institute of Nigeria*, pp. 100-110.

Sidari, M., Mallamaci, C. and Muscolo, A. (2008). Drought, salinity and heat differentially affect seed germination of *Pinus pinea*. *Journal of Forest Research*, 13:326.

Tangahu, B. V., Abdullah, S. R., Basri, H., Idris, M., Anuar, N. and Mukhlisin, M. (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering*, 20(11): 1-31.

Thayer, K. A., Heindel, J. J. and Gallo, M. A. (2012). Role of environmental chemicals in diabetes and obesity: A national toxicology program workshop review. *Environmental Health Perspectives, 120*(6): 779-789.

Togun, A. (1977). A review of the prospect of cashew in Ibadan (Southwestern Nigeria). *Agricultural Industry*, p. 39.

Ventakaramah, T.M., (1976). Cashew nut production and processing: Nigeria agronomic aspect of cashew nut production. *Cocoa Research Institute of Nigeria*, p. 39.

Webber, J. (1981). Trace metals in agriculture. In: Lepp NW, editor. Effect of heavy metal pollution on plants: Metals in the environment. *American Journal of Applied* Sciences, 11:15-184.

Woodroof, J. G. (1967). *Tree nuts: Production, Processing and Products*. Volume 1. Avi Publishing Company Incorporation, U.K.

Zeller, S. and Feller, U. (1999). Long-distance transport of cobalt and nickel in maturing wheat. *European Journal of Agronomy*, 10: 91–98.