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OPTIMIZATION OF MECHANICAL PROPERTIES OF HYBRID BIOCOMPOSITE FROM STERCULIA SETIGERA DELILE FIBRE AND PTEROCARPUS ERINACEUS WOOD DUST EPOXY

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Abstract

Synthetic fibres have been used for the production of high strength composite material of low density for various aerospace and automotive applications. The problem with synthetic fibres includes high energy consumption during processing, high cost, non-biodegradability and environmental pollution during production and disposal. Natural fibre reinforced composites have been explored as potential replacement for synthetic fibres. The research explores the use of wood fibre reinforcement such as *Sterculia Setigera delile* fibre (SSD) sourced from a durable inner tree bark and *Pterocarpus erinaceus* (PTE) wood dust from high quality wood for the production of a hybrid composite material with epoxy as the matrix. The aim is to produce a novel hybrid material with high tensile, flexural and compressive strength, low density and high thermal stability. Taguchi method was used for the design of experiment and the optimization of factors affecting the tensile, flexural and compression strength of the composite. The factors include alkaline treatment (hot, cold and untreated), SSD fibre content (2.5, 5, 7.5, 10, 12.5) wt.%, *Pterocarpus erinacues* wood dust content (0, 2.5, 5, 7.5, 10) wt.% and fibre angular orientation (0, 15, 30, 45, 90°). The result showed that the optimum composite parameters consist of 5% cold alkaline treated 5% SSD fibre with 7.5% PTE wood dust at 0-degree orientation. The thermal stability of the composite was also improved by the addition of the reinforcements. Factors such as fibre angular orientation and alkaline treatment were significant factors. The optimized composite improved the tensile strength of the composite when compared to the epoxy matrix by 105.9% and also improved the flexural strength by 94.91%. The optimized composite has a lower density (1.093g/cm^3) when compared to carbon-epoxy composite (1.6g/cm^3) and S-glass fibre epoxy composite (1.9g/cm^3). The optimized composite would have better biodegradability, lighter weight means lower energy consumption and cost when used in aerospace and automobile components.

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OPTIMIZATION OF MECHANICAL PROPERTIES OF HYBRID BIOCOMPOSITE FROM STERCULIA SETIGERA DELILE FIBRE AND PTEROCARPUS ERINACEUS WOOD DUST EPOXY

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1. Introduction

Natural fibres are a renewable source of reinforcements that are used to improve the mechanical and thermal properties of matrices. This type of fibre is strong, low density, inexpensive and renewable. natural fibres are materials that are currently used as possible replacement for synthetic fibres. The automotive and aircraft industry has been developing interior components using mainly, hemp fibre, flax, sisal fibre and bioresin systems. The high specific properties and low cost make them attractive for various applications (Sanjay et al., 2016). The reinforcements are explored as potential replacement for synthetic fibres. Synthetic fibre production leads to high energy consumption and environmental pollution. It is known to have high cost, hazardous to workers processing it and non-biodegradability. This research involves the investigation of the effect of hybridization of *Sterculia setigera delile* fibre and *Pterocarpus erinaceus* wood dust epoxy composite. *Sterculia setigera delile* is a savannah tree widespread in tropical Africa and is known by its English name Karaya gum tree. It is a hardwood species and is known in Nigeria with local names such as Hausa-Kukuki, and Fulani- Bo'boli. The tree is found in open savannah woodland often characterized with rocky hills (Adelakun et al., 2014). The tree is used for medicinal purposes. It is used by the Fulani's as a saddle on their cows for load carrying and it is known to have good durability which is the reason it is explored as a potential composite reinforcement. *Pterocarpus erinaceus* is a highly-priced wood used for furniture's and belongs to the Fabaceae family. It is known to have one of the best mechanical properties and durability in Africa. It is known with many names such as African Rosewood, African Kino and Senegal Rosewood. It has been over-exploited for its wood by China and India. *Pterocarpus erinaceus* is classified as medium-long and medium fibre hardwood. It is suitable for paper and pulp production. The mechanical test showed that it provided high mechanical properties (Anthonio and Antwi-Boasiako, 2017). *Pterocarpus erinaceus* wood dust could be used for the reinforcement of epoxy matrix. The mechanical properties of *Pterocarpus erinaceus* would be explored and could be combined with other reinforcement mentioned to make composites. It would be an effective use of wood dust. The research involves the hybridization of *Sterculia setigera delile* fibre and *Pterocarpus erinaceus* wood dust and epoxy matrix. Hybridization is known to improve the mechanical properties of composite materials (Hanan et al., 2018; Mehra et al., 2021). This research is focused on the fabrication and optimization of the mechanical properties of hybrid fibre epoxy composite. The tensile, flexural and compressive strength of the composite was optimized using the Taguchi method. Taguchi method was used for the design of experiment and was analyzed using Analysis of Variance (ANOVA). The experiments were carried out to understand the relationship among controllable parameters and identify the significant parameters that influence the properties of the composite. In this research, a novel bio-composite material of low density and good mechanical properties was produced as a potential replacement for synthetic fibres. Its material properties can be used by researchers for simulating components in the aerospace, automotive industry and also in the furniture industry as a potential use of wood dust.

2. Materials and Methods

Taguchi method was used for the design of experiment using Minitab software. The factors considered in the design of experiment include alkaline treatment, SSD fibre Content, *PTE* filler content and fibre angular orientation. The hand lay-up process was used for the fabrication of the composites. Materials used for the manufacturing of the composite include epoxy from EPOCHEM with a density of 1.13 g/cm^3 and hardener from EPOCHEM with a density of 1.04 g/cm^3 purchased from Epoxy Olisev Nigeria. *Sterculia setigera delile* wood bark was obtained from the tree, subjected to drying for 11 days and retting process for 11 days before manual extraction. The wood dust from *Pterocarpus erinacues* was obtained from the wood mill and a sieve of 425 μm was used to attain fine wood dust. The untreated fibre has a density of 1.1924 g/cm^3, hot alkaline treated fibre 1.184 g/cm^3, cold Alkaline treated fibre, 1g/cm^3. The untreated, hot alkaline treated and cold alkaline treated *PTE* wood dust have densities of 0.8, 0.74, 0.75g/cm^3 respectively. The dried fibres were placed on a paper with different fibre angular orientations. The fibres were then placed inside the mould. A mixture of epoxy, matrix and wood dust were thoroughly mixed and spread inside the mould and was subjected to compression using a manual press of a pressure of 23.29 MPa. For the hot alkaline treated fibre, the fibres are subjected to 5% hot alkaline treatment at 95^{o}C for 1 hour. The 5% cold alkaline treatment was conducted for 1 hour by putting the fibres in the alkaline solution for 1 hour. The factors and levels are shown in Table 2. The structure in which the experiments were performed is shown in Table 3. Tensile, flexural and compression test were performed using ASTM standard ASTM D3039, ASTM D790 and ASTM D695. The results were then recorded. The results were transformed into Signal to Noise ratio.

For the larger the better, the formula is shown in equation:

$$S/N = -10 * log(\sum(1/y^2)/n \tag{1}$$

Where y is the experimental result for the given factor level combination and n is the number of responses and n is the number of responses in factor level combinations (Trehan et al., 2013).

Table 1: Design Summary showing the type of orthogonal array, number of factors and number of experiments

Design Summary	
Taguchi Array	L25 (5^4)
Factors	4
Runs	25

Table 2: Design of Experiment data for the Factors affecting the Hybrid Composite Performance and the Levels

No	Contributing Factor	Level 1	Level 2	Level 3	Level 4	Level 5
1	Alkaline treatment	Without	5% Hot Alkaline treated reinforcements	5% Cold Alkaline treated reinforcement	Without	5% Hot Alkaline treated reinforcements
2	SSD Fibre Content (%)	2.5	5	7.5	10	12.5
3	*Pterocarpus erinaceus* filler content (%)	0	2.5	5	7.5	10
4	Fibre Angular Orientation (Degrees)	0	15	30	45	90

Table 3: Orthogonal Table Designed using Taguchi method and Minitab software

Alkaline Treatment (NaOH)	SSD Fibre Content (wt.%)	PTE Filler Content (wt.%)	Fibre Orientation (Degrees)
Without	2.5	0.0	0
Without	5.0	2.5	15
Without	7.5	5.0	30
Without	10.0	7.5	45
Without	12.5	10.0	90
Hot	2.5	2.5	30
Hot	5.0	5.0	45
Hot	7.5	7.5	90
Hot	10.0	10.0	0
Hot	12.5	0.0	15
Cold	2.5	5.0	90
Cold	5.0	7.5	0
Cold	7.5	10.0	15
Cold	10.0	0.0	30
Cold	12.5	2.5	45
Without	2.5	7.5	15
Without	5.0	10.0	30
Without	7.5	0.0	45
Without	10.0	2.5	90
Without	12.5	5.0	0
Hot	2.5	10.0	45
Hot	5.0	0.0	90
Hot	7.5	2.5	0
Hot	10.0	5.0	15
Hot	12.5	7.5	30

Figure 1: Fibre placed at 30˚ angular orientation

Figure 2: SSD fibre/PTE wood dust epoxy composite fibre placed at 0˚ angular orientation

3. Results and Discussion

3.1. Tensile Strength

The tensile test is based on ASTM D3039 (Standard Test Method of Polymer Matrix Composite). For the tensile strength the result in Table 4 shows that the hot alkaline treated 10% fibre with 10% *PTE* wood dust at 0-degree orientation has a tensile strength of 25.82 MPa and a Signal to Noise 28.10, cold alkaline treated 5% fibre and 7.5% PTE wood dust at 0-degree orientation has a tensile strength of 26.90 MPa and Signal to Noise of 28.51 and finally the hot alkaline treated 7.5% fibre and 2.5% *PTE* wood dust at 0-degree has a tensile strength of 28.46 and Signal to Noise ratio of 29.00. The result from Table 4 has clearly shown that alkaline treatment and fibre orientation of 0-degree has a significant impact on the tensile strength of the composite. The ranking table has shown that the fibre orientation has the highest effect on the tensile strength with a delta of 12.21, this is followed by the SSD fibre content of 2.71 and then the alkaline treatment of 2.31. Analysis of variance is a statistical tool used by researchers to know the extent factors influence to the outcome of the experiments and interactions, confidence value and test of significance. The p-value suggests the significance of a factor on desired characteristic. The principle behind significance value is that the p-value should not be less than 0.05 (considering confidence of 95%). The larger the F-value the more significant a factor is. Table 5 shows that fibre orientation has a p-value of 0.000 indicating it as the most significant factor. The p-value 0.000(fibre orientation), 0.239(alkaline treatment), 0.533(SSD fibre content) and *PTE* fibre content shows the significance of these factors on the tensile strength with fibre orientation being the highest at 95% confidence interval. The F-value shows that the fibre orientation has a F-value of 15.62 on the tensile strength followed by the alkaline treatment of 1.65, *SSD* fibre content 0.84 and *PTE* wood dust 0.31 (Trehan et al., 2013; Roy, 2010). The tensile strength of the epoxy was 13.82 MPa. The hybrid composite reinforcement's addition increased the tensile strength of the epoxy by 105.9%. Literatures have shown that fibre orientation is significant in improving the tensile strength of composite. The specimens fail by tensile rupture of fibre followed by debonding along the fibre matrix interface (Mallick, 2007). Fibres oriented at one direction give high stiffness and strength in that direction (Kaw, 2006). Parallel alignment of fibres to the direction of load application improves the mechanical properties of the composite (Varadaraju & Srinivasan, 2019). When fibre is arranged in the direction of the load, the load is transferred to fibre which resists the load thereby improving the composite resistance to failure and the mechanical properties of the composite. Lasikun et al. (2018) researched on the effect of fibre orientation on the tensile and impact properties of zalacca midrib fiber /HDPE composites. Fibres arranged at 0, 15, 30, 45, 60, 75 and 90˚ to produce composites. The tensile and impact strength result showed a decline in strength from 0˚ to 90˚ with 0˚ being the highest tensile and impact strength. Hossain et al. (2013) studied the effect of fibre orientation on tensile strength of Jute-Epoxy composite the result showed that jute fibre arranged at 0 degree orientation has the highest tensile strength. Similar result was observed by Chanamala et al. (2019) that studied the effect of fiber orientation on dynamic mechanical properties of PALF hybridized with basalt reinforced epoxy composites. Ammar et al. (2019) also obtained similar result investigating the effect of Sugar Palm Fibre Reinforced Vinyl Ester Composites at Different Fibre Arrangements on the Mechanical Properties of the composite.

The reason for improvement of tensile properties as a result of Alkaline treatment is that Alkaline treatment removes natural fats, wax, surface debris, pectin, lignin and other impurities of the surface resulting to rougher surface of the fibre thus revealing chemically reactive functional groups like hydroxyl groups and other reactive functional groups on the surface. Sodium hydroxide also reacts with accessible –OH groups reducing the fibres affinity to moisture absorption. The surface of the fibre becoming rough increases the surface area of available interaction with the resin. Good bonding between the fibre and resin improves the load carrying capacity of the composite giving better mechanical properties (Kamath & Bennehalli, 2021; Benyahia et al., 2013).

Table 4: Tensile Strength Result for the hybrid composite with *SSD* fibre and *PTE* filler reinforcement

Alkaline Treatment (NaOH)	SSD Fibre Content (wt.%)	PTE Filler Content (wt.%)	Fibre Orientation (Deg.)	Tensile Strength (MPa)					S/N Ratio
				1	2	3	4	Mean	
Without	2.5	0.0	0	26.92	18.71	25.04	15.47	21.54	26.02
Without	5.0	2.5	15	19.66	11.19	17.70	8.98	14.38	21.84
Without	7.5	5.0	30	7.30	10.55	12.20	12.12	10.54	19.86
Without	10.0	7.5	45	7.21	6.99	7.58	11.10	8.22	17.88
Without	12.5	10.0	90	2.29	2.66	4.21	2.94	3.03	9.00
Hot	2.5	2.5	30	12.38	10.96	9.52	12.38	11.31	20.91
Hot	5.0	5.0	45	12.41	9.68	14.64	7.426	11.04	20.00
Hot	7.5	7.5	90	7.12	6.83	4.64	8.16	6.69	15.91
Hot	**10.0**	**10.0**	**0**	**22.15**	**29.52**	**25.21**	**26.38**	**25.82**	**28.10**
Hot	12.5	0.0	15	11.74	17.51	17.95	25.51	18.18	24.21
Cold	2.5	5.0	90	10.29	9.57	9.46	4.46	8.445	16.89
Cold	**5.0**	**7.5**	**0**	**26.01**	**23.76**	**28.50**	**29.33**	**26.90**	**28.51**
Cold	7.5	10.0	15	25.7	29.70	26.28	22.56	26.06	28.20
Cold	10.0	0.0	30	10.96	10.05	16.67	15.05	13.18	21.83
Cold	12.5	2.5	45	10.02	9.88	5.26	9.19	8.59	17.68
Without	2.5	7.5	15	18.12	18.69	22.16	18.64	19.40	25.68
Without	5.0	10.0	30	25.97	15.54	26.43	19.64	21.90	26.18
Without	7.5	0.0	45	6.87	7.57	8.89	10.48	8.45	18.21
Without	10.0	2.5	90	5.70	7.39	9.79	15.25	9.53	17.97
Without	12.5	5.0	0	23.73	16.77	14.41	20.92	18.96	25.08
Hot	2.5	10.0	45	10.78	17.29	15.23	19.02	15.58	23.23
Hot	5.0	0.0	90	5.43	5.89	6.64	7.62	6.40	15.91
Hot	**7.5**	**2.5**	**0**	**31.48**	**29.44**	**25.55**	**27.36**	**28.46**	**29.00**
Hot	10.0	5.0	15	32.39	20.92	24.34	23.54	25.30	27.74
Hot	12.5	7.5	30	15.23	21.01	24.04	11.52	17.95	24.02
Epoxy				11.98	11.92	15.44	16.64	13.99	22.63

Table 5: Rankings of factors affecting Tensile Strength of Hybrid-Biocomposite using Signal to Noise ratio (Larger is better)

Level	Alkaline treatment	*SSD* Fibre Content (Wt.%)	*PTE* Filler Content (Wt.%)	Fibre Orientation (Deg.)
1	20.77	22.55	21.24	**27.34**
2	22.90	22.49	21.48	25.53
3	22.62	22.24	21.92	22.56
4		22.70	22.40	19.40
5		20.00	22.94	15.13
Delta	2.13	2.71	1.70	12.21
Rank	3	2	4	1

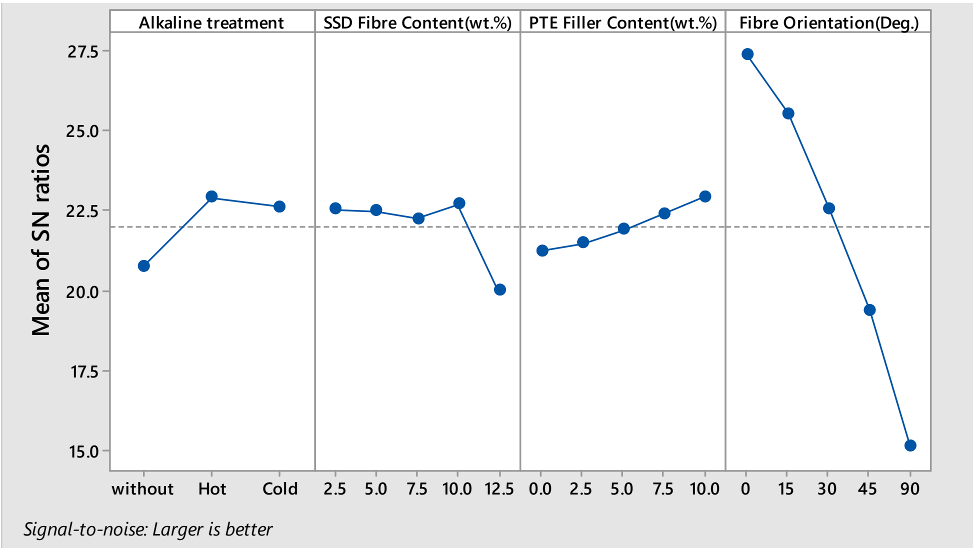

Figure 3: main effect plot for signal to noise ratio of the tensile strength of the hybrid-biocomposite

The Table 5 shows the mean effect of each parameter on the tensile strength of the hybrid-biocomposite. The result for each level of a factor was generated from the average mean effect from each level. For example, for the 0-degree fibre orientation the values were obtained from Table 4 from the signal to Noise ratio column for all values that have 0° orientation. The values used from S/N ratio table of the 0-degree orientations are 26.02, 28.10, 28.51, 25.08 and 29.00. The mean effect of the 0-degree orientation was found by finding the average of the mentioned S/N ratio values. The average value was 27.34 as highlighted in Table 5. The Main Effect plot effect for Signal to Noise Ratio of the tensile strength of the hybrid biocomposite is shown in Figure 3. The main effect plot suggests optimum factors and parameters that would give optimum performance (Roy, 2010).

Prediction of optimum tensile strength for hybrid composite

The optimum composite suggested by the main effect plot suggests that hot alkaline treated fibre with 10% SSD fibre content and 10% wood dust with 0-degree orientation would give the optimum result. In order to predict the optimum tensile strength, the ANOVA table was used to identify the significant factor which in this case is fibre orientation. The highest value of the factor was 27.34. The average value of the S/N ratio was also calculated. The two values were used in order to predict the tensile strength.

The prediction was calculated as follows:

$$EV=AVR+ (A_{opt} - AVR) + (B_{opt} - AVR) + (C_{opt} - AVR) + (D_{opt} - AVR) +.....(nth_{opt} - AVR) \quad (2)$$

$$EV = 21.9944 + (27.34 - 21.9944) = 27.34$$

The value obtained was inserted into equation 1 to attain the tensile strength of 23.28 MPa. The predicted value is y = 23.28 MPa while the experimental value was 25.82 MPa.

Where EV = Expected Response, AVR = Average Response, A_{opt} = mean value of response at optimum setting at factor A, B_{opt} = mean value of response at optimum setting at factor B, C_{opt} = mean value of response at optimum setting at factor C (Okafor et al., 2013; Trehan et al., 2013).

Table 6: Analysis of Variance of the Tensile Strength for the Hybrid Biocomposite

Source	DF	Adj SS	Adj MS	Significance	
				F-Value	P-Value
Alkaline treatment	2	25.219	12.609	1.65	0.239
SSD Fibre Content (wt.%)	4	25.463	6.366	0.84	0.533
PTE Filler Content (wt.%)	4	9.490	2.372	0.31	0.864
Fibre Orientation (Deg.)	4	476.053	119.013	15.62	0.000
Error	10	76.198	7.620		
Total	24	612.422			

The P-value shows the significance of the parameter at 95% Confidence level, a P-value of 0.05 or less shows that a factor is highly significant on the performance of the composite and the F-value shows the level significance of each factor. The higher the F-Value the higher the significance of the factor when compared to other factors.

3.2. Flexural Strength

The flexural test was based on ASTM D790, the standard test method for flexural properties of unreinforced and reinforced plastic. For the flexural strength of the hybrid composite, Table 7 has shown that the cold alkaline treated fibre with 5% *SSD* fibre and 7.5% *PTE* filler content with 0-degree fibre orientation has the maximum flexural strength of 77.39 MPa with an S/N Ratio of 37.40. The table has also shown that hot and cold alkaline treatment improved the flexural strength of the hybrid composite and the angle of orientation was the key to improved performance of the composite. Ranking Table 8 has shown that for the S/N ratio fibre orientation has the highest effect on the flexural strength (3.92) followed by alkaline treatment (1.90), *PTE* wood dust (1.27) and *SSD* fibre (0.95). The fibre orientation has a p-value of 0.000 and alkaline treatment with a value of 0.006. The larger the F-value the more significant a factor is, fibre orientation has an F-value of 17.52, followed by alkaline treatment (8.81), *PTE* filler (0.281) and *SSD* fibre (0.352) (Roy, 2010). The fibre orientation resulted in improved resistance against flexural loading loaded in its direction and the alkaline treatment removed impurities and oils from the natural fibre improving the bonding between the fibre and the matrix thereby improving the flexural strength.

Prediction of flexural strength for hybrid composite

The main effect plot for flexural strength of hybrid composite suggested that cold alkaline treated fibre with 5% *SSD* fibre and 7.5% *PTE* wood dust at 0-degree orientation would give the optimum result. The prediction was calculated as follows:

$$EV = 33.0816 + (34.01 - 33.0816) + (34.98 - 33.0816) = 35.9084$$

The value obtained was inserted in equation 1 in order to attain the predicted optimum flexural strength of 62.43 MPa. Predicted y = 62.43 MPa, Experimental result, y=77.38 MPa.

Table 7: Flexural Strength Result for the hybrid composite with *SSD* fibre and *PTE* filler reinforcement with Epoxy Matrix

Alkaline Treatment (NaOH)	*SSD* Fibre Content (wt.%)	*PTE* Filler Content (wt.%)	Fibre Orientation (Deg.)	Flexural Strength (MPa)					S/N
				1	2	3	4	Mean	
Without	2.5	0.0	0	32.76	57.11	63.06	72.49	56.36	33.77
Without	5.0	2.5	15	44.56	60.45	53.07	52.69	52.69	34.28
Without	7.5	5.0	30	45.44	45.75	39.10	45.08	43.84	32.78
Without	10.0	7.5	45	35.17	33.77	27.76	46.46	35.79	30.65
Without	12.5	10.0	90	41.48	25.64	30.80	45.46	35.85	30.41
Hot	2.5	2.5	30	47.80	42.31	40.44	42.49	43.26	32.67
Hot	5.0	5.0	45	34.90	42.60	43.25	39.86	40.15	31.97
Hot	7.5	7.5	90	54.85	37.10	40.69	32.79	41.36	31.88
Hot	10.0	10.0	0	61.24	51.10	69.64	57.35	59.83	35.38
Hot	12.5	0.0	15	69.04	45.79	59.84	43.37	54.51	34.27
Cold	2.5	5.0	90	47.43	44.65	46.71	40.63	44.86	32.99
Cold	**5.0**	**7.5**	**0**	**76.43**	**64.12**	**68.45**	**100.5**	**77.38**	**37.40**
Cold	7.5	10.0	15	38.26	43.95	64.99	67.99	53.80	33.84
Cold	10.0	0.0	30	45.68	46.67	44.22	60.49	49.27	33.66
Cold	12.5	2.5	45	48.77	52.15	32.36	38.50	42.95	32.19
Without	2.5	7.5	15	67.91	50.48	57.38	60.84	59.15	35.29
Without	5.0	10.0	30	54.29	48.11	37.67	43.94	46.00	33.02
Without	7.5	0.0	45	37.68	20.53	33.83	41.67	33.43	29.46
Without	10.0	2.5	90	27.90	24.67	33.39	23.98	27.49	28.57
Without	12.5	5.0	0	57.76	37.42	69.83	34.82	49.96	32.92
Hot	2.5	10.0	45	42.25	50.81	47.45	43.62	46.03	33.19
Hot	5.0	0.0	90	64.22	55.87	36.82	25.15	45.52	31.42
Hot	7.5	2.5	0	72.70	56.98	49.67	63.78	60.78	35.42
Hot	10.0	5.0	15	50.30	69.43	84.98	59.36	66.02	35.91
Hot	12.5	7.5	30	51.74	40.70	50.62	54.32	49.35	33.70
Epoxy				38.53	44.14	28.97	47.15	39.70	31.50

Table 8: Rankings of factors that influences Flexural Strength of Hybrid-Biocomposite using Signal to Noise ratio (Larger is better)

Level	Alkaline treatment (NaOH)	*SSD* Fibre Content (wt. %)	*PTE* Filler Content (wt. %)	Fibre Orientation(Deg.)
1	32.11	33.58	32.52	34.98
2	33.58	33.62	32.63	34.72
3	34.01	32.68	33.32	33.17
4		32.83	33.79	31.49
5		32.70	33.17	31.05
Delta	1.90	0.95	1.27	3.92
Rank	2	4	3	1

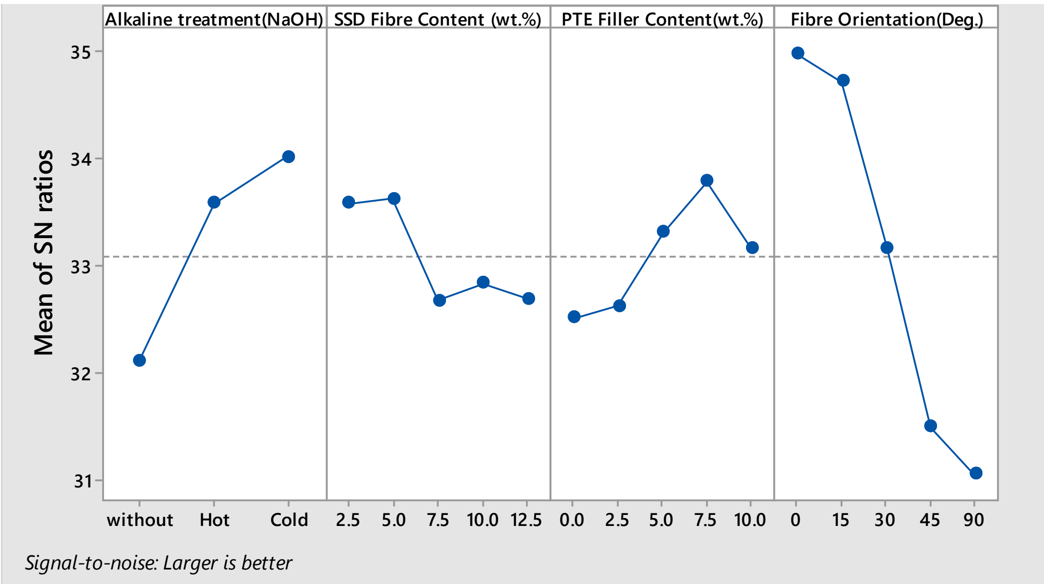

Figure 4: Main effect plot for the signal to noise ratio of factors affecting the flexural strength of the hybrid composite

Table 9: Analysis of Variance of Signal to Noise ratio of hybrid composite

Source	DF	Adj SS	Adj MS	Significant	
				F-Value	P-Value
Alkaline treatment (NaOH)	2	16.218	8.1091	8.81	0.006
SSD Fibre Content (wt. %)	4	4.595	1.1488	1.25	0.352
PTE Filler Content (wt. %)	4	5.427	1.3568	1.47	0.281
Fibre Orientation (Deg.)	4	64.536	16.1341	17.52	0.000
Error	10	9.207	0.9207		
Total	24	99.984			

The Analysis of Variance Table shows that the fibre orientation with a p-value of 0.000 and alkaline treatment with a p value of 0.006 are the most significant factors.

3.3. Compression Strength

The Compressive test was conducted based on ASTM D695. The compressive strength values in Table 10 for the hybrid composite has shown that the cold alkaline treated fibre with 5% *SSD* fibre and 7.5% *PTE* wood dust at a 0˚ orientation had a mean value of 51.39 MPa with an S/N Ratio 34.12. The hot alkaline treated fibre with 10% *SSD* fibre 5% *PTE* wood dust at 15º angular orientation had a mean value of 48.09 MPa with an S/N ratio of 33.40. The ranking Table 11 for the S/N ratio of hybrid composite shows that fibre orientation led to the highest change in the mean effect of compressive strength (2.28), followed by *SSD* fibre (2.10), *PTE* filler content (1.78) and then alkaline treatment. The analysis of variance has shown that all the factors are significant to the compressive strength. The fibre orientation had a p-value of 0.041, *SSD* fibre content (0.044). The two results were at 95% confidence interval. Alkaline treatment has a p-value of 0.070 and the *PTE* wood dust (0.084). For the compressive strength, fibre content, alkaline treatment and *PTE* wood dust were essential in resisting the compressive forces. The main plot graph suggests that the Hot Alkaline treated fibre at 5% SSD fibre and 5% wood dust at the 0º orientation would give the optimum result. The predicted response result found was 52.92 MPa while the confirmation/experimental test had a mean value of 44.62 MPa. The Epoxy matrix had a compressive strength of 53.31 MPa. The compressive strength of the composite was based on ASTM D695-15, the standard test method for compressive properties of rigid plastics.

Table 10: Compressive Strength of hybrid composite with *SSD* fibre and *PTE* filler reinforcement with Epoxy Matrix

Alkaline Treatment (NaOH)	*SSD* Fibre Content (wt.%)	*PTE* Filler Content (wt.%)	Fibre Orientation (Deg.)	Compressive Strength (MPa)					S/N Ratio
				1	2	3	4	Mean	
Without	2.5	0.0	0	55.61	45.81	50.05	40.74	48.05	33.46
Without	5.0	2.5	15	39.76	38.83	37.92	38.50	38.75	31.76
Without	7.5	5.0	30	42.63	33.40	43.08	55.71	43.71	32.39
Without	10.0	7.5	45	36.56	34.84	37.35	39.67	37.10	31.36
Without	12.5	10.0	90	40.53	29.10	43.27	28.55	35.36	30.52
Hot	2.5	2.5	30	35.42	40.67	29.70	47.20	38.25	31.27
Hot	5.0	5.0	45	55.53	49.07	45.53	41.69	47.96	33.47
Hot	7.5	7.5	90	48.39	55.93	46.21	39.54	47.52	33.34
Hot	10.0	10.0	0	43.81	44.54	42.31	44.55	43.80	32.82
Hot	12.5	0.0	15	60.90	48.88	42.53	38.36	47.67	33.19
Cold	2.5	5.0	90	53.37	46.59	47.70	45.73	48.35	33.64
Cold	**5.0**	**7.5**	**0**	**55.85**	**59.29**	**50.25**	**47.59**	**53.25**	**34.43**
Cold	7.5	10.0	15	40.44	47.30	49.76	40.32	44.46	32.85
Cold	10.0	0.0	30	25.20	29.29	24.53	24.42	25.86	28.18
Cold	12.5	2.5	45	29.56	31.41	32.23	23.76	29.24	29.13
Without	2.5	7.5	15	51.94	46.04	52.92	37.32	47.06	33.19
Without	5.0	10.0	30	50.35	46.90	45.76	49.34	48.09	33.62
Without	7.5	0.0	45	20.64	30.79	38.95	23.57	28.49	28.34
Without	10.0	2.5	90	32.66	35.05	25.27	36.89	32.47	29.94
Without	12.5	5.0	0	38.10	51.46	41.61	34.34	41.38	32.05
Hot	2.5	10.0	45	46.79	45.42	43.71	47.13	45.76	33.20
Hot	5.0	0.0	90	50.57	42.40	44.47	40.88	44.58	32.90
Hot	7.5	2.5	0	57.73	47.48	53.28	47.05	51.39	34.12
Hot	10.0	5.0	15	47.63	42.45	42.60	59.66	48.09	33.40
Hot	12.5	7.5	30	53.96	29.73	30.77	42.12	39.14	31.11
Epoxy				**53.25**	**59.46**	**44.85**	**55.53**	**53.27**	**34.39**

Table 11: Rankings of factors that influence Compressive strength of the Hybrid composite using Signal to Noise ratio (Larger is better)

Level	Alkaline treatment	SSD Fibre Content (Wt.%)	PTE Filler Content (Wt.%)	Fibre Orientation (Deg.)
1	31.66	32.95	31.21	33.38
2	32.88	33.24	31.25	32.88
3	31.64	32.21	32.99	31.32
4		31.14	32.69	31.10
5		31.20	32.60	32.07
Delta	1.24	2.10	1.78	2.28
Rank	4	2	3	1

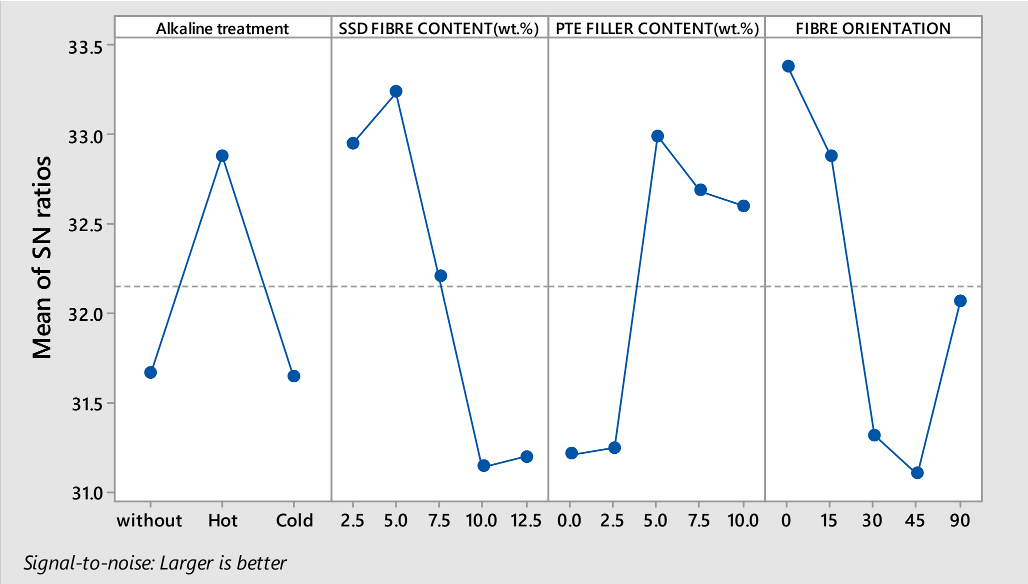

Figure 5: Main Effect plot for S/N ratio for the compressive strength of Hybrid composite

Table 11 shows that fibre orientation has the highest effect on the compressive strength of the hybrid composite. This is observed through its ranking. The main effect plot for the signal to noise ratio of the compressive strength of the hybrid biocomposite shown in Figure 5 suggests that hot alkaline treated fibre, with 5% reinforcement, 5% *PTE* wood dust and 0-degree orientation would give the optimized performance.

Table 12: Analysis of Variance for the compressive strength of Hybrid composite

Source	DF	Adj SS	Adj MS	Significance	
				F-Value	P-Value
Alkaline treatment	2	9.010	4.505	3.50	0.070
SSD Fibre Content (wt.%)	4	18.753	4.688	3.65	0.044
PTE Filler Content (wt.%)	4	14.472	3.618	2.81	0.084
Fibre Orientation	4	19.228	4.807	3.74	0.041
Error	10	12.856	1.286		
Total	24	74.318			

The ANOVA table shows that fibre orientation is still the most significant factor with a p-value of 0.041 followed by SSD fibre content with a p-value of 0.044. The alkaline treatment with a p-value (0.070) and *PTE* filler content (0.084) still affects the compressive strength.

Prediction of optimum compressive strength of hybrid composite

The predicted value of y=52.92 MPa was obtained for the compression strength and a confirmation test was performed in which an experimental value of 44.62 MPa was achieved. The result for the confirmation experiment was for the Hot Alkaline Treated 5% *SSD* fibre and 5% *PTE* Wood 0 degree orientation. This is lower than some of the results achieved in Table 10.

Table 13: Compression Strength of Hot Alkaline Treated 5% SSD fibre and 5% PTE Wood 0 Degree orientation

Force (N)	b (mm)	t (mm)	Compression Strength (MPa)
5719	12.94	9.16	48.24
5130	12.66	7.76	52.20
7182	12.90	11.54	48.24
4362	12.39	9.39	37.53
4942	11.48	11.67	36.90
			Average: 44.62

3.4. Thermogravimetric Analysis

The results for the thermogravimetric analysis for the epoxy matrix, cold alkaline treated 5% *SSD* fibre with 7.5% *PTE* wood dust composite. The tests were performed using Perkin Elmer TGA 4000. The specimens were subjected to heat from 30˚ to 950˚C at a rate of 10˚C/min.

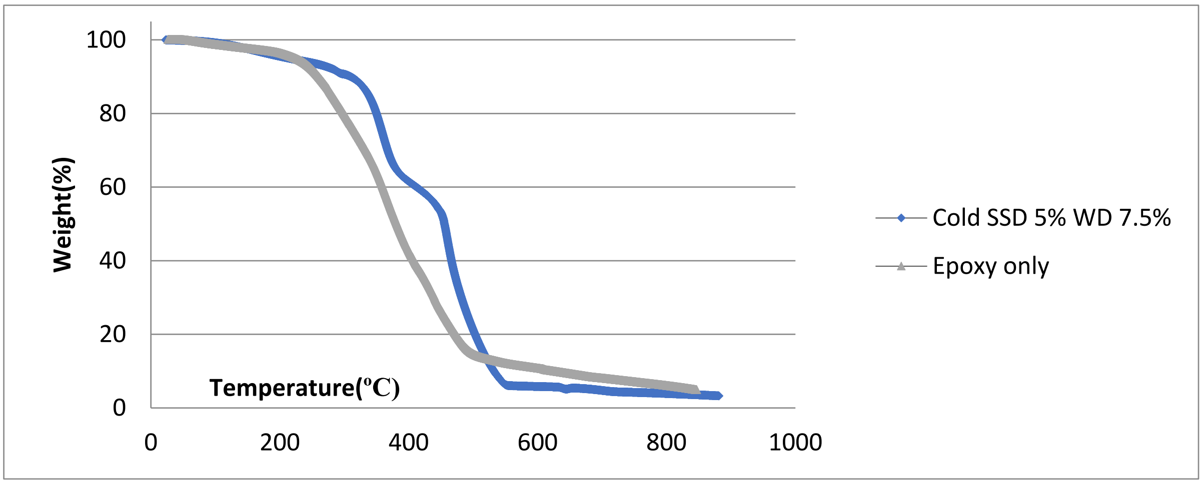

Figure 6: Thermogravimetric analyses of 5% cold alkaline treated fibre with 7.5% PTE wood dust and epoxy matrix

The onset temperature for the Cold Alkaline treated composite material is at 335ºC. The two Derivative Thermogravimetric (DTG) Peaks (Inflection Points) may represent two decomposition processes. The first inflection point was at 412.22 ˚C , the second inflection point was at 464.49˚ and the end temperature of the cold Alkaline treated composite was at 540˚C. The onset temperature for the Epoxy matrix was at 249˚C. The inflection temperature of the Epoxy occurred at about 365.16˚C and an end temperature of 497˚C. Sutrisno, Rahayu, & Adhika (2019) reported that the maximum thermal degradation peak of epoxy occurs at about 350˚C which is similar to the experimental result. The results have shown that the Cold Alkaline treated 5% SSD fibre and 7.5% PTE wood dust improved the thermal stability of the Epoxy by increasing its onset temperature from 249˚C to 335˚C. The thermal degradation peak of Epoxy was attained at a temperature of 365.16˚C while the thermal degradation peak for the Cold Alkaline treated 5% SSD fibre and 7.5% PTE wood dust was attained at 412.22˚C. This showed that the reinforcements improved the thermal stability of Epoxy. This is due to the high cellulose content of the fibre and wood dust reinforcements which improved the thermal stability of the composite. The reinforced composite has higher maximum degradation peaks than matrix such as Polyester (385˚), however matrices such as High Density Polyethylene (HDPE) (515˚C), vinyl ester (419˚C), Polypropylene (PP) (431˚C) and Polyurethane (420˚C) have higher main peak thermal degradation. This is due to the nature of the matrix.

The thermal behaviour for both untreated and treated (Silane and methanol) aspen wood fibre reinforced composites with polypropylene (PP) or maleated polypropylene (MAPP) matrices has been studied by Monteiro et al. (2012). The 20 wt.% untreated fibre/PP composite was associated with the onset range of 307˚C-453˚C and a peak 476˚C while the 20wt.% A1100 silane treated wood fibre/PP composite had an onset range 325˚C-447˚C and a peak of 471˚C. The neat PP had an onset temperature of 459˚C and a peak around 470˚C (Monteiro et al., 2012). Lei et al. (2007) studied the thermal stability of 30 wt. % pine wood fiber reinforcing recyled high density polyethylene (RHDPE) by TGA analysis. Wood fibre composites was treated with two active coupling agents: a maleated polyethylene (MAPE) and Titanium derived mixture (TDM) of chemical agents. The onset degradation temperature for the neat RHDPE occurs at 441˚C and the maximum degradation rate of about 471ºC. The introduction of 30 wt.% wood fibre another intermediate decomposition peak, onset, stages I and II were respectively found: Pure fibre/RHDPE 262, 353 and 469˚C, 1.2% MAPE coupled fibre/RHDPE with 263, 353˚C and 469˚C and 0.9% TDM coupled fibre/RHDPE with 260˚C, 349˚C and 468˚C. The coupling agents seems to have little influence on the thermal degradation of the composite.

In summary the results have shown that the fibre reinforcement in the examined literatures did not have significant improvement in thermal stability, however the cold Alkanine treated 5% SSD fibre and 7.5% PTE wood dust improved the thermal stability of the composite. The use of treated 5% SSD fibre and 7.5% PTE wood dust to reinforce the matrices mentioned could lead to improved thermal stability.

4. Conclusion

Composites of various compositions of fibre content, wood filler content, alkaline treatment and fibre orientation were designed using Taguchi design of experiment tool and fabricated. Novel composite materials have been produced. The composites were subjected to tensile, flexural and compressive tests. The result showed that fibre angular orientation was the most significant factor affecting the mechanical properties of composite and essential in improving the properties. In addition, alkaline treatment was also significant in improving the mechanical properties of the composite. Results showed that for the hybrid composite the 5% NaOH cold alkaline treated 5% SSD fibre with 7.5% *PTE* wood dust have consistently given high Tensile, flexural and compressive strength (26.90 MPa, 77.38 MPa and 51.39) respectively. Results also showed that the addition of wood fibre and wood dust in epoxy improved the thermal stability of the composite. The use of natural reinforcements means lower production costs. The optimized composite produced has a lower density of 1.093 g/cm^3 while carbon-epoxy composite 1.6 g/cm^3 and S-glass fibre composite 1.9 g/cm^3. The hybrid natural reinforced composite would be essential in the aerospace and automotive industry since weight is among the most important factors in the industry. Low weight means lower fuel consumption of aerospace and automotive vehicles; lower weight means lower cost, lower energy usage and lower emission of greenhouse gas. An effective use of wood dust for composite material has successfully been used as a supporting reinforcement. The material properties achieved would be used for finite element simulation purpose for various applications to identify areas of application of the material.

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LITERATURE REVIEW: THE ROLE OF EARTHWORMS IN BIOREMEDIATION OF TREATED WOOD

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Keywords

Eisenia fetida
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Bioaccumulation Factor (BAF)

Abstract

In testing for the efficacy of earthworm's capability to remove heavy metals from wood protected with chromium, copper, and arsenate (CCA) chemical wood preservative, California red worm *Eisenia fetida* was exposed to a substrate made from a mixture of cow dung and wood sawdust made from yellow pine CCA-treated utility pole. The period of exposure of the experiment spanned twelve weeks. The study was done to ascertain whether earthworms bioremediating the substrate into their tissue would have any effect on their reproduction and total biomass development. As the new bioremediation methodology of using earthworm species to remove heavy metals from a medium in ecotoxicology study was also utilised to determine the number of heavy metals *Eisenia fetida* could extract from the mixture of cow dung and CCA-treated wood sawdust.

At the end of the study, the calculation of bioaccumulation factor (BAF) values for chromium, copper and arsenic revealed arsenic to have been significantly bioaccumulated in earthworm tissue than chromium and copper.

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LITERATURE REVIEW: THE ROLE OF EARTHWORMS IN BIOREMEDIATION OF TREATED WOOD

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1. Introduction

The absolute reliance on steel and concrete in structural applications in recent times has been overwhelming. The use of wood for this purpose however cannot be overlooked. Wood is still and will continue to be a vital and versatile raw material element in our infrastructural applications. It is also a de facto renewable natural resource accessible for this function. Notwithstanding all the unique qualities of wood one of its fundamental hindrances is its vulnerability to lose its quality in an inimical situation (Morrell, 2006). Water is the greatest enemy of wood in putting its durability to test. In dealing with this deficiency of wood there have been many ways available to preserve wood for its durability. Some of the long-established known techniques of preserving wood entailed the utilization of naturally resistant heartwood species, supplementary preserved wood of non-durable species and devises to expel water from wood. Wood must either be naturally tenacious or be preserved to be able to prevail against watery prone conditions. The insufficient availability of naturally durable woods in the wood industry has influenced the rise in the use of non-durable species that are preserved with chemicals. These chemicals used in the preservation of wood are of different varieties and greater number of them are melted in water and applied which incorporate heavy metals such as copper, chromium and arsenic (CCA) into wood.

Discarding of CCA treated wood waste has been projected to rise globally as years pass by. The current traditional methods of disposing CCA treated wood products involve incineration which is practice more in Japan and Korea although studies have revealed how a gram of ashes remain from incineration of CCA treated wood material is lethal enough to kill 150 people (Hay et al., 2000). Landfill disposal, which is also common in Australia, and then recycle and reuse. However, recycle and reuse seem to be thwarted by complication in separating and sorting preserved and unpreserved wood waste materials (Hata et al., 2006) which unfortunately makes sorting unacceptable in practical and economical terms.

The information above is clear enough a reason on how serious the method of disposing obsolete treated wood materials pose threats to the environment and its inhabitants. The reprocessing of wood waste is vital for the efficient exploitation of natural resources. This benign emerging innovation that has already begun in other developed countries is new to the developing world. There is a critical necessity to come about with innovation for reusing out of-service CCA treated wood for other useful purposes owing to concerns raised about environmental pollution by disposed CCA treated wood products. In recent time, researchers have used different forms of strong acids which could remove and dissolve CCA compounds that had been fixed in CCA treated wood (Gezer et al., 2006). Using earthworms removing copper, chromium, and arsenic from CCA- treated wood materials could be a good choice and way. Therefore, in this paper, general information about earthworms and their role in bioremediation were reviewed.

2. General Concept About Earthworms

Earthworms are tube-shaped, segmented natural invertebrates of agroecosystem belonging to the class *Oligachaeta*, order *Megadrilacea* and in the phylum Annelida (Haokip and Singh, 2012). They are significant soil creatures making up a sizable proportion of the entire biomass of invertebrates prevailing in soils of temperate and tropical regions. Earthworms are the majority soil invertebrate organisms in tropical, temperate, and subtropical regions (Nainawat and Nagendra, 2001). They are the foremost multicellular organisms without backbone to have thrived in terrestrial locations (Kale and Karmegam, 2010). Earthworms are hermaphrodites however self-fertilization for reproduction does not take place within an organism. They are significant burrowing organisms that increase soil quality in the ecosystem. For this purpose and the significant work done by earthworms in soils they are referred to as ecosystem engineers (Lavelle, 1997). Soil quality, crop yield and plant growth are results of the important activities of earthworm species owing to the relative portly stature and their behavioural feeding pattern. Earthworms are also popularly known as “night crawler”, “anglerworm” (for fishing baits), “dew-worm”, and “rainworm”. Others are also known as megadriles and microdriles because of their literally bigger and smaller body size respectively. Earthworms are active and located in their numbers at places that are wettish as compared to distressed environments.

The burrowing activities of earthworms improve soil physical structure and increase the general qualities of soils. It increases plants moisture availability and uptake. Key processes such as dynamics of chemical processes, soil nutrients unleashing, dynamics of organic matter and microbial activities are also influenced by earthworms casting, channelling activities, feeding behaviour and physical characteristics like permeation and conglomeration. (Sharpley and Syers, 1976; Lavelle et al., 1983; Bostrom, 1987; Bouche et al., 1987; Scheu, 1987; Scheu, 1990; Lavelle and Martin, 1992; Pashanasi et al., 1992; Edward and Bohlen, 1996). The above processes all aid to provide plant growth catalysts and also facilitate the conversion of soil nutrients into absorbable state for plants use. Barois and Lavelle, (1986); Lavelle et al., (1995) has all reported on the existence of an interdependent relationship between earthworm's species and microorganisms which aid in the conservation of microbial diversity and soil productivity.

Earthworms are the main organisms in charge of blending and distribution of soil particles. They contribute to soil quality by taking halfway decayed litter from the topsoil, digesting it, and moving it to other locations in the soil. These traits of earthworms make them suitable creatures for monitoring the effects of soils pollution (Fischer and Koszorus, 1992). Their presence in soil is evidence of the calibre of the soil and its fertility. The impact of earthworms on soil activities varies between species and environmental types. Earthworms’ activities such as casting, and burrowing can impact on the health and functionality of soil. Undecomposed organic materials are often seen on the topsoil of soils without earthworms which affects the soil structure. The availability of earthworms in their numbers in soils is a sign of the presence of other soil organisms that contribute to improve soil fertility.

Earthworms are significant source of food for different organisms of all types. The popular one among them is the use of earthworms as bait for fishing. They are also a source of medium through which contaminants are passed on to more advanced trophic levels. Earthworms are extensively used as pointers of soil contamination and health owing to the significant role they play in breaking down organic materials in soils which contribute to soil development and maintenance of soil structure (Fischer and Koszorus, 1992; Edwards and Bohlen, 1996). The earthworm family *Lumbricidae* are known for the enhancement of macro porosity and aggregation of soil (Lee, 1985). The casts and burrows produced by this group of earthworms contain a lot of macronutrients which improves the growth of plants roots in the soil (Edwards and Bater, 1992). They also improve the fertility of soils and coexist with other soil fauna organisms (Fragoso and Lavelle, 1992).

According to Fragoso and Lavelle, (1992) earthworm species in the tropics are predisposed to certain factors in their locations. These factors may include soil condition (pH, nutrients content), seasonal changes and temperature. Their population is also affected by the annual rainfall observed in a typical rainforest zone. Annual rainfall of 3000mm is ideal for their survival with an amount of 4000mm and 2000mm being too wet and dry for earthworms’ existence respectively (Fragoso and Lavelle, 1992). Lavelle, (1988) also reported that earthworms are hardly seen in arid zones populated with termites and receive annual rainfall amount of 900mm or lower with temperatures above 35ºC and a longer dry season. Nevertheless, they are able to withstand dry seasons by moving vertically lower and deeper into the soil causing periodic perpendicular changes in earthworms’ distribution in the soil (Fragoso and Lavelle, 1992). However, lateral circulation of earthworms in the soil according to Decaëns and Rossi, (2001) is haphazard and organized at different spaces. Earthworms have also been used in many waste managements and ecotoxicological studies in which they have been used as bioaccumulation factor of a medium (Langdon et al., 2003; Leduc et al., 2007; Nahmani et al., 2007; Mench and Bes, 2009; Pattnaik and Reddy, 2011). And in all the studies, the ecological characteristics of earthworms were considered for the remediation of contaminated soils or other forms of contaminated media. For instance, the earthworm species Eisenia fetida is widely known to be the approved and recognized earthworm species for research in ecotoxicological test (Langdon et al., 2005; Peijnenburg and Vijver, 2009). They are also bred as baits for fishing and for vermicompost purposes. They dwell only in organic-rich materials environs like composts and dung heaps and are barely found in soils (Edwards and Arancon, 2004). These ecological variations of earthworms have substantial outcomes on the accumulation, sensitivity and exposure of heavy metals to earthworms.

For example, some features of earthworms like their place of abode and preferred mode of feeding can impact on the extent to which they get into contact with contaminated soil particles which may result in variations of earthworms’ exposure to heavy metals. Earthworms have special means of adjusting themselves to the exposure of heavy metal which aid them to dynamically control heavy metal bioconcentration in their body tissue (Hopkin, 1989). A report by Spurgeon and Hopkin (2000) confirmed a steady amassing of Cu and Zn in bodies of Eisenia fetida which signified a substantial removal of these metals and showing their distinct capability to accommodate heavy metals. These earthworms also have the capacity to hold themselves against lethal impacts of heavy metals by storing excess heavy metal, removal and detoxification of heavy metals (Vijver et al., 2004). The type of heavy metals and earthworm species influences the attachment of heavy metals to proteins such as metallothionine just as reported in eco-physiological research (Spurgeon and Hopkin, 2000; Vijver et al., 2004). However, Spurgeon et al., (2000) and Nahmani et al., (2007) reported that the accrual of heavy metal levels is different regarding earthworm species but reactivity of Eisenia fetida to heavy metal was found lesser than other species.

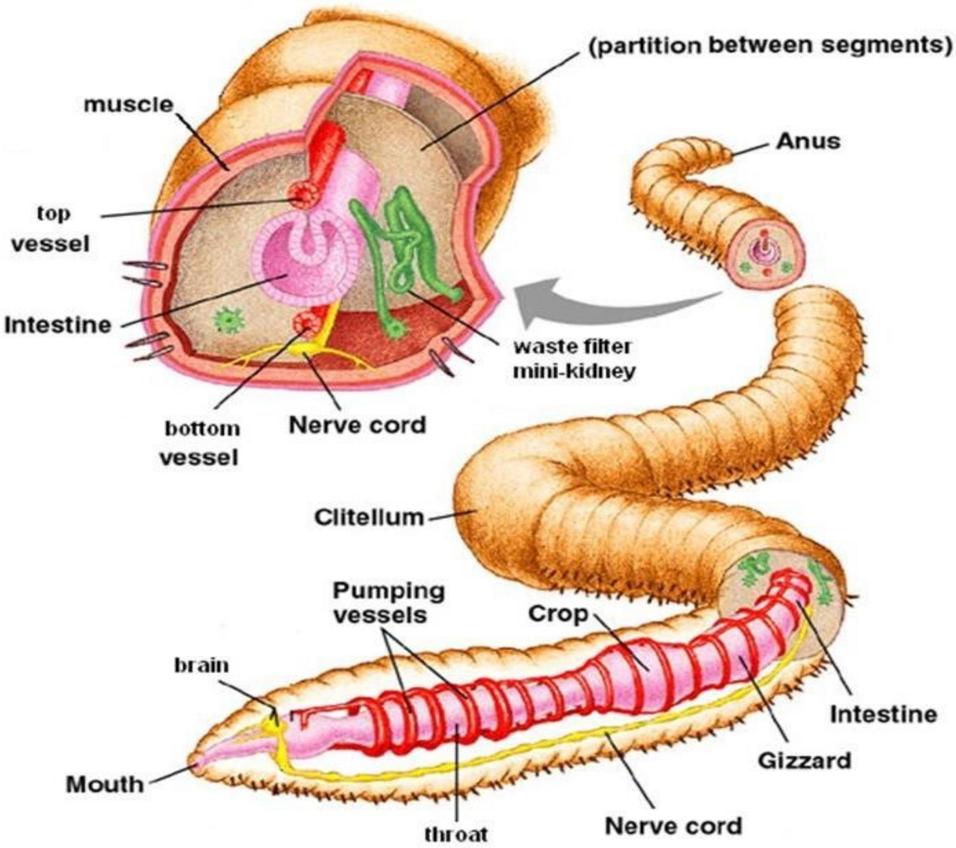

Figure 1: A labelled diagram of an earthworm (Copyright C 2002 Pearson Education, Inc., publishing as Benjamin Cummings)

2.1. Categories of Earthworm

There are three types of recognized earthworms with regards to their environmental purposes, location of habitat and bodily characteristics such as body size, color and shape. In respect of the above three ecotypes of earthworms have been classified by researchers as follows;

- *Anecic* earthworms
- *Epigeic* earthworms
- *Endogeic* earthworms (Bouché, 1977).

Each of these earthworm categories constitutes earthworm ranges with distinctive features. The *epigeic* earthworms like other fragile soil organisms are principally responsible for soil litter modification. On the other hand, the *endogeics* and *anecics* earthworm species impact on ecosystem layer processes and activities. They also contribute significantly to soil health condition and their impact on soils is great and influences soil properties and processes at the ecosystem level (Lavelle, 1997). Also, all the three ecotypes of earthworms identified above are used in one way or the other for burrowing purposes. Whiles *endogeics* are suitable for horizontally deep burrowing, *anecics* are used for general burrowing purposes and *epigeics* which are popularly known as ground dwellers are used for the purpose of tillage with little burrowing (Hickman and Reid 2008).

Epigeic earthworms are typically located in the topmost part of the soil while the *endogeic* earthworm species are found in the first 10 to 20 cm close to the surface of the soil. On the other hand, the *anecics* reside in the lowest alcove of the soil with a very scanty number in the tropics due to the location of their habitat (Barois et al., 1999). The Figure 2 illustrates categories of earthworms in their ecological habitats and location in the soil.

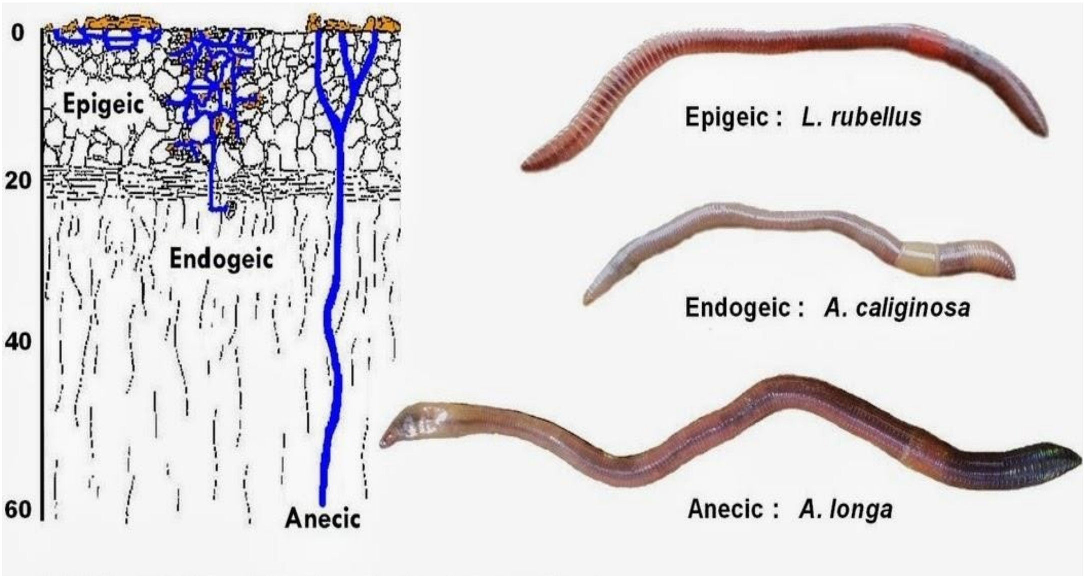

Figure 2: Earthworm ecotypes in their respective location in the soil. The figure was adapted from Qiu et al., (2013), and slightly modified.

The *Anecic* Earthworm

The *anecic* earthworms are mostly dominant in biomass of soils of many temperate regions (Lavelle, 1983). They are primarily vertical burrowing species and they carry organic matter from the upper part of the soil to their fixed abode deep down the soil (Butt, 1993; Domínguez, 2004). The transferred organic matter and surface litter are fed on by *anecics* at the basal part of the soil. *Anecics* are large in body form and burrows to the upper layer of the soil solely for feeding purposes (Paoletti, 1999). Their presence in an area can be noticed by a feature known as 'middens' which is mostly found at the openings of burrows. This mound ring-shaped trait is mostly a combination of leaves, soil and other organic matters. The activities of *anecics* earthworm species in soils may extremely alter soil essentials up to a depth of 1m or more. Earthworm species of *anecic* group may include *Lumbricus terrestris and Aporrectodea longa.*

The *Epigeic* Earthworms

The *epigeic* earthworm species are normally smallish in body form and dwell in, comminute and feed on materials found on the surface of the soil. These materials are mostly made of organic humus and litter devoid of soil particles (Lavelle, 1988). They are also found attached to plant root because they ingest leaf litter on the surface of the soil (Domínguez, 2004). The feeding manner and mechanism used by *epigeic* earthworm species to refine litter offers rich amount of nutrients to the soil. They have a short period of guts transfer which make them rely on other microorganisms to assist them in the disintegration of litter in their guts. Some of the earthworm species belonging to this group of earthworms' ecotypes include; *Lumbricus rubellus* and *Eisenia fetida*.

The *Endogeic* Earthworm

Endogeic earthworm species are commonly found in the tropics and the sole class of earthworm prevalence in biomass of the agroecosystems (Lavelle, 1983). They nourish themselves with soil minerals of the soil subsurface and dwell therein as their abode. They are geophagous species of earthworms that are hardly found in the layer of the topsoil (Bouché, 1977). *Endogeics* feed on varied kinds of soil minerals and produce greater quality nutritious ground casts. These casts are mostly of two types; large and compacted cast and small but loose cast which contain some quantities of NH_3 and other substantial nutrients (Barois et al., 1999) as compared to undigested soil organic minerals. *Murchieona muldali, Aporrectodea caliginosa, Pontoscolex corethrurus, Aporrectodea rosea, Allolobophora chlorotica, Lampito mauritii* and *Aporrectodea icterica* are all members of *endogeic* earthworm species.

2.2. Distribution of Earthworms

The earthworm ecotypes and categories discussed above are prevalent all over Europe and can also be found in other part of the world such as North America (Hendrix and Bohlen, 2002). There are about 6,000 species of earthworms discovered and about 120 of those species are widely distributed around the globe. Most of these earthworm species are of global and foreign origins. Different types of earthworm species have been discovered in different countries from different origins. The highest recognized earthworm species is reported to be in Australia. 650 indigenous species of earthworms with 75 other exotic species can be found in Australia. Also, a total of 182 earthworm species of 12 families have been reported in the North of Mexico, USA, and Canada sixty taxa out of them have been exotically introduced.

2.3. The California Red Worm (*Eisenia fetida)*

There are two recognized families of *Eisenia fetida* available and these have been divided into two species. These two species include; *Eisenia foetida* and *Eisenia foetida andrei*. They are morphologically similar, but the former has an intersecting and crosswise stripping on their segment whereas the later has a variegated reddish color (O.E.C.D., 2016).

The California red worm is an earthworm species that belongs to the *epigeic* earthworm ecotype and lives in the upper layer of the soil or in 10inches of the topsoil under the litter layer. It has a dark red color skin with external structures of 35-130mm in length which is greater than 70mm, 3-5mm in diameter, 80-120 segments and weighs about 1.4g. California red worm just like any other species of worm does not tolerate solar light because exposure to solar light results in their death within few minutes. *Eisenia fetida* is one of the earthworm species that has demonstrated a lot of prospects for the purposes of vermicompost. The environmental requirements and biology of *Eisenia fetida* have been broadly studied and reported in different forms (Kaplan et al., 1980; Hartenstein et al., 1981; Reinecke and Venter, 1987; Edwards, 1988).

It is an earthworm species that multiplies massively (Hartenstein et al., 1979) and develops very fast (Neuhauser et al., 1980). It has also shown an inherent sign and promising mechanism for waste management (Hartenstein, 1981). It lives approximately four and a half years and is able to multiply and reproduce about 1,300 new earthworms annually under favorable conditions (vermiculture manual). Any medium that harbors *Eisenia fetida* earthworm species must have an adequate climatic condition. Severe heat or cold impedes the activities of *Eisenia fetida* species. However, the water content of the soil harboring them is very significant for the burrowing purpose of the earthworm to commute easily through the soil (Kooijman and Cammeraat 2010; Owojori and Reinecke 2010). With optimum temperatures at a particular place, it is possible to raise this type of earthworm species at any location. They are strained under 30^0C and can easily die under temperatures above 40^0C. They are also not responsive when the temperature is below 7^0C though still generating humus in smaller amount. They reach their maximum reproduction ability when they live between temperatures 14^0C and 27^0C. Their reproductive activeness for a significant longer period than it had been considered possible is a wide spread phenomenon (Venter and Reinecke, 1988).

Normally matured California red worm produces humus of quantity of their weight and consume an equivalent quantity of food every day. This implies that their activeness is dependent on the amount of food available to them (Klok, 2007). They are able to live through well-defined soil conditions. The multiplication of earthworm population was reported to have been as a result of the application of manure and waste water sludge at a polluted area (Tejada 2009; Eijsackers 2010; Tejada and Masciandaro, 2011). California red worm also avoids extreme soil conditions. Their activities and survival are impeded by conditions such as high content of salt, organic contamination, high concentration of heavy metals and severe pH which also changes the formation of their habitat (Lapied et al., 2001; Eijsackers, 2010; Irmler, 2010; Kooijman and Cammeraat, 2010; Owojori and Reinecke, 2010). They die in extreme alkaline or acidic conditions. For this reason, their food and environmental pH is between 6.2 and 7.8 but a pH of 7 is ideal.

Eisenia fetida simultaneously advances, eats, and makes tunnels in soils. It is during this process it leaves the excreta behind and turns the soil in its terrain much more fertile than the fertile soil from any artificial fertilizer. The excretes of California red worm are many times richer in nitrogen, potassium, phosphorus and calcium than artificial fertilizer.

Figure 3: Earthworms at different stages of their life cycle; hatchling cocoon, juvenile, clitellum-matured and bunch of earthworms

2.4 The Life Circle of California Red Worm (*Eisenia fetida*)

California red worms are hermaphrodites with both male and female reproductive organs in a single organism. A matured *Eisenia fetida* has a thick, light-colored pigment and saddle-like ring found in the skin of the worm known as clitellum. For the purposes of copulation earthworms place themselves in a horizontal position on the ground with their head pointed in corresponding directions. In this position they secrete mucus that sticks their bodies together. Earthworms then interchange sperms which are placed onto their skin. These sperms are later moved into an opening slightly apart from the clitellum where they are stored for a short while. During this process earthworm also expels their own eggs separately into openings near the openings of the sperms on their skin.

After this process they move away from each other and produce extra gummy mucus all over their clitellum which desiccates into a solidified band to strengthen their bodies. Afterwards the earthworms withdraw from the solid band of mucus by sagging themselves out of it. The sperms and eggs pores are picked up from the gluey base as the solid mucus band moves over the opening keeping them. After earthworms have successfully withdrawn from the mucus band the end closes and it becomes a minute rigid cocoon with egg and sperm in it. The sperms obtained during the exchange period of sperms are utilized to produce more cocoons till it finishes. The fertilization of earthworms' eggs and sperms happens within the cocoon for juvenile worms to be reproduced. *Eisenia fetida* has an incubation period ranging from 32 to 73 days. It takes about 32 to 109 weeks for freshly hatched juveniles of worm mature and reproduces cocoons. They are able to produce cocoons in substantial numbers annually. However, this will depend on environmental conditions such as; moisture, temperature, and availability of food. When earthworms are fed well with three times of their weight of food, at least 75% of moisture availability and available temperatures between 20 and 25, their biomass double up within 3 to 4 months.

According to Klok (2007), earthworms need enough food to be active. However, Neuhauser et al., (1980), demonstrated the available relationship between earthworms' rate of weight gain and particle size of the food they consume; a tinier particle size of food leads to a higher growth of earthworms. Also, promising signs of population build-up and reproduction was observed in earthworms after several weeks of experimentation especially in the abundance of earthworm cocoons (Morgan, 2011).

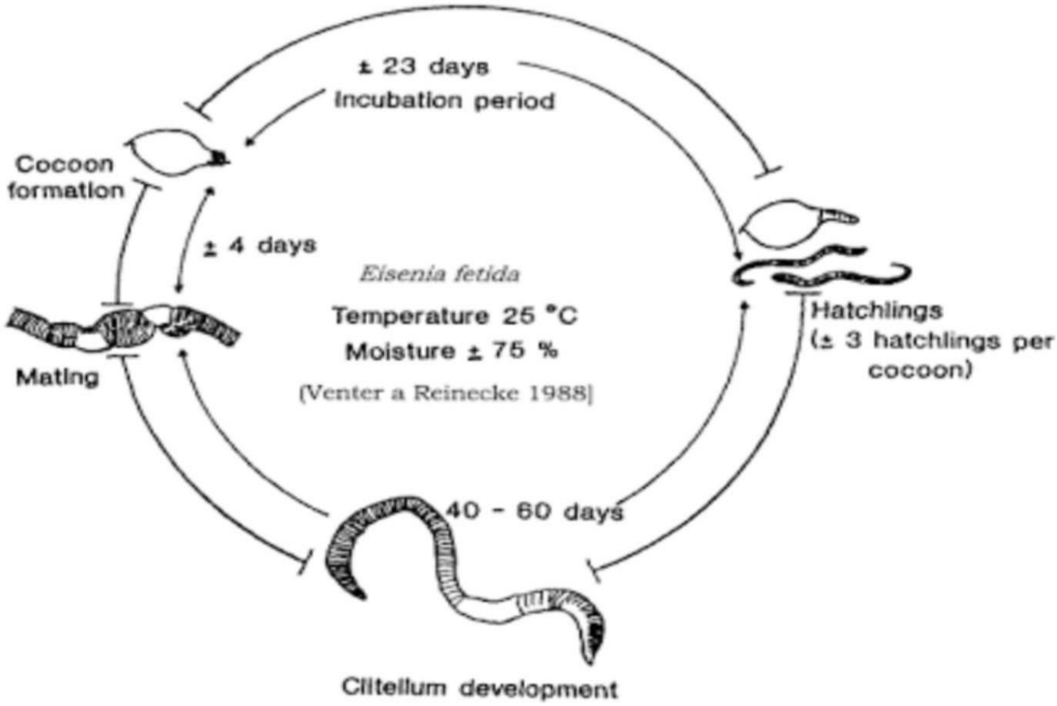

Figure 4: The life cycle California red worm *Eisenia fetida* (Morgan, 2011)

Figure 5: *Eisenia fetida* laying side by side in exchange of sperms for reproduction

2.5 Metal Accumulation by California Red worm *(Eisenia fetida)*

There is no doubt about the substantial evidence available on the potential of the three eco-physiological categories of earthworms accumulating several essential and non-essential metals from plant growth media. The *epigeic* ecotype of vermicomposting earthworm species like *Eisenia fetida* and *Dendrobaena veneta* are the most popular species that have been used for metal accumulation purposes. They are able to accumulate heavy metals from different contaminated soils. Many studies have reported the ability of this ecotype of earthworms to accumulate heavy metals from soils of metallic polluted and unpolluted soils used as control due to anthropogenic activities (Morgan et al., 1993; Peijnenburg, 2002;

Peijnenburg and Vijver, 2009). The fragile and highly permeable body walls of earthworms coupled with their bio-metal accumulation characteristics reflect their detritivores lifestyle. However, earthworms bioaccumulate heavy metals and store it in a non-lethal form but die of it when it reaches a critical concentration (Morgan and Morgan, 1988).

Mountouris et al., (2002) defined bioaccumulation as the mechanism by which organisms concentrate chemicals from their immediate habitat or environment into their body tissues. There are oodles of factors that regulate the accumulation of heavy metals into tissues of earthworm. These factors are known to be the characteristics of its habitat. Some of these factors include; oxides of manganese, aluminum, iron (Shea, 1988; Young and Harvey, 1991; Bryan and Langston, 1992; Bendell-Young et al., 1994; Janssen et al., 1997), acid volatile sulphide (Chapman et al., 1998) and organic carbon content (Mahony et al., 1996). According to Luoma and Rainbow (2005), bioaccumulation by earthworms is mostly driven by physiological and physicochemical parameters. The environmental conditions, characteristics of metalloid and heavy metals and the intended species all put together regulate bioaccumulation dynamically. Vermeulen et al., (2009), have demonstrated in their study the influence of environmental factors on accumulation of Pb, Cu and As in earthworms' tissues. However, pH level and organic matter content has also been reported to be a significant facilitator of concentration of the heavy metals Cd and Pb in tissues of organisms (Peijnenburg, 2002).

The targeted metal of concern in the usage of CCA wood preservative is widely known to be arsenic. However, its availability to earthworm species also varies with respect to earthworm's location in the soil. According to Langdon et al., (2003), arsenic is more available to earthworm species that are not found deep in the soil due to the strong bond between arsenic and both organic and mineral matter in the soil.

Bioaccumulation factor (BAF; also cited as uptake factor, UF, Concentration factors, CF, and bioconcentration factor, BCF depending on whichever context) is the ratio of the total concentration of heavy metal in tissue of an organism to the total concentration of heavy metal in its immediate environment of habitat (substrate or medium used for a study). As used in many studies (Mountouris et al*.,* 2002; Leduc et al., 2008; Pattnaik and Reddy, 2011; Singh et al., 2017) to ascertain the inherent concentration of heavy metals in an organism, the following formula was used to extrapolate the proportion of these metals' allocation in organism and its environment;

$$\mathbf{BAF = C_{organism}/C_{habitat}} \tag{1}$$

Where;

$\mathbf{C_{organism}}$ means the total metal concentration in the tissues of the organism (ppm; mg/kg).

$\mathbf{C_{habitat}}$ means the total metal concentration in the organism's habitat (ppm; mg/kg).

Pattnaik and Reddy, (2011), also interpreted the results calculated from the above formula as the comparison of the obtained answer to a unit (i.e., one); stating that when heavy metal concentration in the organism (earthworm tissues) is higher than that of its habitat (substrate), the bioaccumulation factor (BAF) will be more than one whiles it's less than one when the metal concentration in the organism's habitat is higher than that of the organism.

2.6 Bioaccumulation of Heavy Metals by Eisenia fetida in the current study

Heavy metals are also known to display different bioaccumulation behaviors in earthworms (Sample et al*.,* 1998). Unfortunately, the bioaccumulation factor of several metals in organisms of water prone area (McGeer et al*.,* 2003; DeForest et al*.,* 2007) and earthworms (Neuhauser et al*.,* 1995) have been reported to show strong inclinations in the direction of inverse relationships with extrinsic metal accumulation. Nieboer and Richardson, (1980), also stated that the bioaccumulation capacity of a given metal is not described by its bonding attractiveness or metabolic significance.

As reported by Morgan (2011), in relation to the utilization of bioaccumulation factor for risk evaluation, it is important for the calculated value to imply the integral characteristics of the chemical of concern. And it should not also be variegated with the variations in environmental conditions. It is also important for differences between bioconcentration, and accumulation be known prior to analysis and interpretation of data from toxicity study. Bioconcentration or bioavailability in toxicity testing is not a broad feature in ecotoxicity studies as it can be species-inclined and/or organism-inclined (Giller et al*.,* 1998). The accumulation of metals by an organism does not necessarily suggest bioconcentration (Morgan, 2011) due to the analytical approaches employed, the variations in lifestyle between species and the mechanism as well as duration of exposure to contaminants. In relation to this, bioaccumulation information on water prone organisms is used with vigilance in risk evaluation on the basis of specific cases (McGeer et al*.,* 2002).

California red worm, Eisenia fetida has been the international number one earthworm species for chemical toxicity tests (OECD, 2016). Although Eisenia fetida is not an inbred deep soil-inhabit organism (Booth and O'Halloran, 2001), it is considered as a representative of other earthworm species due to its sensitivity to heavy metals and other chemicals. Little information is available on how Eisenia fetida responds to heterogenous metal contaminants like CCA even though this group of earthworms has been employed in standard ecotoxicity chemical trials (OECD, 2016). Many studies have also concentred on earthworms' toxicity and single metal accrual (Weltje, 1998). However, specific growth and reproductive outcomes were achieved in a study where earthworms exposed to leachate from wood preservative made of multiple metal (Cu, Cr, As-CCA) as compared to single metal leachate (Cu-ACQ) (Leduc et al,. 2008).

The reactions of Eisenia fetida to heavy metals in studies are available in large quantities of data. Eisenia fetida has also been the choice and a point of reference in the international toxicity test (ISO 1993; 1998; OECD 2004). It is strong and can easily be grown in copious numbers in the laboratory as it develops within eight weeks, profusely reproduces and has lesser generation period as compared to other species and it is receptive to an extensive array of toxicants (Nahmani et al,. 2007). Nonetheless, Eisenia fetida has been a source of criticism in accumulation and toxicity studies, predominantly for the reason that it is not an inbred soil-inhabitant earthworm species. It resides mostly in organic rich environments like manure piles and dung (Bouche, 1972).

Eisenia fetida has also shown the capacity to tolerate heavy metals which are non-lethal to its body through results of many studies (Leduc et al., 2008). They tend to accommodate more nutrients for a lengthier period without any adverse influence on the environment. While consuming soil and organic substance they absorb heavy metals with the help of their fragile skin and intestine thereupon accumulating these metals in their body tissues (Hand et al., 1988; Singh and Sharma, 2002). Moreover, Cortet et al., (1999), reported that copper, zinc, lead and cadmium are accumulated and bioconcentrated in earthworms within certain environmental conditions. However, Spurgeon and Hopkin (2000) stated in their work that zinc in many cases was the only grave toxic metal to these organisms.

Many studies have reported variations in heavy metals accumulation by different species of earthworms. To date there are records of increases, decreases and no changes in heavy metals accumulated in earthworms' tissues. The three heavy metals, arsenic (As), chromium (Cr) and copper (Cu) which were investigated in this study revealed varying results of bioaccumulation in California red worm (*Eisenia fetida)* after 12 weeks of exposure. There is sufficient evidence on earthworms' high capacity to accrue lethal chemicals from variety of medium substrate such as metal contaminated soils, sludges, kitchen waste, farm waste and thermal power plant fly ash.

Nonetheless, the extent and scale of accrual depends on the type of metal or chemical and physiognomies of the substrate (Langdon et al*.,* 2003). In this study arsenic showed the highest and significant bioaccumulation in earthworms' tissues at all treatment levels and particle sizes of sawdust used. This might be that earthworms bioaccumulated arsenic in their tissues and sequestrated them in a form that could not be easily eradicated from their system as suggested by Meharg et al*.,* (1998). Langdon et al*.,* (2003), also reported that the closeness of earthworms to organic and mineral matters allows it to easily accumulate arsenic in both solid and aqueous state because organic and mineral matters are arsenic bound. Also, the effect of organic matter was reported by Ezemonye et al., (2006) to have increased copper bioaccumulation in soil amended with organic substrates like poultry, pig and cow manure. Although cow dung was used in this study a significant bioaccumulation of copper was not achieved as in a study by Iordache and Borza, (2012). Relatively the results on high arsenic bioaccumulation by *Eisenia fetida* in the present study could be associated to the feeding preference of earthworms on arsenic bound sawdust and cow dung thereby accumulating more arsenic via their feeding habit and fragile skin. This also attested to the fact that earthworms are prone to specific contaminants based on features such as ecological properties, location, mobility behaviour and food preference (Tomlin, 1992).

Higher initial concentrations for both Cr and Cu than As was observed in the current study. Heavy metals percentage removal in substrate after weeks of exposure also showed a slightly high percentage values for Cr and Cu at all treatment levels and particle sizes of the sawdust used than for As. However, computation of *bioaccumulation factors* in earthworms' tissues showed higher values for arsenic as compared to Cr and Cu which implied the intrinsic amount of As in earthworm tissue and also contrasted the suggestion that high level of Cr and Cu contaminants affect the absorption and metabolism of As (Meharg et al., 1998). This could be an indication that substantial number of heavy metals is still in the substrate after the period of exposure. This can be that earthworms had reached their threshold for heavy metals bioaccumulation or the time of exposure was not enough to absorb all the heavy metals from the substrate used for the study. Also, if bioaccumulation dependence upon the degree of contamination and the characteristics of substrate is anything to be considered then the low BAF values recorded for Cr and Cu in this study could be associated to the pH and the content of heavy metals in the substrate used. Heavy metals are known to be mobile under acidic conditions (Ekperusi et al., 2016). The pH value of the substrate used in this study was 9.30 which represented an alkaline nature of the substrate before the exposure and this may have contributed to the mobility of some heavy metals thereby making the metals bioavailable for uptake by *Eisenia fetida.* Earlier studies like Leduc et al., (2008), have also confirmed the influence of pH values specifically on Cu bioaccumulation in earthworms' tissues. They said higher pH values led to lower Cu bioaccumulation in *Eisenia fetida* which was same for this study.

On the other hand, findings from the current study supported the account of Langdon et al*.,* (2003); detection of metals in earthworms' tissues may necessarily not insinuate metal biomagnification in tissues of earthworm species. Numerous studies have shown varying values of bioaccumulation factor in earthworm species. In all these studies alike the same category (epigeic) of earthworms but different species (*Aporrectodea rosea, Eisenia fetida and Lumbricus rubellus*) of earthworms were used in heavy metal accumulation test. Studies involving species like *Aporrectodea rosea* and *Lumbricus rubellus,* showed no biomagnification of arsenic in their tissues (Yeates et al*.,* 1994; Geizinger et al., 1998) but studies with species like *Eisenia fetida* showed higher bioaccumulation for arsenic in earthworms' tissues (Fischer and Koszorus, 1992). And this was verified in the outcome of this study. In spite of the fact that same category of earthworm species was used in different studies for heavy metals accumulation test, it can be deduced from the above findings that bioaccumulation for Cr, Cu and As in this study were species-driven (Suthar et al*.,* 2008). The lower values of bioaccumulation factor and the level of absorptions of Cu and Cr in tissues of earthworms in relation to this study also confirmed the fact alluded by Hopkin (1989) that earthworms might have the latitude to control metals in their bodies but the mechanism and bioaccumulation for metals like Cu may be species-specific. A metal accumulation and toxicity study in different earthworm species under the same exposure concentrations also revealed *Eisenia fetida* accumulation of Cu and Ni being lower as compared to *Lumbricus rubellus* (Qiu et al., 2013).

The span and duration of exposure in heavy metals bioremediation studies have proved to be a major factor that influenced differences in heavy metals concentrations in earthworms (Hopkin, 1989; Pattnaik and Reddy, 2011). Variations in bioaccumulation of As, Cr and, Cu in tissues of earthworms were noticed in this study and these differences in metal accumulation in *Eisenia fetida* tissues could among other reasons be ascribe to the duration of the study as described in other studies of heavy metal toxicity test by earthworms (Jamaludin and Mahmood, 2010; and Pattnaik and Reddy, 2011). Even though the continuous bioaccumulation factor of heavy metals in this study was not monitored. The consistency in the observed decrease in As, Cr and Cu concentration in substrates within the experimental time intervals could equally be predicted for BAF values of heavy metals in earthworms' tissues along the same time intervals. However, BAF values for As increased along all the substrate levels except at substrate level T_4 where the trend was decreased while fluctuations were observed for Cr and Cu. The high value of bioaccumulation factor for As in most of the substrate levels and particle sizes of the sawdust used in this study signified the concentrations of As in the tissues of *Eisenia fetida* surpassing the concentrations in the substrates on many occurrences.

The reduction of metal availability to earthworms due to metal attachment to organic matter has been reported by Lukkari (2006). In the current study, Cr and Cu were the least accumulated heavy metals in earthworms' tissues according to the values of BAF relative to both treatment levels of substrates and particle sizes of the sawdust used. The low bioaccumulation of Cr and Cu in earthworms' tissues may be that Cr and Cu were clinched to organic matter in the experimental substrates and were not bioavailable in absorbable form for earthworm's uptake. Furthermore, findings from the present study showed an independent relationship between metal concentrations in substrate used for the experiment and metal concentrations in earthworms' tissues as compared to the findings by Pattnaik and Reddy (2011). The heavy metals concentrations in substrate especially for Cr and Cu manifested not in the tissues of *Eisenia fetida* after 12 weeks of exposure. However, Gupta et al., (2005); Suthar et al., (2008) and Pattnaik and Reddy, (2011) reported direct dependent relationship between metal concentrations in substrate and metal concentrations in earthworms' tissues. If the above evidence is anything to go by then results and findings from the current study differ because Cr and Cu had the highest concentration in substrate as compared to As but metal bioaccumulation in earthworms was higher for As than Cr and Cu. This further defies the hypothesis that metal availability in substrate influences tissue-metal levels. The metals of interest and of great concern in this study followed a similar bioaccumulation pattern for other studies especially for the results of As and Cr which are related in terms of metal accumulation in remediation studies.

3. Conclusion

Recent studies and investigations related to CCA chemical wood preservative have focused on chemical remediation to get rid of Cu, As and Cr heavy metals out of CCA contaminated media of different forms (Kartal and Kose, 2003; Clausen, 2004; Kazi and Cooper, 2006; Kakitani et al., 2006; Gezer et al., 2006). Chemical remediation is the most common method used in ecotoxicological studies. However, it's implementation has not been safe and cost effective. In other words, chemical remediation method has not been a panacea for the very important problem at stake. In scope of this, it is quite unique to state that, this is one of the rare studies where California red worm (Eisenia fetida) may be used to explore the likelihoods of extracting chromium, arsenic and copper from CCA preserved wood. In line with this, the current study investigated with the aim of assessing possibilities of Eisenia fetida extracting and accumulating heavy metals from a substrate mixture of cow dung and sawdust from CCA-treated wood. And as a matter of fact, CCA-treated sawdust has not been popular in ecotoxicology research as a medium for earthworm exposure.

However, on the account given above, Eisenia fetida earthworm species has shown with reasons and evidence on how they are capable of bioaccumulating heavy metals especially the heavy metal (Arsenic) of utmost concern in our environment. This shows that earthworms can aid in utilizing out-service utility poles and other wood product that have been preserved with lethal preservatives by remediating them of the lethal content for other important purposes. On the other hand, earthworm production for the purposes of fertilizer and manure for amending soil fertility has gained much attention and has become important in recent years. Earthworms are known to produce manure that is highly rich in nutrients for agricultural purposes and for that matter they are easily accessible from dealers in fertilizers (Nahmani et al., 2007). The transformation of biowaste by means of earthworms' species into manure for agricultural use is also fast becoming popular around the globe (Rajkhowa et al., 2015). For these reasons, the use of earthworms for heavy metals extraction has been predicted to be an antidote to ecotoxicology as economically viable, socially acceptable and ecologically sound technology (Sharma et al., 2005).

Finally, arsenic from CCA-treated wood products is a known heavy metal of concern in most ecotoxicity test. In this study earthworms *Eisenia fetida* showed a significant bioaccumulation of arsenic in their tissues which presents a clear indication of the importance of *Eisenia fetida* in bioremediation of arsenic-contaminated environments. The findings from this study could also be suitable for the wide range of various results from similar topics with different kinds of substrates and methodologies.

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