International Journal of Engineering Processing & Safety Research
Published by Cambridge Research and Publications
Vol. 28 No.5
June, 2023.

EFFECT OF ANIMAL BONE ASH AS A PARTIAL REPLACEMENT FOR CEMENT IN THE PRODUCTIO OF SELF HEALING CONCRETE

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Abstract

The study presented is part the ongoing study which is aimed at finding more suitable, economic and cost effective materials to be used in the construction industry, while continuing to stay on the cutting edge of constructional technology. It involves the use of Animal Bone Ash (cattle) as a partial replacement for cement in the production of self-healing concrete. For over two decades the rise and return of self-healing concrete in the research of world of civil engineering has been on an increase. This has been tested and tried with several methods, one of such methods is the use of microbes for the precipitation of calcite particles, with the aim of sealing up the cracks that could be present in concrete due to various reasons. This project takes on the challenge of finding a more accessible and environmentally friendly manner in carrying out the goal of self-healing incocnrete. It involves the use of Animal Bone Ash at 0%,3%,6%,9% and 12% to replace cement in concrete production. A total of 90 cubes of 50x50x50mm and 30 beams of 100x100x400mm beams were produced to test the mechanical properties of the concrete, such as the compressive strength, flexural strength and water absorption. From the research carried out it was seen that the concrete sample with 12% replacement had the best compressive and flexural strength values, and that of 3% replacement had the least values in compressive and flexural strength, but the highest water absorption values. From the FTIR test carried out, it was seen that the highest present constituent material in the Animal Bone Ash used was CaO which was quite useful in calcite precipitation, though it was found to be on the low side in comparison (done by MICP testing) to a primary calcium source (calcium lactate) in the production of self-healing concerte. Though the use of Animal Bone Ash has its promises, it also has its limitations which can be further reviewed in future works to come.

Key Words: Effect , Animal Bone Ash , Partial, Replacement , Cement Production , Self-Healing, Concrete

Introduction

Concrete is a composite material made of fine and coarse aggregate that is joined by fluid cement (cement paste) that eventually solidifies (cure).

Concrete is the most often used building material and the second most used substance in the world after water (Gagg, 2014).

Following the creation of Portland cement in the early 19th century, concrete became the most popular building material used worldwide. In addition to water and aggregates like rock, sand, or gravel, cement serves as the concrete's binding agent. Concrete is the preferred building material because it offers a low cost as well as a number of desirable qualities, such as workability, great compressive strength, and durability. Up to 23% more concrete is expected to be produced globally by 2050, and in the early 21st century. (maddisosn, 2018) Although concrete can be quite advantageous and readily integrated in various areas of civilized living, concrete still has its various limitations, which can prove rather harmful to the structure created. Even and especially after the structure has been built, the rectification of the effects of these limitations may still be expensive, time-consuming, and energy-consuming for the parties engaged in the construction project. One of these restrictions is the tendency of concrete constructions to develop cracks. A concrete structure may develop cracks for a variety of reasons, the majority of which might shorten the building's service life. Service life is typically determined by how important a structure is. The design service life is typically raised to more than 100 years in more complicated structures, compared to 50 years for common structures. Because of this, materials and building systems need to be able to function well enough to last for such a long time, but they also need accurate assessments of their mechanical and durability capabilities over the long period in order to design practical maintenance strategies. Cracks are common in many reinforced concrete structures, and depending on the steps taken during the structural design, they may be acceptable. In general, combined axial, shear, and bending loads will be applied to concrete elements. (Gagg, 2014)Formation of cracks on concrete structures can cause a significant increase in the expense of maintaining and repairing concrete structures. It is widely acknowledged that the emergence of micro cracks in concrete enables the entry of many substances, including water, carbon dioxide, chloride ions, and others. This intrusion may subsequently result in carbonation, sulfate-related concrete degradation, and probable corrosion of traditional reinforcing. Due to this degradation, concrete structures must be repaired and maintained in order to maintain their structural integrity, which comes at a cost to the economy and society. (Hizami Abdulah M., 2018) For the purpose of the elongation of the service life of concrete structures, and also for the reduction in cost consumption of maintenance and repairs of issues arising from cracking of already constructed concrete structures, several methods, means and materials have been taken into consideration, most of which still involve the need for external human intervention, in order to solve these issues. Such solutions however are still considered expensive, time and energy consuming. In this research, the use of bacterial made self healing concrete would be explored as a possible option for the solution of some of the afro mentioned problems.

Self-healing concrete is a form of concrete that has the ability to fix gaps/cracks on its own without assistance from outside sources (Marta Roig Flores, 2021). This indicates that concrete can apparently recreate itself while fixing internal cracks during the self-healing process. It is possible for autonomous or autogenous healing to produce this mechanism. The natural process of autogenous healing is brought on by carbonation and/or ongoing hydration. In order to produce self-healing, specific agents are used in autonomous healing. These agents can be added directly to the concrete matrix, contained within capsules, injected through vascular networks or as part of the concrete mix. Superabsorbent polymers, crystalline admixtures, sodium silicate in microcapsules, and microbes are a few examples (Mugahed Amran 1, 2022). In this review, the autonomous self-healing process involving the use of bacteria as the healing agent was studied hence, enabling the concrete to be environmentally friendly. Concrete self-healing through microbial-induced calcium carbonate precipitation has become a promising technology in the last two decades for increasing the durability and sustainability of concrete structures (Bagga M, 2022). The self-healing process involved in bacterial self-healing concrete is based on the ability of the bacteria used to precipitate insoluble $CaC0_3$ (calcium carbonate/ limestone) as a result of its consumption of a calcium based nutrient source, in the presence of oxygen, an example of such a nutrient source is Calcium lactate ($CaC_6H_{10}O_6$). Tziviloglou E et al (2016) which used 200g/l of calcium lactate in the bacterial mixing solution. The effect of nutrient such as calcium lactate, calcium nitrate and urea is to form Calcium Carbonate due to bacterial metabolic conversion. This is further proved by a study that immersed cracked mortar in calcium lactate and calcium gluconate solution. The result indicates that calcium lactate and calcium gluconate increased the self-healing kinetic of mortar by increasing the availability of calcium and carbonate ion in the cracks. Hence, seen from previous statement above, the presence of calcium based nutrient source is needed by the bacteria present for the precipitation of calcium carbonate. As is known, cement is a hydraulic binder formed by the grinding process of cement clinker, as intermediate product, which is produced by baking the lime-clay raw material mixture to the sintering temperature. The basic raw materials for cement production are limestone and clay. The limestone represents the dominant component, most often 75%, clay and corrective raw materials remaining 25% of the raw blend (paul rudolph, 2022). Due to the high amount of calcium from the limestone, present in the cement, the major source of calcium nutrient for the bacteria first worked with in the production of self-healing concrete was the calcium present in the cement used for the production of said concrete. This source however was both unreliable and insufficient for the task at hand and thus other sources were looked to, hence the addition of calcium nutrient source supplies. The use of ordinary Portland cement has been in existence for a long time, but its production process has been found to be unhealthy for the environment, hence alternative materials have been implemented as a partial replacement for the production of cement, to increase its sustainability. One of such materials is animal bone ash. With its high amount of calcium quality, it can serve not only as a good partial replacement material for concrete, but also as a partial nutrient source for the bacteria present. In this paper the effects of using animal bone ash as a partial replacement for cement in the production of self healing concrete was investigated.

Materials and Methods

This study of consists of the proposed methodology and the necessary materials used in carrying out this research. It also involves the adaptation of processes used by previous researchers of similar works. In this research, test was carried out on the effectiveness of using an alternative material as a partial replacement for cement in the production of self-healing concrete, to see if it met the necessary requirements as shown by the control and main material.

This study visualizes into carrying out tests to determine the mechanical and chemical properties of cattle bone ash when used as a partial replacement for cement in self-healing concrete, as well as its effects on the healing rate of the concrete, the nutrient provision for the bacteria healing agents, and its abilities to meet other requirements, relating to previous publications and research as summarized by other authors and stated in chapter two. A number of concrete cubes, ranging between "50 – 70" of standard 50mm x 50mm x 50mm will be used to determine the compressive strengths of the concrete cubes.

Available research present on this topic, though limited, has been directed mainly towards developing a standard mix which utilizes sustainable and cost effective alternatives to cement to ascertain reliability and usability compared to conventional self-healing concrete.

The details of the materials, mix proportioning, specimen preparation and even test methodology and procedure are briefly discussed in this chapter.

Materials:

The materials to be used in this experiment include-

i. Fine aggregates
ii. Coarse aggregates.
iii. Ordinary Portland cement
iv. Water
v. Animal bone ash (cattle)
vi. Bacteria (*Bacillus Sp.*)
vii. Nutrient agar.
viii. Nutrient broth.
ix. Calcium lactate.

Methods:

Collection and preparation of materials:

Concrete is most basically a combination of mineral material, cement as a binder, and water to create a rock like formation when hardened as a result of chemical reactions between its sub materials. Self-healing concrete at its core follows the same concept of what concrete is. The only difference is its ability to close up its cracks after they occur, the main difference being the addition of a few extra sub materials to the original conventional concrete combination. Besides the selection of optimum contents of the ingredients to give standard mix, selection of the right materials is also an important aspect to achieve desirable outcome.

Cement:

The cement used had a minimum compressive strength at different ages of curing, following specifications from IS specification. In this study, ordinary Portland cement that was used was kept in sacks of approximately 50kg as in local use. These cement sacks or bags were kept in air tight conditions to avoid dampness, with a minimal distance away from the walls and also a height from the floor. To be stacked on top one another, and ensuring that not more than 10 bags stacked.

Fig: Ordinary Portland Cement

Water:

The main purpose of water in the concrete mixture is to propel hydration process. It also aids the lubrication between the coarse and fine aggregates present in the concrete mixture. Optimum water content is required for concrete and its mortar. The water that was used was portable, meaning that it was odorless, tasteless and colorless; therefore the water used was suitable for drinking. The water that was used was free from impurities which could dampen the setting time or strength and durability of the concrete being produced.

The water for curing however was identical to the water that was used for concrete production.

Coarse aggregate:

Coarse aggregates are larger sized mineral material. The coarse aggregates used was hard, strong and free from impurities and coatings, alkali and organic matter capable of reducing or altering the strength of the concrete in general. The aggregate that was used did not have a loose mass, and it should be elongated to enable perfect bond with the binding agent. It serves majorly the same function as fine aggregate.

Fig: Coarse aggregate

Fine aggregate:

The fine aggregate that was used was natural sand, such as river washed sharp sand that was locally sourced from rivers and free from impurities such as salt impurity. The size of the fine aggregate that was used was determined for both concrete and mortar production. Sand is classified in many ways, through the use of various criteria's such as the mineralogy, source, size etc. based on its source the sand that was used in this research was natural sand, i.e. it occurs as a product of natural breaking down process of rocks to create the sand, and manufactured sand.

Bacteria (*Bacillus sp*.):

The bacteria that were used were cultured on the grounds of Landmark University, by the efforts of the lab technicians in microbiology department of Landmark University. Wherein it was cultured first, using a media of nutrient agar inside a Petri-dish. After it was cultured, the spores of the bacterial organism were isolated and prepared into a broth for a larger quantity to be obtained and then added as a liquid solution into the concrete mix.

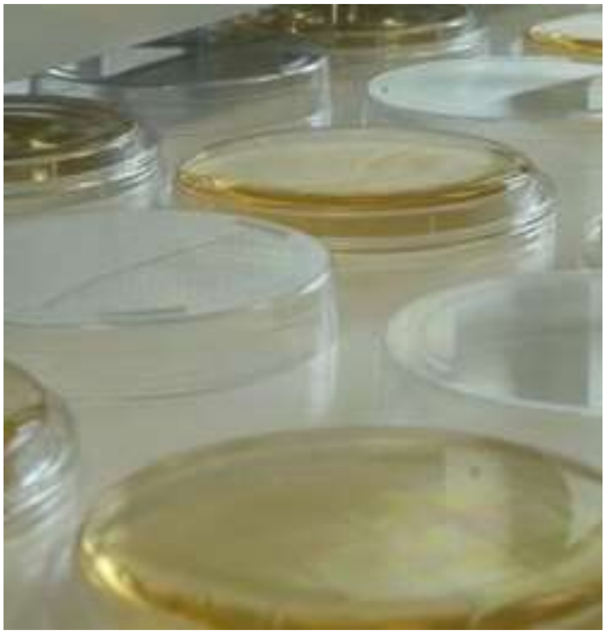


Fig: bacterial culture plates

Animal bone ash (Cattle):

After collecting two sacks of cattle bone from a local vendor, the bones were sundried by removing tissues and other detached wastes. The bone residue were then cleaned, crushed by hand hammering to the maximum size of 25 mm that is suitable for disk milling. The prepared bone sample were burned at range of 600-900^{0}C for 3 hours, at a heating rate of 10^{0}C per minute. The burned bone was then milled by a disk milling machine to get 75 micrometer sized bone ash.

Fig: cattle bone ash

Calcium Lactate:

Calcium lactate is also known as calcium salt pentahydrate and the chemical formula is $C_6H_{10}CaO_6$. It looks like white powder and it possesses efflorescent odor. This calcium lactate powder is produced by reacting lactic acid with calcium carbonate or calcium hydroxide. Calcium lactate can be easily dissolved in ethanol and the melting point of calcium lactate is 239^0 C. In this research calcium lactate was added in 10% by weight of cement. The physical properties of calcium lactate included in the table as shown below;

PHYSICAL PROPERTIES	
Chemical formula	$C_6H_{10}CaO_6$
Appearance	White powder
Density	1494 kg/m3
Solubility in water	7.7 g/100 ml
Odor	Efflorescent
Solubility	Very soluble in ethanol
Molar mass	308.1 g/mol

Fig: calcium lactate

Test on materials:

A plethora of factors can affect the strength and durability of conventional concrete, ranging from constituents materials, method of casting, mix design, water content, climatic factor etc. However, in the production of self healing concrete, the factors which could affect its workability and effectiveness and efficiency in healing can include, but are not limited to temperature, pressure and healing time, size of the crack, presence of water and air, age of concrete and its crack, concrete materials composition, healing mechanism and Ca^{2+} ions concentration (which could be due to the calcium nutrient source present). Various test and precaution can be undertaken on the constituents of the concrete being produced to either check for the credibility of the constituents, ensuring they are up to standard or to curb the possible effects of said factors. Factors such as climactic factor, method of obtaining and processing of construction materials vary from place to place, hence the various parameters of the materials will be determined, based on their location; in this case, it will be the local areas of Omu-Aran. Some of the tests that were carried out on the aggregates to be used include: sieve analysis, specific gravity and bulk density was done on coarse aggregate material. Test on the reactivity between the bacteria used and the base calcium nutrient source (calcium lactate), as well as a substitute calcium nutrient source (animal bone ash) was also carried out. The test on the concrete itself included compressive strength test, flexural strength test and water absorption test.

Sieve analysis:

The degree to which the sizes of the particles in an aggregate are evenly distributed is determined by the aggregate grading. Every size of particle was fairly represented in a well-graded aggregate, which had a good range of particle sizes.

By using a sieve analysis technique, the distribution of aggregate particle size was determined. To establish the sizes of the aggregates to be utilized, ranging between 12.5mm and 9.5mm of coarse aggregate, according to ASTM 136, the method involved the use of various numbers of standard sieves. The aggregates were cleaned, allowed to air dry, and then hand graded using sieves arranged in descending order. Each sieve's retention of different aggregate sizes was examined in accordance with the requirements of ASTM 136

Bulk density:

This is determined by deriving the ratio of dry weight of aggregate to the volume of water. This will be done by first weighing and recording the weight of a container of known volme, such as a beaker of 1000ml (as was used in this project study), said container is then filled with the coarse aggregate sample, and weighed (W_2). The bulk density is the derived as;

$$Bulk\ Density\ (kg/m^{\wedge}3) = \frac{W2 - W1}{V}$$

Where;
W1 = weight of beaker
W2 = weight of beaker + aggregate
V = volume of baker (1000ml)

Specific gravity:

Specific gravity is the ratio of weight of a given volume of aggregate to weight of equal volume of water. An empty beaker of known volume is first weighed (W1). Into said beaker an amount of coarse aggregate is added and then weighed again (W2), after which water is used to fill up said beaker and the weight is then recorded (W3). The contents of the beaker are thrown out and replaced with only water to occupy the volume of said beaker and the weight is recorded once more (W4). The weights obtained in conjunction with the volume of the beaker are then used for calculations. It can be gotten as;

$$Specific\ Gravity\ (Gs) = \frac{(W2 - W1)}{[(W4 - W1) - \ (W3 - W2)]}$$

Where;
W1 = weight of beaker
W2 = weight of beaker + aggregate
W3 = weight of beaker + aggregate + water
W4 = weight of beaker + water.
Volume of said beaker used was = 1000ml.

FTIR test on animal bone ash:

Fourier Transform Infrared Spectroscopy, which is commonly referred to as FTIR Analysis or FTIR Spectroscopy, is a method of analysis employed to recognize organic, polymeric, and, on certain occasions, inorganic substances. This analytical technique involves employing infrared light to scan and examine test samples, allowing for the observation of their chemical characteristics (RTI laboratories, 2019). Compared to other methods of characterization analysis, FTIR is widely preferred and popular. It offers several advantages such as rapidity, high accuracy, and a relatively high level of sensitivity (Asep Bayu Dani Nandiyanto, 2019). In FTIR analysis, the instrument emits infrared radiation within a range of approximately 10,000 to 100 cm^(-1) through a sample. During this process, some of the radiation is absorbed by the sample, while the rest passes through. The absorbed radiation causes the sample molecules to convert the energy into rotational and/or vibration energy. As a result, a spectrum is generated and detected, typically ranging from 4000 cm^(-1) to 400 cm^(-1). This spectrum acts as a molecular fingerprint of the sample, wherein each molecule or chemical structure produces a unique spectral pattern. This characteristic makes FTIR analysis an excellent tool for chemical identification (RTI laboratories, 2019). This procedure was used in this study in order to determine the chemical compounds present in the animal bone Ash used for partial replacement of cement.

Test procedure:

Bacterial isolation and replication:

The bacteria specimen (*Bacillus Sp.*) used during this project study was cultured in the laboratory of the Microbiology department of Landmark university and was isolated for culturing form the soil beside the teaching and research farm of Landmark University Omu-Aran Kwara state. After culturing, it was replicated at the central laboratory present in the new college building in Landmark University. The bacteria specimen was replicated in urea free agar and broth solutions. To prepare these media, the solid components of nutrient agar and nutrient broth were added to separate conical flasks containing de-ionized water of volumes 250ml each and mixed well. The mixtures were then gently heated, and brought to a boiling temperature. Later, it was autoclaved for 15 min at 15 psi pressure at 121 °C. After autoclaving, it was cooled to 50–55 °C. The nutrient agar medium was then immediately poured into approximately 10 culture plates in a pre-sterilized work station using both surface sterilization with ethanol and also atmospheric sterilization, with the use of a spirit lamp. This was done to reduce the risk of contaminating the culture growth media prior to the introduction of the *Bacillus Sp.* cultures.

The details of the compositions of the agents used in the preparation of the growth mediums are given in the tables below;

Chemical composition of the nutrient broth;

Composition	**Quantity**
Peptone	5 g/lit
Sodium Chloride (NaCl)	5 g/lit
Yeast extract	1.5 g/lit

Beef extract	1 .5g/lit

Fig: Bacteria present in nutrient agar and broth

Chemical composition of the nutrient agar;

Composition	**Quantity**
Peptic digest of animal tissue	5 g/lit
Sodium Chloride (NaCl)	5 g/lit
Yeast extract	1.5 g/lit
Meat extract	1 .5g/lit
Agar	15g/lit

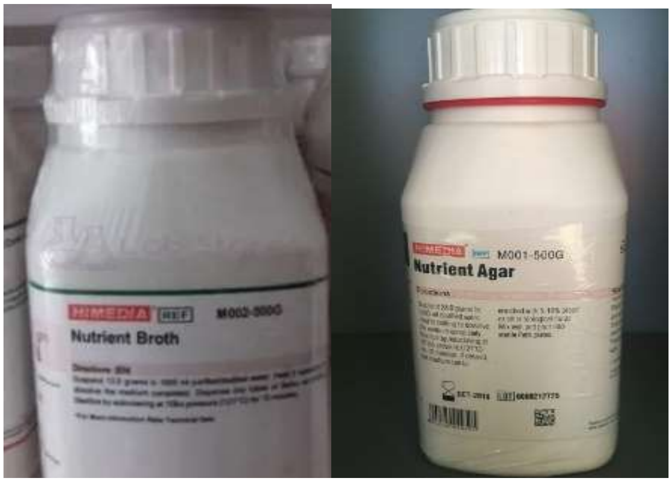


Fig: nutrient broth and nutrient agar

One loopful of pure colony was taken from the stock bacteria and streaked onto each culture plate, as well as the broth containing conical flask. The plates and the flask were then inverted and incubated for 18–24 h at 30 °C in an incubator. After incubation, 40-ml batches of the bacteria culturing solution were transferred from the culture flask to a series of 50-ml culture tubes. Each culture tube was centrifuged at 3000 rpm for 20 min to separate the suspension from the supernatant. The supernatant was removed by pouring it into a separate flask, and the remaining bacterial pellet was then used for the bacterial test procedure in order to obtain the Colony count of the bacteria samples. Measurement of turbidity or optical density (OD) is not a direct measurement of bacterial numbers, but an indirect measurement of cell biomass that includes both living and dead cells. Hence, to quantify viable the cells, a plate-count method was used and cell counting by hemocytometer under microscope was also performed to have an accurate estimate.

Further along the line, after sufficient bacterial growth had been observed more broth mediums were prepared which were then used in the production of the concrete samples.

Concrete sample preparation (mixing and casting):

The concrete samples, consisting of cubes of 50x50x50 mm and beams of 100x100x400mm, were produced with using a control mix (of sample ID; A) of grade M15, i.e. 1:2:4 of cement, fine aggregate and coarse aggregate respectively. With water cement ratio (w/c) of 0.3. the subsequent samples (of sample IDs; B,C,D,E), had a percentage, i.e. 3%, 6%, 9% and 12% respectively of their cement content replaced with Animal Bone Ash (cattle). Calcium lactate was also added in the mix at 0.1% by weight of the total binder content. The bacteria used was added in form of broth at 10% to the total water content of the concrete mix. In the mix procedure, the dry materials, such as the cement, fine aggregate, coarse aggregate and Animal Bone Ash (when added) were first mixed together to until a consistent mixture was obtained. The wet materials, consisting of water, nutrient broth of bacteria and calcium lactate where then added to the mix to attain the fully mixed paste-like-state concrete.

The experimental mix design used during this is as shown in the table below;

Mix id	**Cement (%)**	**Coarse aggregate (%)**	**Fine aggregate (%)**	**Animal bone ash (cattle) %**	**Calcium lactate (%)**	**Water (%)**	**Bacteria (%)**
A	100	100	100	0	0.1	100	10
B	97	100	100	3	0.1	100	10
C	94	100	100	6	0.1	100	10
D	91	100	100	9	0.1	100	10
E	88	100	100	12	0.1	100	10

After mixing, the concrete in its paste-like form, the inner walls/sides molds to be used were first oiled and the concrete paste was then poured into the molds of the cubes and beams with the use of a hand trowel, tamping was done during this process so as to allow for proper settlement of the concrete, and to remove air voids that could possibly form. The weights of the materials used mix design for the production of the concrete samples were gotten via an electric weighing balance.

Fig: weigh and mixing of concrete samples

Batching process:

To achieve the desired chemical and mechanical properties of the concrete samples, its constituents were carefully selected and proportioned. To attain proper workability, the mix ratio for the various ingredients in this study was 1:2:4 (one-part cement to two parts fine aggregate to four part coarse aggregate). Five distinct mixes (0%, 3%, 6%, 9% and 12%) were created utilizing various amounts of Animal Bone Ash, with 0% serving as the control mix devoid of additive (Animal Bone Ash). An electronic weighing balance was used to batch the concrete according to weight. The quantities of the materials used were based on the following calculations and compiled in table shown in the apendix;

De-molding of concrete samples:

This process was carried out 24 hours after the concrete samples were casted. This involved the removal of the concrete samples from their various molds, in order for them to be cured.

Fig: De-molded concrete samples

Curing:

This was done 24 hours of casting the samples, with the samples removed from their molds and immediately placed in clean water for the process. The samples were cured for 7 days and 28 days, done in accordance with the IS 516 code. The tanks used for curing were filled with clean water. Curing is one of the most essential phases and process of concrete testing, helping to improve the strength and other properties hence, it stimulates concrete in actual construction because most cast concrete are subjected to atmospheric condition like rain fall, wind and other natural element which is modeled in curing but at a smaller rate. Curing is the process where concrete is protected from loss of hydration and kept within required temperature. It will increase the strength and reduce permeability. All samples were kept immersed well underneath the water surface at a depth of about 10cm seen above the top of the sample.

Concrete testing:

Compressive strength test:

This gives an overall classification of characteristics of concrete. It is the value of uniaxial stress reached when a material fails completely. Cubes of sizes 50mm x 50mm x 50mm were used, and tested using a compression machine of 2000 KN capacity, with the loading being applied at a rate of 10N/s. the machine was calibrated to required values and standards. The test was carried out on cubes of cast concrete of different grades. This test had been done after the curing stage after 7 days, and 28 days, after which the cubes samples were wiped clean and transferred beneath the head of the machine and loaded centrally. The average values obtained from this test were then used for subsequent calculations.

$$Calculated\ as\ compressive\ strength\ (Cu)\ given\ in\ N/mm^\wedge 2\ or\ Mpa = \frac{Force\ reading\ obtained\ from\ the\ machine\ (KN)\ x\ 1000}{Net\ area\ of\ the\ concrete\ cube\ (mm^\wedge 2)}$$

Fig : compressive strength test on cocrete samples

Flexural strength test:

This checks the tensile strength in concrete, hence determining the extent and size of cracking in structure. Beams of specific dimensions (400mm x 400mm x 100mm) were used. These beams were tested by applying load placed at appoint along the span. The rate of loading was predetermined, as 10N/s as the beams were continually loaded until failure occurred. The flexural strength of the samples was estimated from the type of failure, appearance of failure and fracture load. As is known, concrete is weak in tension hence this test is of utmost importance. Similar to compressive strength test, this test was carried out after curing, wherein the samples were subjected to loading continuously without shock until breakage. The flexural strength test was conducted on concrete cured for 7 and 28 days.

$$Calculated\ as\ flexural\ strength\ given\ in\ N/mm\text{^}2\ = \frac{3\ x\ Maximum\ loading, obtained\ from\ the\ machine\ (N)\ x\ length\ of\ the\ beam}{2\ x\ breadth\ of\ the\ beam\ x\ depth\ of\ beam\text{^}2}$$

Fig : tensile strength thest on concrte beams

Bulk density test on concrete:

This test is done on concrete, with the aim of determining the average ratio of weight of the concrete cube samples to the net area of said concrete cubes. In this test, the samples are first removed from the curing water at 7 and 28 days, and then dried for a minimum of 45 minutes to 1 hour to remove the moisture. The cubes are then weighed and that weight is recorded and used in appropriate calculations in order to obtain the bulk density of the concrete samples.

$$Bulk\ density\ (kg/m\ \text{^}3)\ = \frac{weight\ of\ concrte\ cubes\ (kg)}{area\ of\ concrte\ cube\ (mm^2)}$$

Microbial induced calcite precipitation test:

This was a laboratory test which was carried out in order to investigate and examine the ability of the bacteria used (*Bacillus Sp.*) to precipitate/ produce calcite particle when provided with different calcium sources i.e. calcium lactate and Animal Bone Ash. It was also carried out in order to have a basic comparison between the rate of calcite precipitation with calcium lactate as the primary source of calcium and Animal Bone Ash as the primary source in separate mediums. Calcite or calcspar is the mineral name of the low-pressure, hexagonal form of calcium carbonate, $CaCO3$. It is observed that calcite is the principal constituent of limestone, marble, and chalk. The ability of the bacteria (*Bacillus Sp.*) to precipitate said calcite particles is the base line to determine whether or not the concrete samples produced posses the ability to heal, s well as the possible rate at which said healing will occur. In this test, a fresh batch of the bacteria specimen (*Bacillus Sp.*) was prepared by inoculating a portion of it into a plate of pre-prepared agar. After 18-24 hrs visible growth the fresh bacteria was seen in the petri dishes. To carry out the calcite precipitation test, all the needed equipments were first sterilized in an autoclave at a temp 121^{0}C for a time period of about 15 minutes. Into a conical flask filled with 200ml of de-ionized water, a solution of nutrient broth was made, using 2.6g of nutrient broth. Said solution after being properly stirred and allowing for the broth particles to dissolve, was then poured into two other conical flask leaving each with 100ml of nutrient broth. Into broth A, a diluted portion of calcium lactate was added, and into broth B, a dissolved amount of Animal Bone Ash (cattle) solution was added, both at concentration of 25Mm. The broth samples were then autoclaved once more at 121^{0}C for 15 minutes after which they were allowed to cool to room temperature before a solution of diluted urea was supplemented at 2% of each of the broths total volume i.e. 2ml each, into the broth samples, this was done in order to aid urease production and to hasten calcite precipitation time as well as to aid bacterial growth in the solution. The bacteria was then inoculated into the broth samples using an inoculating loop that was sterilized by a spirit lamp in a work space in which surface sterilization was done to prevent contamination. The solutions were left to rest at room temperature for 24-48 hrs before testing, was carried out. The test was carried out through optical microscopy.

Fig: MICP testing

Fig : Use of optical microscope to study bacterial growth and calcite precipitation

Results And Discussion

In this study, the details of all the test, experiments and investigations carried out during the various aspects of the project process are highlighted. These include the results of the test carried out on the mechanical properties of hardened concrete i.e. compressive strength test, flexural strength and water absorption test. As well as the results of the material test carried out on the materials used, such as the FTIR test carried out on the animal bone ash, and also the test carried out on the aggregate used, such as sieve analysis, bulk density and specific gravity test. This chapter also discusses the results of the Microbial Induced Calcium Carbonate Precipitation (MICCP) test, carried out on the bacteria.

Test on materials:

Several tests were carried out on the materials used during the production of the concrete samples. The results of said tests are discussed below;

Test on coarse aggregate:

The tests carried out on coarse aggregate help to understand the characteristics and properties of the coarse aggregate used during the production of the concrete samples, which in turn can help to understand the characteristic behaviors of the concrete samples when subjected to certain tests.

Sieve analysis and physical properties:

The outcomes of the sieve analysis conducted on the aggregates employed in this research are provided in figure 4.1. The aggregate's fineness modulus was determined as 2.43 based on the test. The coefficient of uniformity (Cu) and coefficient of curvature (Cc) are calculated to be 0.72 and 1.39 respectively. The obtained results indicated that the aggregate had a uniform grading as the Cu value was below 4.0, which aligns with the requirement stated in ASTM D-2478 (2000). This also suggests that a significant portion of the aggregate particles conform to a specific size range. This characteristic can enhance compaction and result in improved performance when applied in various applications.

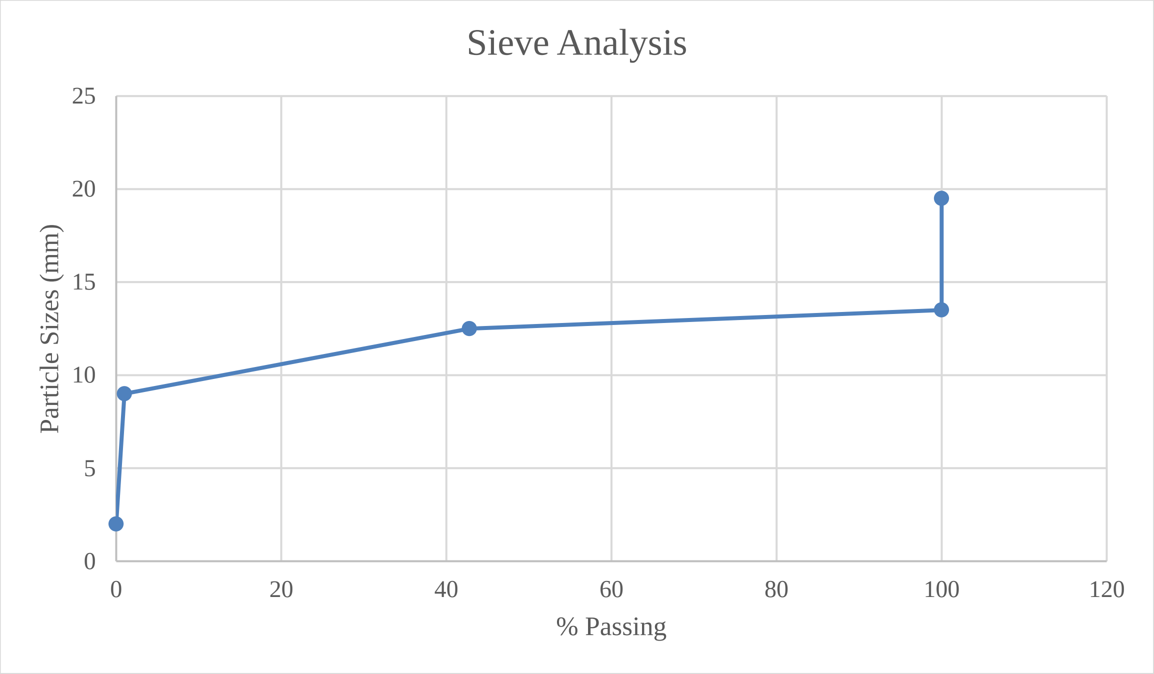


Figure 4.1: Particle size distribution for the Aggreegates

Bulk density test:

The bulk density test done on the aggregate samples was in accordance to BS 1881-114, which gives an allowable range of 1200-1750 kg/m^3 as the bulk density of the coarse aggregate. The bulk density test result obtained was:

$$Bulk\ density\ (\frac{kg}{m^3})\ =$$

$$\frac{2.036 - 0.389}{0.001}$$

= 1647 kg/m^3

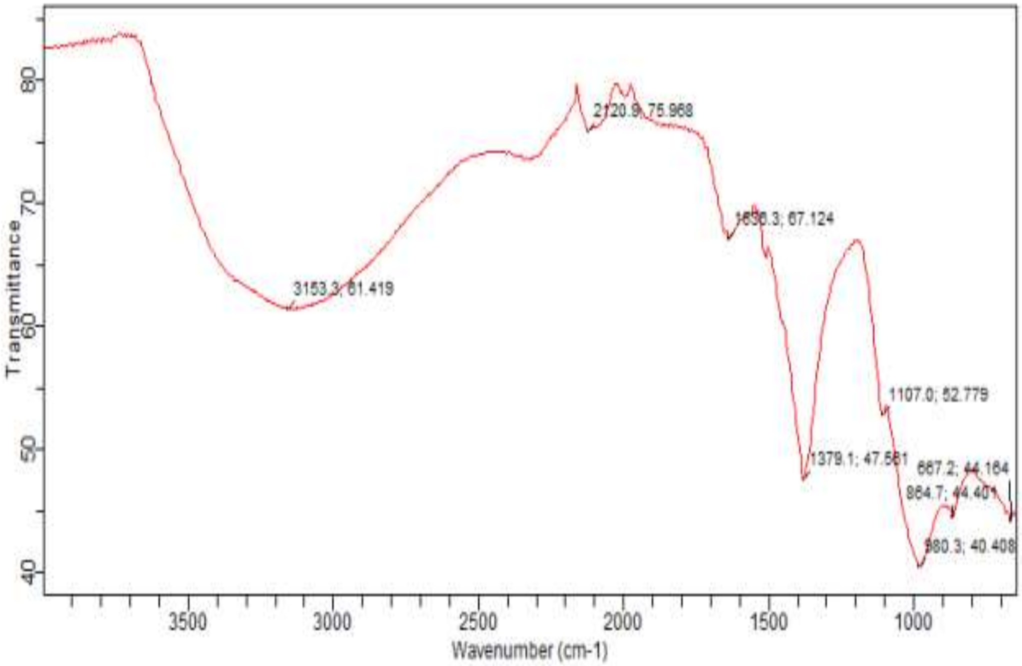
Specific gravity test:

This test was carried out with respect to IS: 2386 (Part III) - 1963, which provides a range of 2.5-3.0 for specific gravity of coarse aggregate. It was done in order to give certain characteristics of the aggregates. It was gotten to be;

$$Specific\ gravity\ ()\ = \frac{(0.930 - 0.389)}{[(1.342 - 0.389) - (1.667 - 0.930)]}$$

$$= 2.50$$

Test on Animal Bone Ash (FTIR):



From the results shown in the FTIR graph above, it can be seen that

Test on hardened concrete:

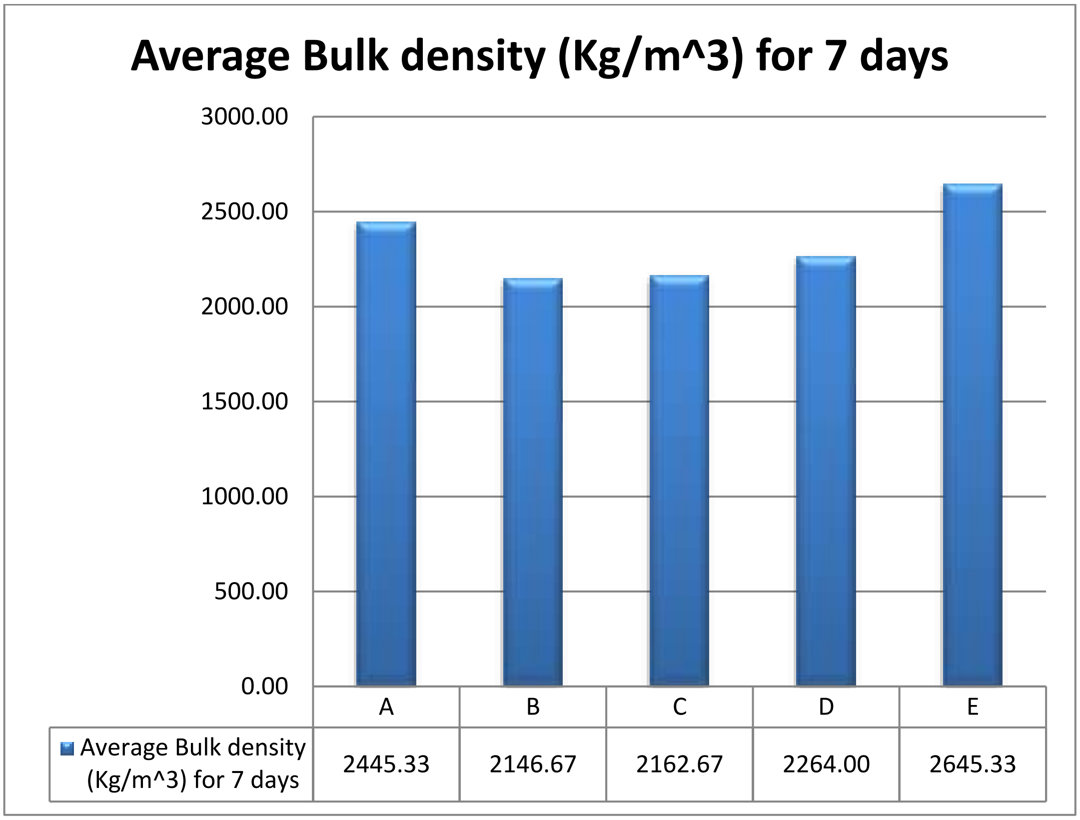
The tests carried out on hardened concrete, were done in order to determine the behavioral characteristic of post cast concrete, after 7 and 28 days of curing. Said test were carried out to simulate the per formative behavior of concrete samples when subjected to certain conditions, such as different types of loading (compressive and flexural strength test), water absorption etc.

From previous works, done it has been shown that the highest present particle in Animal Bone Ash is the CaO content. As seen in the FTIR spectrometer above, it has a value of 3135.3 transmission to wavelength. Though it has a relatively high calcium content as compared to ither contents it is still considered low as compared to primary calcium sources e.g. Calcium Lactate.

Bulk density:

The bulk density test carried out on the hardened concrete gave the following results:

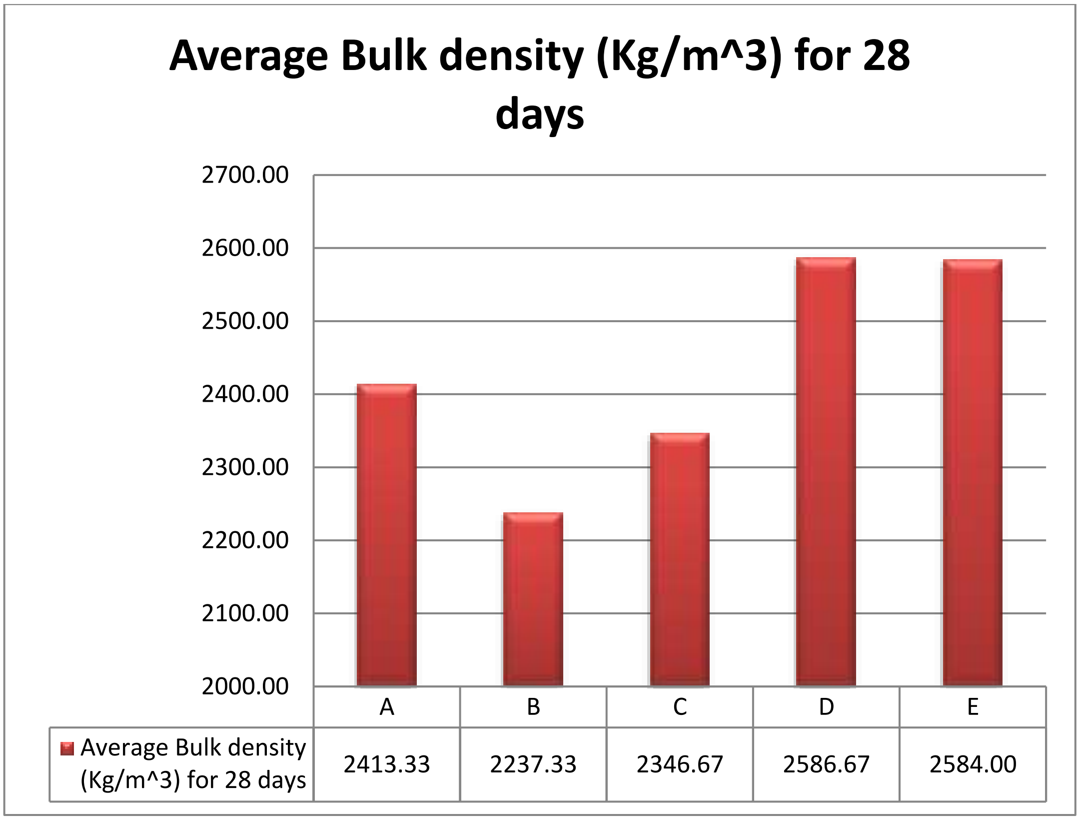
For 7 days;



	A	B	C	D	E
■ Average Bulk density (Kg/m^3) for 7 days	2445.33	2146.67	2162.67	2264.00	2645.33

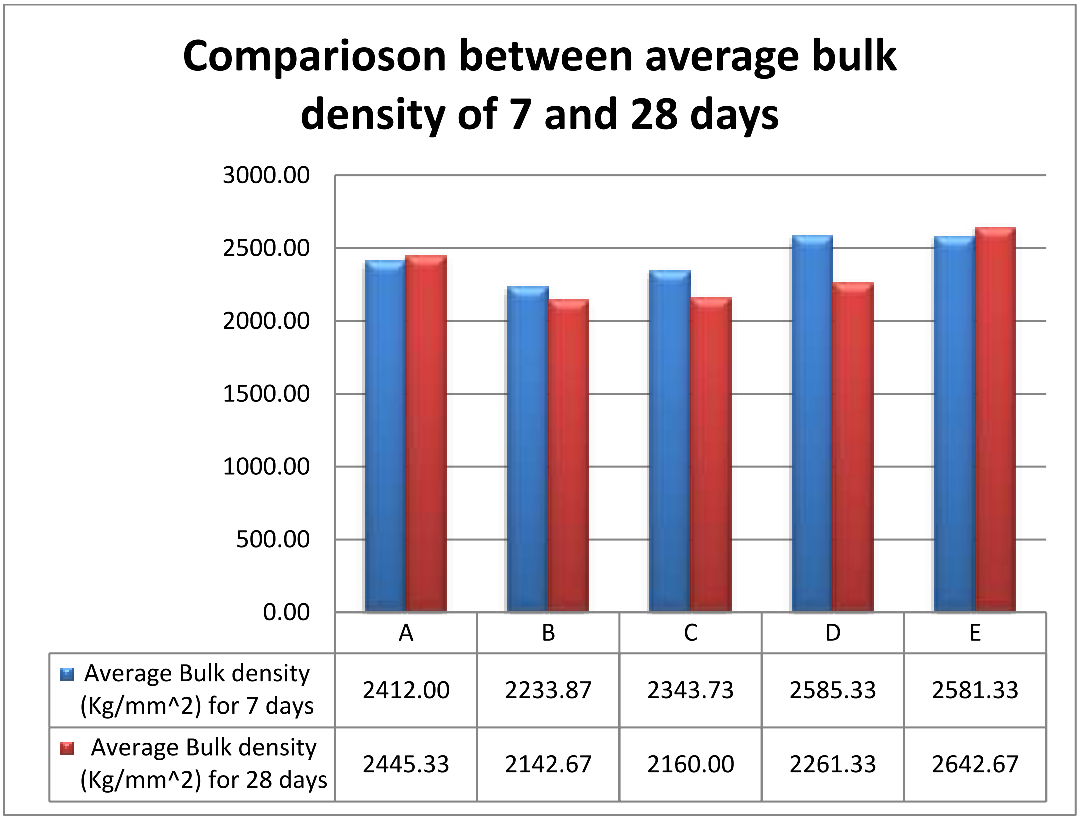
As can be seen, the figure above, the average bulk density of the concrete samples examined during this test have varying average bulk density values with respect of the percentage of cement replaced by Animal Bone Ash in each of the samples produced. The 12% cement replaced sample yielded the greatest average bulk density of 2645kg/m^3, which was relatively close to the average bulk density of the control sample which had 0% cement replacement and gave an average bulk density of 2445.33kg/m^3. The least average bulk density value was recorded from the 3% cement replacement sample which was 2146.67kg/m^3. From the results, it shows that an initial replacement cement by Animal Bone Ash (Cement) in the concrete mix will lead to an initial decrease in bulk density, however further replacement of the cement with Animal Bone Ash leads to an increase in the concrete bulk density after 7 days. Though from previous works the average bulk density of concrete reduces with an increment in the percentage replacement of cement with Animal Bone Ash, it can be speculated that the increase is largely in part due to the presence and action of the bacteria (*Bacillus Sp.*) in the concrete samples which has been recorded from previous works to precipitate calcite particle which helps to fill the void spaces present in the concrete, hence causing an increment in the bulk density value and a possible increment in the compressive strength. This is because in the samples with increased bulk density values, the possibility of sufficient CaO (Calcium Oxide) for the bacteria to feed on is increased and C_3S (Tricalcium Silicate) to be formed, which is has been noted to be responsible for early onset strength and density.

For 28 days;



As can be seen, the figure above, the average bulk density of the concrete samples examined during this test have varying average bulk density values with respect of the percentage of cement replaced by Animal Bone Ash in each of the samples produced. The 12 and 9% cement replaced sample yielded the greatest average bulk density of 2584.00kg/m^3 and 2586.67kg/m^3 which were relatively close to the average bulk density of the control sample which had 0% cement replacement and gave an average bulk density of 2413.33kg/m^3. The least average bulk density value was recorded from the 3% cement replacement sample which was 2237.33kg/m^3. From the results, it shows that an initial replacement cement by Animal Bone Ash (Cement) in the concrete mix will lead to an initial decrease in bulk density, however further replacement of the cement with Animal Bone Ash leads to an increase in the concrete bulk density after 28 days. Though from previous works the average bulk density of concrete reduces with an increment in the percentage replacement of cement with Animal Bone Ash, it can be speculated that the increase is largely in part due to the presence and action of the bacteria (*Bacillus Sp.*) in the concrete samples which has been recorded from previous works to precipitate calcite particle which helps to fill the void spaces present in the concrete, hence causing an increment in the bulk density value and a possible increment in the compressive strength. This is because in the samples with increased bulk density values, the possibility of sufficient CaO (Calcium Oxide) for the bacteria to feed on is increased and C_3S (Tricalcium Silicate) to be formed, which is has been noted to be responsible for early onset strength and density.

Average bulk density comparison between 7 and 28 days yielded;



	A	B	C	D	E
🟦 Average Bulk density (Kg/mm^2) for 7 days	2412.00	2233.87	2343.73	2585.33	2581.33
🟥 Average Bulk density (Kg/mm^2) for 28 days	2445.33	2142.67	2160.00	2261.33	2642.67

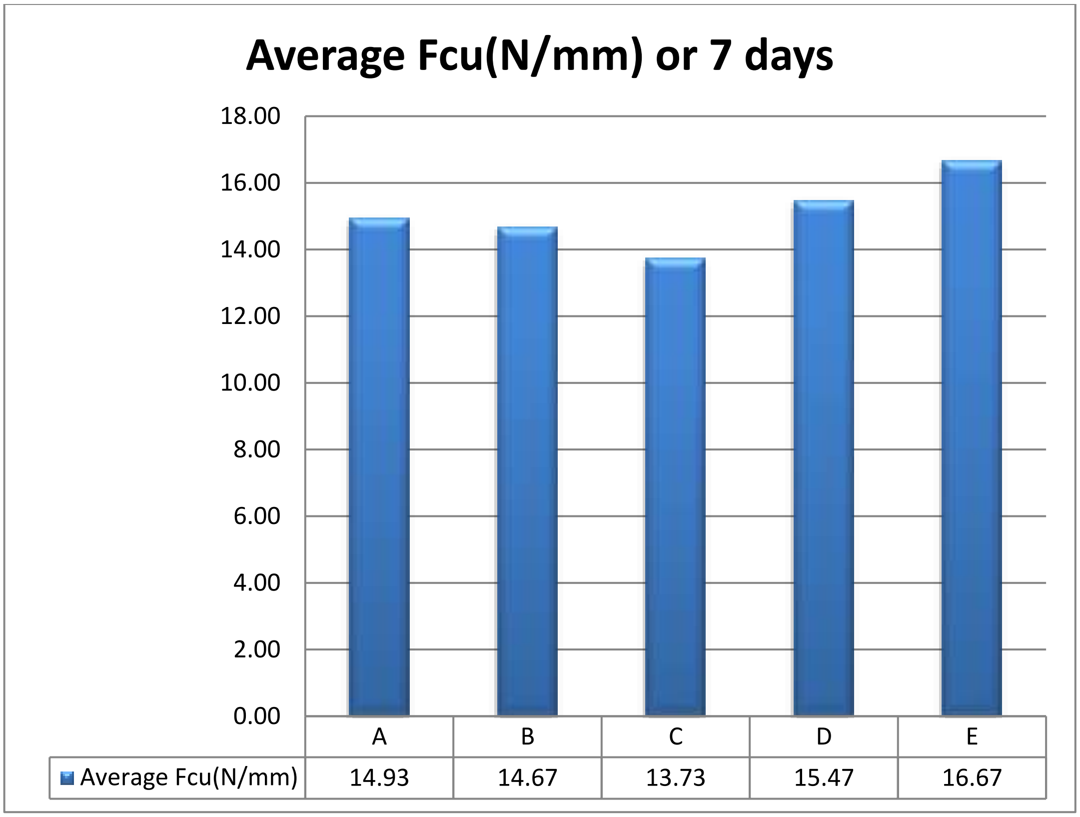
From the results obtained, it can be deduced that there is an initial decrease in the average bulk density values with the first replacements of cement by Animal Bone Ash in concrete production, however futher addition of the Animal Bone Ash ce be seen to lead to a later increase in the average bulk density value. Although such is true, the bulk density results obtained for the 0% cement replaced samples and the 12% cement replaced samples have lesser values at their 28 days curing stage as compared to their 7 days curing strength indicating a sort of discrepancy in the results.

Compressive strength test:

The compressive strength yielded the following results;

For 7 days;

The compressive strength test for the concrete cubes when tested after 7 days gave the following results -

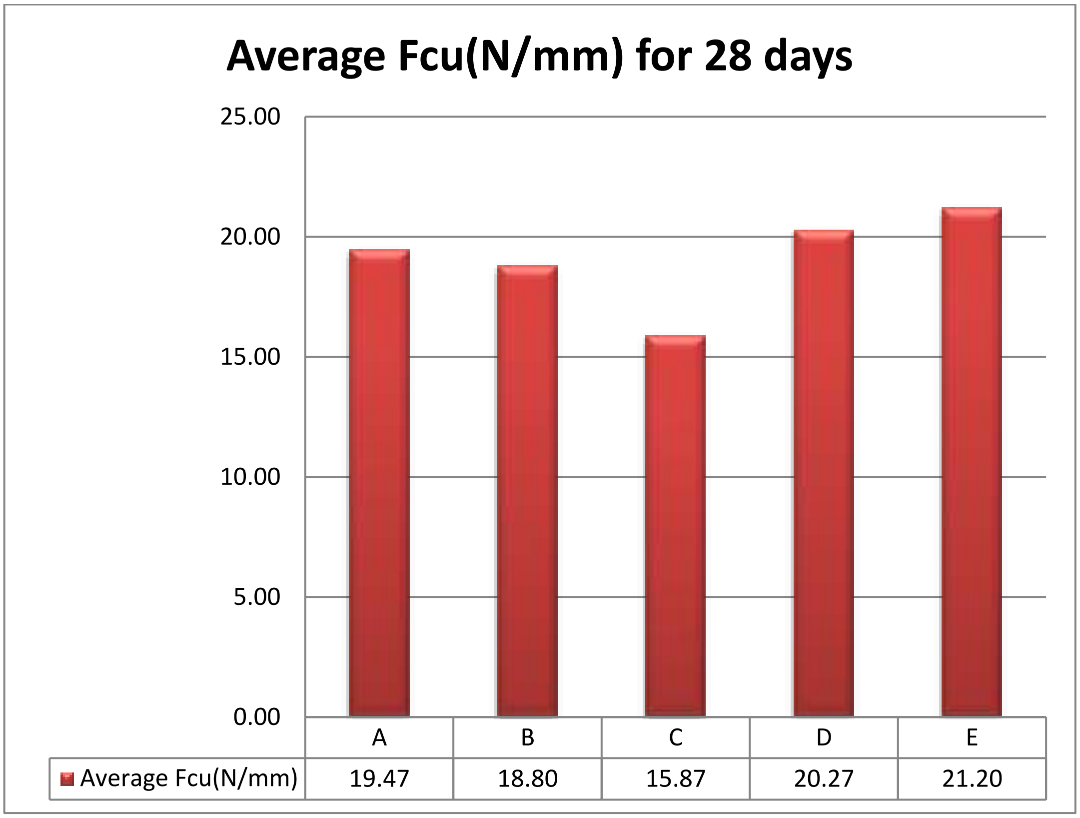


	A	B	C	D	E
■ Average Fcu(N/mm)	14.93	14.67	13.73	15.47	16.67

From the figure above it can be seen that the average compressive strength of the concrete cubes decrease between 0-6% partial replacement of cement with Animal Bone Ash, with the 3% replacement sample yielding a 1.74% decrease in strength, and the 6% replacement sample a 8.04% decrease. While an increase between in the average compressive strength o the concrete samples having 9-12% replacements of cement using Animal Bone Ash was observed with the 9% replacement samples showing an average increase of 3.62%, and the 12% replacement samples show an increase of 11.65%. The average compressive strength at 12% replacement gave the greatest value of 16.67N/mm^2, while that of the 6% replacement gave the least value of

13.73N/mm^2. The values obtained from the control sample (0//5 replacement) and the 3% replacement samples are closest to each other, with 0/5 replacement samples (control) having an average compressive value of14.93N/mm^2 and the 3% replacement samples having an average compressive value of 14.67N/mm^2. The second greatest average compressive strength value was obtained from the 9% replacement samples which gave 15.47N/mm^2. Hence from the results, it can be deduced that an intial increase in the percantge replacement of Animal Bone Ash (cattle) leads to an initial decrease in compressive strength, however, futher increase in the percentage replacement of cement by Animal Bone Ash leads to an increase in the average compressive strength values after 7 days. The percentage values are not only a result of the animal bone ash replacement, which typically causes an initial decrease in compressive strength, followed by a drastic increase as further replacement occurs, but also as a result of the action of the bacteria present. Showing that the bacteria presents also aids in increasing the average compressive strength of the concrete samples, as compared to the similar works. (1.J.O. AKINYELE, 2016) (Bih, 2022)

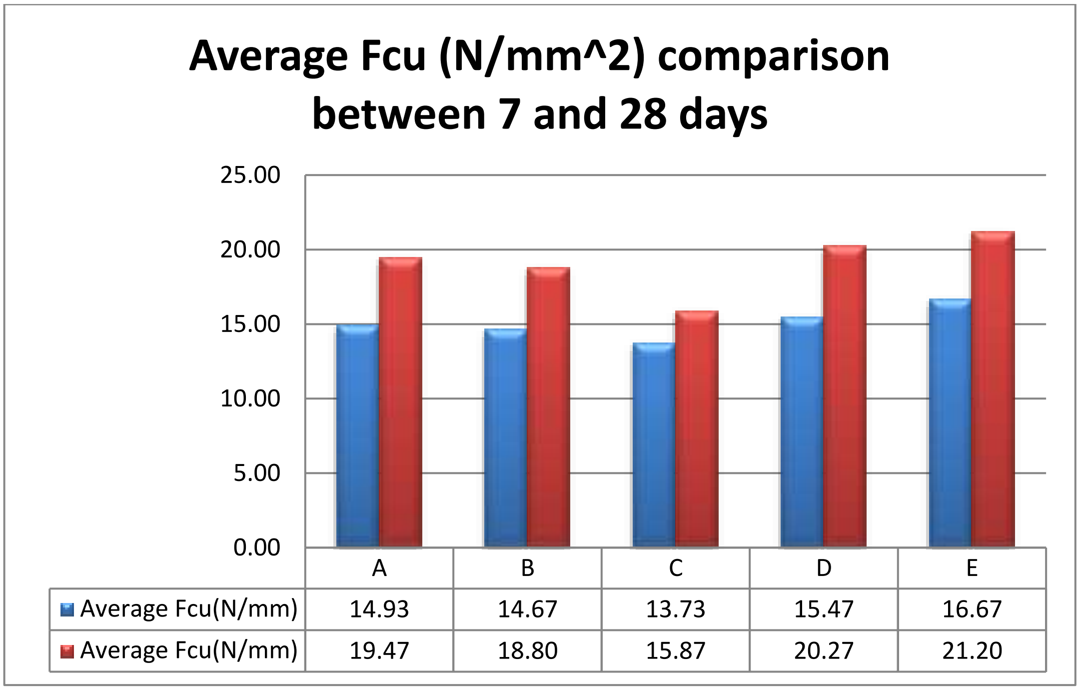
For 28 days;



From the figure above it can be seen that the average compressive strength of the concrete cubes decrease between 0-6% partial replacement of cement with Animal Bone Ash, with the 3% replacement sample yielding a 3.44% decrease in strength, and the 6%

replacement sample a 18.49% decrease. While an increase between in the average compressive strength o the concrete samples having 9-12% replacements of cement using Animal Bone Ash was observed with the 9% replacement samples showing an average increase of 4.11%, and the 12% replacement samples show an increase of 8.89%.%. The average compressive strength at 12% replacement gave the greatest value of 16.67N/mm^2, while that of the 6% replacement gave the least value of 13.73N/mm^2. The values obtained from the control sample (0//5 replacement) and the 3% replacement samples are closest to each other, with 0/5 replacement samples (control) having an average compressive value of14.93N/mm^2 and the 3% replacement samples having an average compressive value of 14.67N/mm^2. The second greatest average compressive strength value was obtained from the 9% replacement samples which gave 15.47N/mm^2. Hence from the results, it can be deduced that an intial increase in the percantge replacement of Animal Bone Ash (cattle) leads to an initial decrease in compressive strength, however, futher increase in the percentage replacement of cement by Animal Bone Ash leads to an increase in the average compressive strength values after 7 days. The percentage values are not only a result of the animal bone ash replacement, which typically causes an initial decrease in compressive strength, followed by a drastic increase as further replacement occurs, but also as a result of the action of the bacteria present. Showing that the bacteria presents also aids in increasing the average compressive strength of the concrete samples, as compared to the similar works. (1.J.O. AKINYELE, 2016) (Bih, 2022)

In comparison for 7 and 28 days;

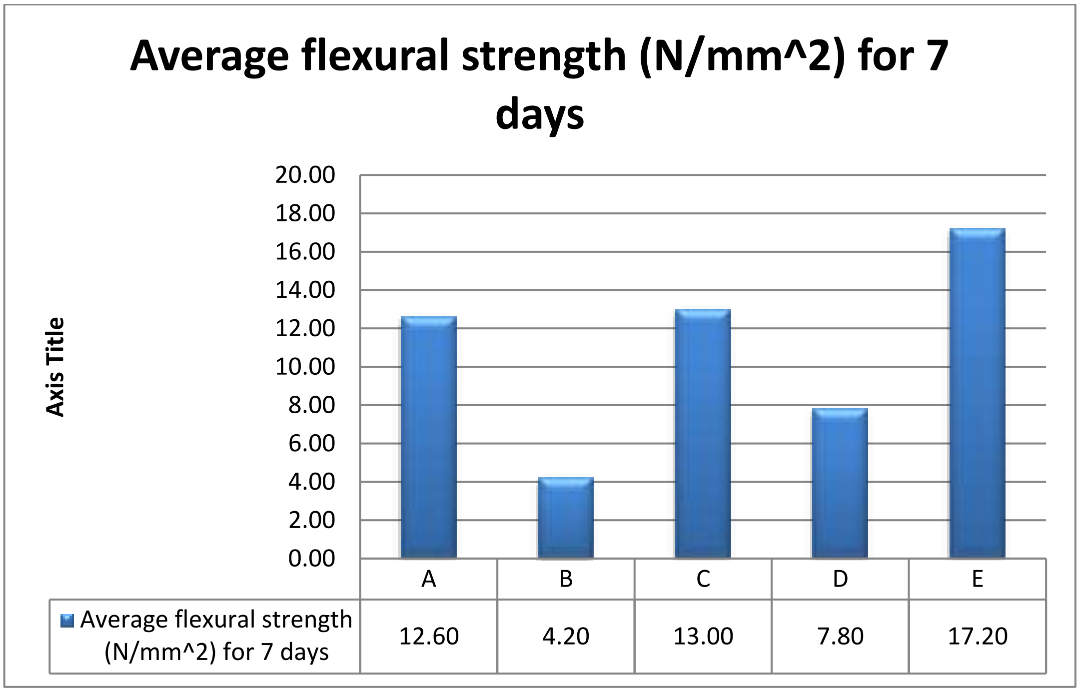


	A	B	C	D	E
🟦 Average Fcu(N/mm)	14.93	14.67	13.73	15.47	16.67
🟥 Average Fcu(N/mm)	19.47	18.80	15.87	20.27	21.20

As can be seen above, from the comparative analysis between the average compressive strength of the concrete samples between after 7 and 28 days of curing, the average compressive strengths of the concrete sample when tested after 28 days have greater values than their 7 days tested counterparts.the highest values for of the testing days were seen in the concrete samples which had 12% partially replaced by cement, with the 28 days sample having an average compressive strength of 21.20N/mm^2 and that of 7 days as 16.67N/mm^2. The results obtained were in accordance with previous similar research work (1.J.O. AKINYELE, 2016) (Bih, 2022), though the average compressive strength values are collectively higher than the those of previous research works, concerning the use of cattle bone ash as a partial replacement for cement in concrete production this is in great part due to the presence and action of the bacteria (*Bacillus sp.*) which reacts with not just the calcium lactate present in the cement samples but also the other calcium sources, i.e. cement and Animal Bone Ash (cattle).

Flexural strength test:

For 7 days;

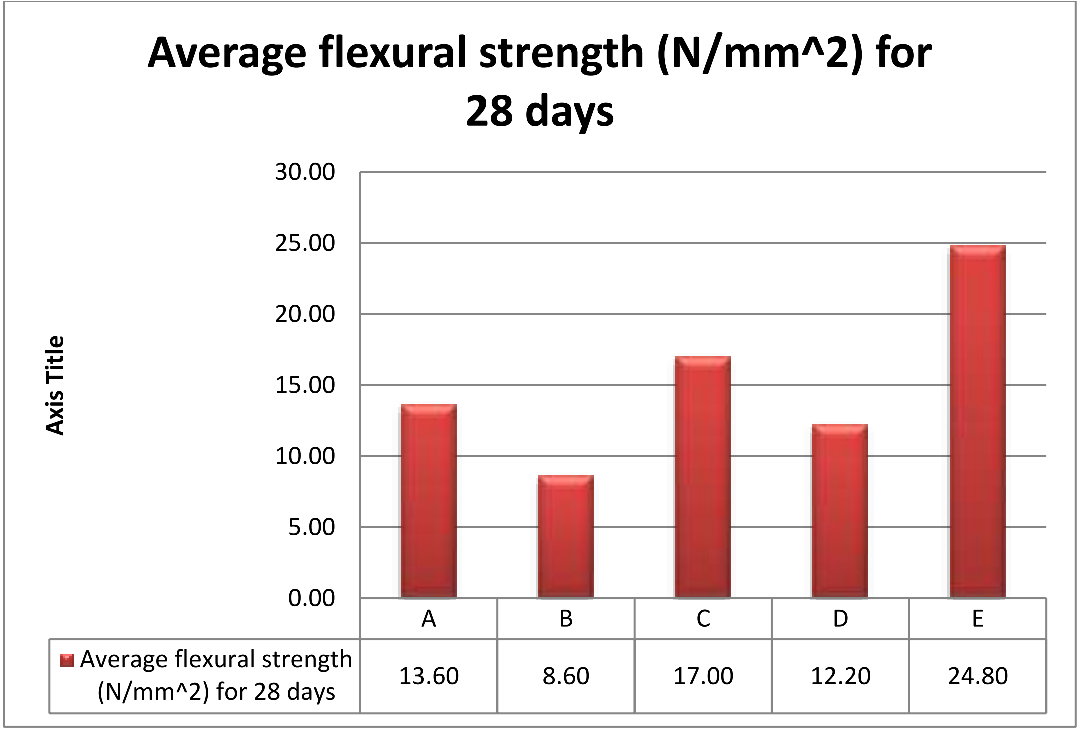


	A	B	C	D	E
■ Average flexural strength (N/mm^2) for 7 days	12.60	4.20	13.00	7.80	17.20

From the provided figure above, it can be deduced that the average flexural strength of the concrete samples has an initial decrease from 0-3% replacement of cement with Animal Bone Ash, and an increase in from 3-6%, followed by another decrease between 6-9%, and yet another increase between 9-12%. The percentage decease for the 3% cement replaced sample was 66.67%, and the decrease of the 9% cement replaced samples was 38.10%. However, the percentage increases gotten from the 6% and 12%

cement replaced samples however were 5.17% and 36.51% respectively. The highest flexural strength recorded was at 12% cement replacement, with an average flexural strength of 17.20N/mm^2 after which comes the 6% cement replacement sample with an average of 13.00N/mm^2, while the least was at 3% with an average flexural strength of 4.20N/mm^2, the results obtained from the control sample and the 6% cement replacement are closest to each other, with average flexural strength values of 12.60N/mm^2 and 13.00/mm^2 for the 0%(control) and 9% cement replacement samples respectively. Hence, it can be stated that an increase in the amount of cement replaced y Animal Bone Ash (cattle) will lead to a slight increase around the 6% replacement mark, though it reduces at around 9%, there is a significant increase at around 12% replacement after 28 days. The results obtained are in alignment with previous studies done on similar works. The variations present on the flexural strengths can be speculated to be due to the effects of the p

For 28 days:



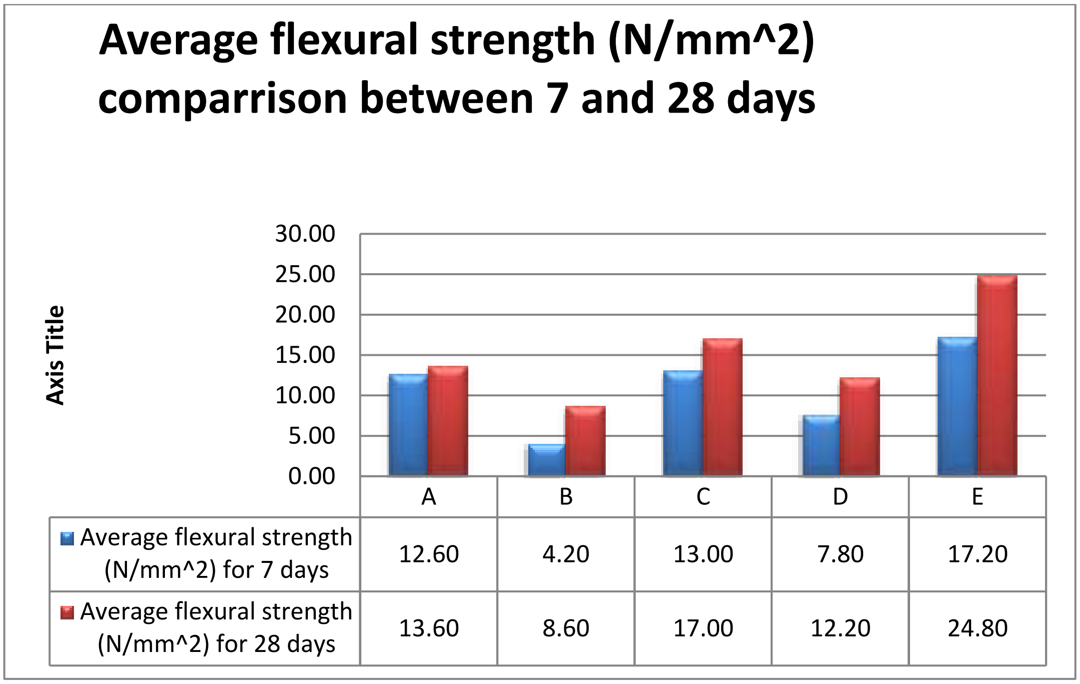
Based on the provided figure, the analysis reveals the following trends regarding the average flexural strength of the concrete samples with varying percentages of cement replacement with Animal Bone Ash, an initial decrease seen in the flexural strength shows an initial decrease when cement is replaced with Animal Bone Ash in the range of 0-3%. A subsequent increase, which is seen between 3-6% cement replacements, the flexural strength demonstrates an increase. However, between 6-9% replacements, another decrease is observed. A drastic increase can be seen notably, between 9-12%

cement replacements, there is a significant and remarkable increase in flexural strength. Specific percentages and changes: The 3% cement replaced sample experiences a decrease of 34.56% in flexural strength, while the 9% cement replaced sample decreases by 10.29%. In contrast, the 6% and 12% cement replaced samples exhibit increases of 25% and 82.35% respectively.

From the figure, the highest recorded flexural strength is observed at 12% cement replacement, with an average of 24.80N/mm^2. The sample with 6% cement replacement follows with an average of 17N/mm^2. The lowest flexural strength is observed at 3% cement replacement, with an average of 8.60N/mm^2. The control sample (0% replacement) and the 9% cement replacement sample show similar values, with 13.60N/mm^2 and 12.20N/mm^2 respectively.

Hence, it can be concluded that an increase in the amount of cement replaced by Animal Bone Ash (cattle) results in a slight increase around the 6% replacement mark, followed by a decrease at around 9%. However, there is a significant increase in flexural strength at around 12% replacement after 28 days. These findings align with previous studies on similar topics. The variations observed in flexural strengths may be attributed to the presence and actions of the bacteria (Bacillus sp.) in the concrete samples.

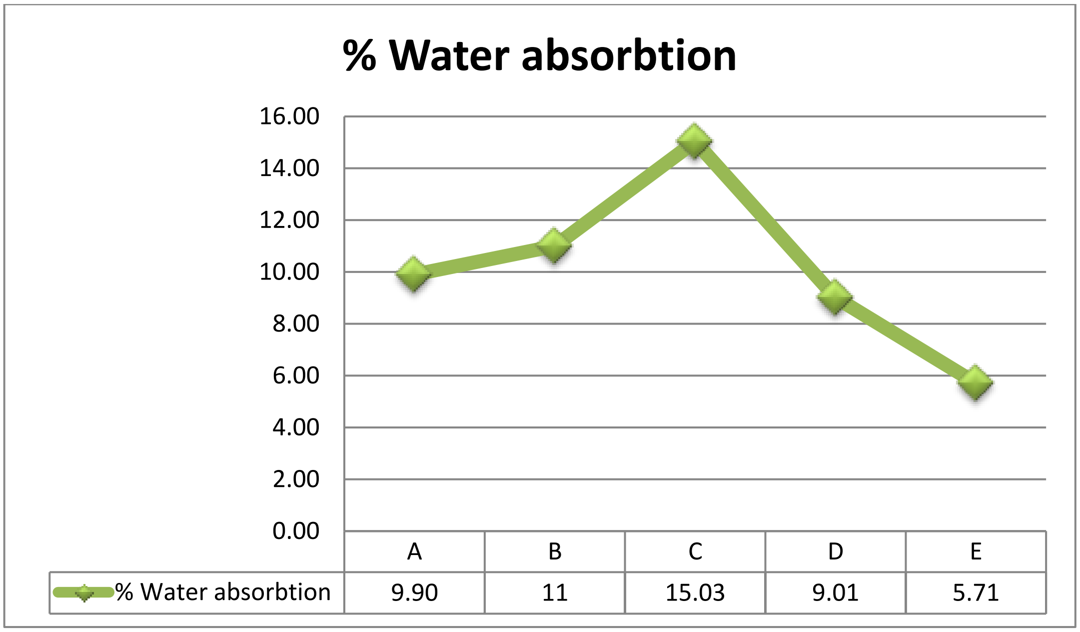
In comparison between 7 and 28 days;



	A	B	C	D	E
🟦 Average flexural strength (N/mm^2) for 7 days	12.60	4.20	13.00	7.80	17.20
🟥 Average flexural strength (N/mm^2) for 28 days	13.60	8.60	17.00	12.20	24.80

As can be seen above, from the comparative analysis between the average flexural strength of the concrete samples between after 7 and 28 days of curing, the average flexural strengths of the concrete sample when tested after 28 days have greater values than their 7 days tested counterparts. The highest values for of the testing days were seen in the concrete samples which had 12% partially replaced by cement, with the 28 days sample having an average flexural strength of 24.80N/mm^2 and that of 7 days as 17.20N/mm^2. The results obtained were in accordance with previous similar research work (1.J.O. AKINYELE, 2016) (Bih, 2022), though the average compressive strength values are collectively higher than the those of previous research works, concerning the use of cattle bone ash as a partial replacement for cement in concrete production this is in great part due to the presence and action of the bacteria (*Bacillus sp.*) This reacts with not just the calcium lactate present in the cement samples but also the other calcium sources, i.e. cement and Animal Bone Ash (cattle).

Water absorption test:



From the figure above, it can be seen that be that the samples with the greatest %water absorption are the samples with 3% Animal Bone Ash (cattle) replacement for cement, which yielded an average %water absorption of 15.03%, while the samples which gave the least values for % water absorption was recorded at the 12% cement replacement with Animal Bone Ash (cattle) with an average % water absorption of 5.71%. The control samples however have an average %water absorption of 9.01% which was noticeably close to the results obtained from the samples with 9% which had average % water absorption of 9.90%. The trend recorded from the results of this test shows that there is an initial increase in the increase of the percentage replacement of cement, with

Animal Bone Ash (cattle), as shown in the climb from 0-6% replacement sample results. However, a further increase in the percentage replacement of cement with Animal Bone Ash leads to a drastic decrease in the average % water absorption values as can be seen in the descend shown in between 6-12% replacement sample results. The trends seen form the results obtained can be as a result of the action of the bacteria (*Bacillus Sp.*) on the concrete samples, i.e. due to the action of the bacteria present, there is a possible increase in the calcite precipitation in samples with higher replacement, hence a reduction in void spacing therefore a causing a drastic reduction in the water absorption especially seen in the 12% cement replaced samples.

Microbial Induced Calcite Precipitation test:

The test on this procedure was conducted using a light microscope. This test shows the prescene and possible growth of the bacteria specimen as well as the possible precipitation of calcite particles in the different growth mediums. The specimens are transferd to a microscope plate through the use of an inoculateing loop which has been sterilized.

Under the light microscope, it was obsereved that the bacterial particles which are known to apper in rod like shapes had higher growth in the medium of pure calcium lacteate and lesser in that of Animal Bone Ash. There ws also noticeable calcite precipitation in the calcium lactate medium, and less in the Animal Bone Ash medium. This can be due to the concentration of Ca ions present in each.

Conclusion and Recommendations

Conclusion:

Based on the information obtained from the experimental study carried out, on the various combinations used to produce the different samples of concrete, with the aim of producing concrete with significant strength of grade M15 concrete with probable self healing capacities, with potential economical and environmental impacts in view, the following deductions can be drawn;

- The compressive strength values obtained from the control sample, 3% , 6%, 9% and 12% replacement of cement by Animal Bone Ash yielded 19.47 N/mm^2, 18.87 N/mm^2, 15.87 N/mm^2, 20.27 N/mm^2 and 21.20 N/mm^2 respectively, after 28 days of curing.
- The flexural strength values obtained from the control sample, 3% , 6%, 9% and 12% replacement of cement by Animal Bone Ash yielded 13.60N/mm^2, 8.60N/mm^2, 17.00 N/mm^2, 12.20 N/mm^2 and 24.80 N/mm^2 respectively, after 28 days curing.

- The total average bulk density of the aggregate used was about 2433.6kg/m^3 for 28 days, and that of 7 days was 2332.8kg/m^3, indicating a total average increase in the average bulk density of the concrete samples.
- The value obtained from the average bulk density values indicates that the concrete samples have good bulk density.
- The 3% replacement samples have the least desired properties in terms of compressive strength, flexural strength and water absorption, however the 12% replacement samples attained the most desired mechanical properties, hence can be advised to be used in a case of construction.
- The presence and action of bacteria (*Bacillus Sp.*) and calcium lactate present in the concrete samples aided in the precipitation of calcite particles, which reduced void spaces, and help to improve the average engineering properties of the concrete samples.
- From the Microbial Induced Calcite Precipitation test (MICP), the presence of turbidity in the nutrient broth culture flasks, shows that there was successful bacteria growth as well calcite precipitation as seen using the microscope. This is further supported by the successful replication of the bacteria (*bacillus Sp.*) which can be visibly seen in the agar growth plates, and was be further tested using colony count system.

In conclusion, a replacement of cement using Animal Bone Ash up 12% can be quite profitable in the presence of calcite producing bacteria (*Bacillus Sp.*), having the highest values in the average bulk density, compressive strength and flexural strength and the least value in average % water absorption.

Recommendations:

Although the study provides us with an insight full look into the action and effects of the various materials used in the production of the concrete samples used in different mixes, the study still leaves room for many questions to be answered and hence it is recommended that;

- Other percentage values for the partial or total replacement of cement can be examined as to obtain the optimum or best possible percentage replacement to be used.
- Further investigations should be carried out on the effect of bacteria specimen (*Bacillus Sp.*) on the calcite precipitation process using Animal Bone Ash as its main calcium source e.g. use of SEM, XRF test to obtain further information.
- Other mix ratios can be used in order to verify the claims of this study.

- A handful of experiments aimed at determining other structural properties of the concrete should be carried out to further validate the results obtained from this work.
- Further experimentation be carried on other alternative cementious materials, with an aim of discovering the best possible material in terms of economic management, environmental friendliness and mechanical properties of prepared concrete.

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