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Effects of Spent Industrial Calcium Carbide on Soil Microbial and Physicochemical Properties

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Abstract
The effects of different concentrations of spent calcium carbide on soil microbial and physiochemical properties were studied using standard microbiological and biochemical techniques. Soils were contaminated with varying concentrations (5 g/kg, 25 g/kg, 50 g/kg, 100 g/kg, 200 g/kg, and 400 g/kg) of spent carbide with the control having 0 g/kg of spent carbide. The effects of spent carbide were monitored weekly for four (04) weeks. Soil pH varied from 6.10±0.00 to 12.40±0.01. Total nitrogen, organic carbon, phosphorus and moisture ranged from 0.02±0.00 to 0.23±0.02 %, 9.16±0.05 to 48.76±1.00 %, 11.30±0.05 to 31.11±0.60 % and 2.88±0.06 to 6.91±0.20 % respectively. Cation exchange capacity (CEC) increased from 1.53±0.01 to 5.03±0.30 meq/100g and electrical conductivity (EC) from 36.5±1.05 to 390.2±3.00 µs/cm. The bacterial isolates which survived the harsh spent calcium carbide waste at 400 g/kg after 4 weeks include Bacillus subtilis, Acinetobacter calcoaceticus, Pseudomonas putida, Micrococcus luteus and Bacillus pumilis while the fungal isolates include Penicillium chrysogenum, Aspergillus niger and Rhizopus stolonifer. The total viable bacterial count decreased from 4.27×10^5±17.0 to 1.99×10^3±22.0 cfu/g and the fungi from 2.04×10^4±18.0 to 2.19×10^2±21.0 cfu/g after 4 weeks. Spent carbide waste is mainly composed of calcium oxide (Ca0) which constitute about 44.99±1.50 % of the waste. The most abundant heavy metal in the waste was zinc with a concentration of 10.11±0.62 mg/kg and the least was mercury (0.01±0.00 mg/kg). The results obtained in the study revealed that spent calcium carbide had adverse impact on both soil physiochemical and microbial numbers.

Keywords: Calcium carbide, soil, concentration, heavy metals, microorganisms

Introduction
A sustainable soil ecosystem is a major determinant for environmental sustainability as soil is the key component of natural ecosystem. Soil is a complex, heterogeneous and a living-dynamic part of the environment that consists of living organisms and dead organic and inorganic matter which developed as a result of pedogenic processes during and after weathering of rocks [1, 2]. Soil represent the interface of three material states: solids (geological and dead biological materials), liquids (water) and gases (air and soil pores). Each soil is a unique product of the combination of geological parent material, glacial and geomorphological history. Soils are the foundation of all terrestrial ecosystems and home to a vast diversity of bacteria, archaea, fungi, insects, annelids and other invertebrates as well as plants and algae [2]. Microorganisms are found in large numbers in soil with bacteria and fungi being the most prevalent. It is stated that usually more than 10^9 microorganisms are present per gram of soil representing 4000 to 7000 different genomes and biomass of 300 to 3000 kg per ha [3]. Soil varies greatly depending on climate, organisms, land form and parent material. Over time, these factors interact so that soils develop characteristic horizons, which are, the A,B,C and in addition an organic horizon may be present especially in forest region, called the O-horizon.[4]. The A-horizon is the topsoil or surface soil. The top soil is subjected to marked leaching and has the highest density of soil microbes and plant roots and is the site of considerable organic matter decomposition and humification in soil [5]. It is also the reservoir of microbial food and it receives the greatest impact from pollutants.

Calcium carbide is a chemical compound with the chemical formula of CaC_2 with a relative molecular mass of 64. Its main use industrially is in the production of acetylene and calcium cyanamide [6]. The pure material is colourless, however, pieces of technical grade calcium carbide are grey or brown. Calcium carbide residue (CCR) or calcium carbide waste (CCW) or spent calcium carbide (SCC) is a by-product from the use of calcium carbide to generate acetylene gas. The acetylene gas is widely used in ripening of fruits in agriculture, industrial welding and fabrication work while the waste after welding is usually discarded carelessly in the environment which sooner or later gets incorporated into the soil and pose a threat to it. Spent calcium carbide is whitish or grayish in colour and has high alkaline pH. It is composed mainly of calcium compounds such as calcium hydroxide ($Ca(OH)_2$) which reacts over time with atmospheric carbon dioxide to form non-toxic calcium carbonate [7]. Research on air-exposed waste has shown that such waste does not change into calcium carbonate for at least a decade and even over 13 years [8]. The pH of such waste remains strongly alkaline, which is the cause of toxicity [9]. Only dilution at a waste-to-water ratio of 1:1000 causes decrease in pH sufficient for it to be harmless to the environment [7].

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Higher organisms depend on microorganisms and their by-products for growth and development. Any factor that affects soil microorganisms will also affect soil health since soils influence most ecosystem services on which we depend. The aim of this study was to investigate the effects of spent calcium carbide on soil physiochemical and microbial properties.

Material and Methods

Collection of Samples

Spent calcium carbide was obtained from the waste dumpsite of calcium carbide used by welders for their welding work in vehicle maintenance shop in Ugbowo, Benin City, Nigeria. Spent calcium carbide was sun dried and milled into finer particles and weighed into 10 g, 50 g, 100 g, 200 g, 400 g and 800 g respectively. Loamy soil was dug up from a farm in Ugbowo, Benin City, Nigeria with no recorded incidence of spent carbide pollution.

Soil Preparation

Loamy soil was dug from a depth of 1-30cm; it was bulked, homogenized and filtered through a 2 mm mesh sieve. Two thousand gram (2000 g) of the soil was weighed using an analytical weight balance (XPE Mettler-Toledo, Ohio, USA) into 7 experimental buckets each having 0 g, 10 g, 50 g, 100 g, 200 g, 400 g and 800 g of spent carbide and the spent carbide and soil sample were mixed thoroughly. The buckets were labeled according to the quantity of spent carbide they contain. Thus the concentrations were 0 g/kg, 5 g/kg, 25 g/kg, 50g/kg, 100 g/kg, 200 g/kg and 400 g/kg respectively. The impact of spent calcium carbide contamination was monitored weekly for a period of four weeks at room temperature (28±2 ^{0}C).

Physicochemical Analyses

Determination of pH

Twenty grams (20 g) of the soil sample was weighed into a 100 ml glass beaker. Twenty milliliters (20 ml) of sterile distilled water was added and the suspension was stirred continuously for 30 min. The mixture was allowed to stand for another 30 min to settle. Prior to usage, the Hanna microprocessor electrode was calibrated using freshly prepared pH buffer, 4.0, 7.0, and 9.0 solutions respectively. A Hanna microprocessor pH meter was dipped into the solution and steady readings noted [10].

Determination of Electrical Conductivity

Twenty grams (20 g) of the fresh sample was weighed into a 100 ml glass beaker. Twenty milliliters (20 ml) of sterile distilled water was added and the suspension was stirred continuously for 30 minutes. The mixture was allowed to stand for another 30 minutes undisturbed to allow it settle. A Digital conductivity meter (Lab 254, Labtech International, India) was used in determining soil conductivity by dipping the sensitive electrode into the mixture and a steady reading taken [10].

Determination of Total Organic Carbon Content

Total organic carbon content of each soil sample was determined using the method described by [11].

Determination of Total Nitrogen

The total nitrogen content of the soil samples were determined using micro Kjeldahl digestion and colorimetric method [12]. The solution was read colorimetrically at 630 nm, using a spectrophotometer (Jenway 6305, Bibby Scientific, UK).

Determination of Available Phosphorus

This was carried out using the method of [13] with five (5) grams of the soil sample. The absorbance of the resulting solution was read at 660 nm using a spectrophotometer (Jenway 6305, Bibby Scientific, UK) [13]

Determination of Moisture Content

An aluminum dish was pre-weighed using a sensitive weigh balance (XPE Mettler-Toledo, Ohio, USA). Ten (10) grams of the soil sample was transferred to the dish and weight of both the dish and soil was recorded. The dish containing the soil sample was placed in a hot air oven at 130^{0}C and dried to obtain a constant weight. The dish was immediately transferred to a desicator and allowed to cool for 30 min. The resultant weight was taken. The moisture content was calculated and recorded as a percentage by weight of the respective soil sample [14].

Determination of Cation Exchange Capacity

Five (5) grams of air dried soil was weighed into a plastic bottle. One hundred (100) milliters of neutral 1 M ammonium acetate was then added to the soil and the mixture shaken with the aid of a mechanical shaker for 30 min. The concentration of the exchangeable cations (Na, Ca, K and Mg) in the filtrate was determined using a flame photometer (Jenway PFP7, Bibby Scientific, UK). The blank utilized was ammonium acetate [15,16].

Quantitative Determination of Oxides and Heavy Metals in Spent Calcium Carbide

One gram (1 g) of the soil sample was weighed into a beaker for acid digestion. Analysis was done using Atomic Absorption Spectrophotometer (Analyst 200 Pelkin Elmer, Massachusetts, USA) [14, 15,16].

Isolation and Enumeration of Total Heterotrophic Bacterial and Fungal counts

Serial dilution and pour plated methods [17] were used to determine the total viable microbial counts of the soil sample. Peptone water was prepared, sterilized and used as diluent. Commercially available nutrient agar (NA) and potato dextrose agar (PDA) (Oxoid, UK) were prepared and sterilized according to the manufacturer's

instructions and used in the enumeration of the total heterotrophic bacterial and fungal counts respectively. Ten (10) grams of sample was mixed with ninety (90) ml of sterile distilled water to obtain the stock solution of the sample. The sample was serially diluted and using pour plate procedure, 1ml aliquots from each dilution was dispensed into the plate in triplicates on sterile nutrient agar for total heterotrophic bacterial count and potato dextrose agar (PDA) for total heterotrophic fungal counts. The nutrient agar plates were incubated at 28±2^{0}C for 24–48 h for bacterial counts while the PDA plates were incubated at (28±2 ^{0}C) for 72 h. After incubation, the resultant discrete microbial colonies obtained from culture plates were counted and recorded and multiplied by the dilution factor and expressed as colony forming unit (CFU).

Characterization and Identification of Microbial Isolates

Representative bacterial and fungal colonies were sub-cultured by surface streak method onto freshly prepared sterile agar plates of NA and PDA and slants using sterile inoculating wire loop under aseptic conditions [17] and incubated at 28±2 ^{0}C. Biochemical tests were conducted to ascertain the identity of the subcultured bacterial isolates [17]. The results of the tests were compared with reference tables in Bergey's manual of determinative bacteriology [18]. Portions of the sub cultured fungal mycelia were picked with the aid of a sterile inoculating needle and placed on grease free microscope slide and stained with lactophenol-cotton blue reagent [17]. Mycelia observed were compared with standard identification keys [19].

Statistical analysis

Reported values are averages from three independent trials. All experimental data were subjected to statistical analysis of variance (ANOVA). The significant value was evaluated using t-distribution (α= 0.05) using appropriate computer software.

Results and Discussion

Physiochemical analyses

The results of the physiochemical analyses are presented in Tables 1 and 2. Variations in pH were observed with increase in pH values in all the contaminated soils with spent calcium carbide (SCC). The pH ranged from 6.10±0.00 to 7.40±0.00, 7.90±0.00 to 8.80±0.00, 9.30±0.00 to 10.30±0.01, 9.60±0.01 to 10.80±0.00, 10.10±0.00 to 11.20±0.20 and 11.30±0.02 to 12.40±0.01 for the 5, 25, 50, 100, 200, and 400 g/kg spent carbide contaminated soils respectively over a period of 4 weeks. However the control soil without carbide had a relatively stable pH of 5.20±0.00 to 5.30±0.00 which was significantly different ($p < 0.05$) from those of the SCC contaminated soils.

The concentration of total nitrogen was adversely affected by the pollution with spent calcium carbide. The control soil sample had a nitrogen content which ranged between 0.19±0.00 to 0.27±0.00 %. There was no significant difference ($p>0.05$) in soils which received 5 and 25 g/kg SCC as they had nitrogen content which were comparable to the control (Table 1) Total nitrogen ranged from 0.15±0.00 to 0.23±0.02 % and 0.11±0.01 to 0.23±0.01 % for 5 and 25 g/kg SCC contaminated soils. With 50, 100, 200 and 400 g/kg SCC, the total nitrogen within four weeks was low and in the range 0.02±0.00 to 0.09±0.00 % and significantly different ($p < 0.05$) from the control.

Organic carbon increased with the increase in the concentration of SCC in the soil samples. The mean organic carbon ranged from 9.16±0.05 to 12.87±0.00 % (5 g/kg), 24.77±0.00 to 27.76±0.30 % (25 g/kg), 32.37±1.00 to 38.250.00 % (50 g/kg), 34.09±0.20 to 40.150.50 % (100 g/kg), 34.55±0.00 to 46.33±0.02 % (200 g/kg) and 35.18±0.15 to 48.761.00 % (400 g/kg) in the spent carbide contaminated soils. These values were significantly different ($p < 0.05$) from the control sample with 2.73±0.00 to 2.78±0.00 %. Phosphorus ranged from 11.30±0.05 to 15.90±0.10 % (5 g/kg), 14.90±0.00 to 19.13±0.07 % (25 g/kg), 17.67±1.00 to 21.70±0.00 % (50 g/kg), 20.96±0.00 to 26.93±1.00 % (100 g/kg), 21.53±0.00 to 29.48±0.40 % (200 g/kg), 23.77±0.60 to 31.11±0.60 % (400 g/kg) in the spent carbide contaminated soils and 7.83±0.00 to 8.10±0.00 % (0 g/kg) in the control soil sample (Table 1). The control values were lower and significantly different from those of SCC contaminated soils.

The cation exchange capacity (CEC) ranged from 1.53±0.01 to 2.55±0.02 meq/100g (5 g/kg), 1.86±0.00 to 3.14±0.01 meq/100g (25 g/kg), 2.27±0.00 to 3.86±0.50 meq/100g (50 g/kg), 2.53±0.00 to 4.14±0.00 meq/100g (100 g/kg), 2.88±0.01 to 4.76±0.70 meq/100g (200 g/kg), 3.92±0.05 to 5.03±0.30 meq/100g (400 g/kg), in the spent carbide contaminated soil and 1.45±0.00 to 1.60±0.00 meq/100g (0 g/kg) in the control soil sample. Electrochemical conductivity (EC) ranged from 36.5±1.05 to 40.9±0.03 µs/cm (5 g/kg), 66.8±0.00 to 77.5±0.07 µs/cm (25 g/kg), 174.9±1.00 to 196.3±1.00 µs/cm (50 g/kg), 194.3±0.00 to 234.3±0.00 µs/cm (100 g/kg), 251.3±2.50 to 301.7±0.80 µs/cm (200 g/kg), 344.7±1.00 to 390.2±3.00 µs/cm (400 g/kg) in the spent carbide contaminated soil and 32.3±0.10 to 32.7±00 µs/cm (0g) in the control soil sample (Table 1). Moisture content was limited by the presence of spent carbide. Moisture content ranged from 5.29±0.00 to 6.91±0.20 % (5 g/kg), 4.03±0.1 to 5.87±0.01 % (25 g/kg), 3.94±0.04 to 5.25±0.00 % (50 g/kg), 3.30±0.05 to 4.92±0.00 % (100 g/kg), 3.19±0.20 to 4.64±0.02 % (200 g/kg), 2.88±0.06 to 3.72±0.00 % (400 g/kg) in the spent carbide contaminated soils and 9.47±0.00 to 10.09±0.05 % (0 g/kg) in the control soil sample (Table 1).

Table 1: Physicochemical Parameters at different concentrations of spent carbide contaminated soil

Parameters								
Cont-amin-ation period (Weeks)	SCC Concen-tration (g/Kg)	pH	N (%)	**Org. C (%)**	**P (%)**	**CEC (meq/100g)**	**EC (µs/cm)**	**Moisture content (%)**
	0	5.30±0.00[a]	0.27±0.00a	2.73±0.00a	8.10±0.00a	1.60±0.00a	32.50±2.00a	10.09±0.05a
1	5	6.10±0.00[b]	0.23±0.02a	9.16±0.05b	11.30±0.05b	1.53±0.01b	36.50±1.05b	6.91±0.20b
	25	7.90±0.00[bc]	0.15±0.00b	24.77±0.00c	14.90±0.00c	1.86±0.00c	66.80±0.00c	5.87±0.01c
	50	9.30±0.00d	0.09±0.00c	32.37±1.00d	17.67±1.00d	2.27±0.00d	174.90±1.00d	5.25±0.00d
	100	9.60±0.01d	0.07±0.00c	34.09±0.20d	20.96±0.00e	2.53±0.00d	194.30±0.00e	4.92±0.00e
	200	10.10±0.00e	0.05±0.00d	34.55±0.00d	21.53±0.00f	2.88±0.01e	251.10±2.50f	4.64±0.02f
	400	11.30±0.02f	0.03±0.00d	35.18±0.15d	23.77±0.60g	3.92±0.05f	344.70±1.00g	3.72±0.00g
	0	5.20±0.00a	0.24±0.02[a]	2.73±0.00a	8.03±0.00a	1.53±0.00a	32.30±0.10a	9.91±0.10a
2	5	6.60±0.00b	0.21±0.00b	10.14±0.00b	11.90±0.00b	1.82±0.02b	39.70±0.00b	6.37±0.06b
	25	7.90±0.00bc	0.23±0.01b	25.74±0.06c	15.53±0.00c	2.01±0.00c	67.10±0.05c	5.10±0.00c
	50	9.60±0.01d	0.07±0.00c	33.35±0.10d	17.99±0.00d	2.63±0.00d	180.30±0.00d	4.98±0.20d
	100	9.90±0.00d	0.05±0.00c	34.56±0.20d	23.58±1.00e	2.90±0.01e	198.50±2.00e	4.41±0.00e
	200	10.50±0.01e	0.04±0.00cd	36.01±0.00d	23.77±0.30e	3.04±0.00f	270.10±0.00f	4.13±0.05f
	400	11.70±0.00f	0.03±0.00d	38.38±1.00e	25.31±1.00f	4.17±0.05g	355.30±5.00g	3.56±0.00f
	0	5.20±0.00a	0.21±0.02a	2.73±0.00a	8.01±0.00a	1.49±0.00a	32.70±0.00a	9.83±0.00a
3	5	6.90±0.00b	0.17±0.00b	11.03±0.05b	12.30±0.00b	2.37±0.00b	40.30±0.03b	5.81±0.01b
	25	8.10±0.00c	0.14±0.01c	26.09±0.00c	17.01±0.00c	2.96±0.00c	69.30±0.06c	4.67±0.04c
	50	9.90±0.01d	0.06±0.00d	35.52±1.00d	19.32±0.20c	3.07±0.01c	184.70±0.00d	4.31±0.10d
	100	10.10±0.00e	0.03±0.00e	39.04±0.00e	25.09±0.10d	3.58±0.00d	210.80±0.05e	4.01±0.03e
	200	10.90±0.00e	0.03±0.00e	41.50±0.00f	27.35±0.00d	3.89±0.02e	292.60±1.10f	3.63±0.00f
	400	11.90±0.03f	0.02±0.00e	43.91±2.00f	28.78±0.05de	4.62±0.01f	377.80±3.00g	3.07±0.02g
	0	5.30±0.00a	0.19±0.00a	2.78±0.00a	7.83±0.00[a]	1.45±0.00a	32.30±0.00a	9.47±0.00a
4	5	7.40±0.00b	0.15±0.01b	12.87±0.00b	15.90±0.10[b]	2.55±0.02b	40.90±0.03b	5.29±0.00b
	25	8.80±0.00c	0.11±0.01c	27.76±0.30c	19.13±0.07c	3.14±0.01c	77.50±0.07c	4.03±0.10c
	50	10.30±0.01d	0.04±0.00d	38.25±0.00d	21.70±0.00d	3.86±0.50d	196.30±1.00d	3.94±0.04d
	100	10.80±0.00d	0.03±0.00d	40.15±0.50d	26.93±1.00[d]	4.14±0.00e	234.30±0.00e	3.30±0.05e
	200	11.20±0.20e	0.02±0.00d	46.33±0.02e	29.43±0.40e	4.76±0.70f	301.70±0.80f	3.19±0.20f
	400	12.40±0.01f	0.02±0.00d	48.76±1.00f	31.11±0.60f	5.03±0.30g	390.20±3.00g	2.88±0.06g

Values with different letters within a column indicate significant difference ($p<0.05$)
Key: EC: Electrical conductivity, CEC: Cation Exchange capacity Org.C: Organic Carbon, N: Total Nitrogen, SCC:Spent calcium carbide

The moisture content of the control was higher than the SCC contaminated soils and significantly different ($p < 0.05$) from them during the 4 weeks experimental study.
The chemical composition of spent calcium carbide and associated heavy metals is shown in Table 2. The result showed that spent calcium carbide is composed mainly of calcium and silicon compounds in the form of calcium oxide (CaO) and silicon oxide (SiO_2) which made up 44.99±1.50 % and 10.09±0.01 % of the total waste volume respectively. The heavy metals associated with the waste include lead, zinc, copper, nickel, iron, arsenic and mercury. Zinc and copper had the highest concentration of 10.11±0.62 and 4.49±0.05 mg/kg respectively. The lowest was mercury with 0.01±0.00 mg/kg (Table 2).

Microbiological analyses

The results of the microbial analyses of spent calcium carbide (SCC) treated soils are shown in Tables 3 and 4. The total heterotrophic bacterial count of the control was stable in the range 4.17×10^5±23.0 to 4.47×10^5±15.0 cfu/g during the 4 weeks study. Considerable reductions in the bacterial populations were observed in the spent carbide contaminated soils during this period. The viable population in 5, 25, 50, 100, 200 and the 400 g/kg SCC contaminated soil were in the range 1.35×10^5±21.0 to 2.19×10^5±20.0 cfu/g, 4.89×10^4±22.0 to 7.08×10^4±14.0 cfu/g, 3.80×10^4±21.0 to 6.03×10^4±24.0 cfu/g, 1.66×10^4±17.0 to 2.69×10^4±15.0 cfu/g, 2.82×10^3±14.0 to 4.68×10^3±23.0 cfu/g and 1.99×10^3±20.0 to 2.95×10^3 ±19.0 cfu/g respectively. These values are significantly different ($p < 0.05$) when compared with the control.

Table 2: Chemical Composition of spent calcium carbide and associated heavy metals

Parameter	**Composition (%)**
SiO_2	10.90±0.01
Al_2O_3	6.31±0.20
P_2O_5	0.01±0.00
PbO	6.10±0.00
CuO	7.60±0.01
K_2O	1.60±0.00
CaO	44.99±1.50
MgO	7.1±0.01
TiO_2	1.30±0.00
Cr_2O_3	5.00±0.02
MnO	3.00±0.01
Fe_2O3	3.60±0.03
Others	2.41±0.00
Heavy metal	
Pb (mg/kg)	0.93±0.05
Zn (mg/kg)	10.11±0.62
Cu (mg/kg)	4.49±0.05
Ni (mg/kg)	0.29±0.00
Fe(mg/kg)	0.66±0.01
As (mg/kg)	0.06±0.00
Hg (mg/kg)	0.01±0.00
Cd (mg/kg)	2.45±1.05

The effect was more with the 200 and 400 g/kg SCC where no growth was recorded for the first two weeks of contamination (Table 3). The mean bacterial population at the end of 4 weeks study in the control and all spent carbide contaminated soils were significantly different ($p < 0.05$) from each other.

The toxicity of SCC on fungal population followed a similar trend as the bacteria. While the control uncontaminated soil had a stable fungal population of 1.91×10^4±21.0 to 2.04×10^4 ±23.0±18.0 cfu/g over four weeks, the population of viable fungi decreased as the concentration of the SCC increased with the concomitant result of 0 to 2.19×10^2 ±21.0 cfu/g in the 400 g/kg contaminated soil sample after 4 weeks. This value was significantly different ($p < 0.05$) from the control.

The bacterial isolates which survived the harsh SCC waste at 400 g/kg after 4 weeks include *Bacillus subtilis, Acinetobacter calcoaceticus*, *Pseudomonas putida*, *Micrococcus luteus* and *Bacillus pumilis* while the fungal isolates include *Penicillium chrysogenum, Aspergillus niger and Rhizopus stolonifer*. These isolates were present in the control soil sample, however others were inactivated or inhibited and could not survive (Table 5). The ability of these organisms to survive environmental stress created by SCC, points to the likely possession of *sur*- genes or metabolic plasmids as reported by Bennett [20].

It has been accepted widely that soil influences most ecosystem functions in the sustenance of life on earth as soil is the key component of natural ecosystem [1]. Environmental pollution is one of the most serious threats faced by mankind today, as the importance of soil to continue survival has been taken for granted by human practices. The soil is being contaminated by pollutants which have significant deleterious consequences on the ecosystem as this can result in changes in soil which can manifest in the alteration of the metabolism of endemic soil microorganisms and also reduces the soil's biodiversity [2].

Spent calcium carbide caused a decrease in the population of heterotrophic microorganisms in the various concentrations of the waste used. The higher the concentration of the waste, the greater the reduction of microbial load in the soil samples. The bacterial populations were more decimated compared to the fungi. The resurgence of microbial population with treatment of 400 g/kg spent calcium carbide waste in the soil after 2 weeks following an initial decimation can be attributed to their wide distribution and their ability to adapt in extreme stress conditions. This is in concord with [21] who reported that spent calcium carbide is highly toxic but loses its toxicity within a short period of time. However the stabilization of microbial numbers in spent calcium carbide contaminated soil will require not just days but weeks [22].

Table 3: Changes in Total Heterotrophic Bacteria Counts (cfu/g) of Spent Carbide Treated Soils

Concentration of spent calcium carbide (g/kg)	Period of CaC_2 impact (Week)			
	1	2	3	4
0	$4.27\times10^5\pm17.0$a	$4.27\times10^5\pm17.0$a	$4.47\times10^5\pm15.0$a	$4.17\times10^5\pm23.0$a
5	$2.19\times10^5\pm20.0$b	$1.91\times10^5\pm19.0$b	$1.62\times10^5\pm13.0$b	$1.35\times10^5\pm21.0$b
25	$7.08\times10^4\pm14.0$c	$5.25\times10^4\pm18.0$c	$4.90\times10^4\pm15.0$c	$4.89\times10^4\pm22.0$c
50	$6.03\times10^4\pm24.0$c	$4.68\times10^4\pm19.0$c	$3.80\times10^4\pm21.0$d	$4.17\times10^4\pm25.0$d
100	$2.69\times10^4\pm15.0$d	$2.24\times10^4\pm12.0$d	$1.66\times10^4\pm17.0$e	$1.95\times10^4\pm19.0$e
200	NGe	$4.68\times10^3\pm23.0$e	$2.82\times10^3\pm14.0$g	$3.16\times10^3\pm18.0$f
400	NGe	NGf	$2.95\times10^3\pm19.0$f	$1.99\times10^3\pm22.0$g

NG = no growth
Results are expressed in cfu/g
Values are expressed as mean ± standard deviation. n = 3
Values with different letters within a column indicate significant difference ($p<0.05$)

Table 4: Changes in Total Heterotrophic Fungal Counts (cfu/g) of Spent Carbide Treated Soils

Concentration of spent calcium carbide (g/kg)	Period of CaC_2 impact (Week)			
	1	2	3	4
0	$1.99\times10^4\pm23.0$a	$1.91\times10^4\pm15.0$a	$2.04\times10^4\pm18.0$a	$1.91\times10^4\pm21.0$a
5	$1.38\times10^4\pm24.0$b	$1.20\times10^4\pm16.0$b	$1.05\times10^4\pm18.0$b	$6.3\times10^3\pm20.0$b
25	$1.02\times10^4\pm16.0$c	$5.37\times10^3\pm21.0$c	$1.05\times10^4\pm18.0$b	$6.3\times10^3\pm20.0$b
50	$7.4\times10^3\pm15.0$d	$3.24\times10^3\pm17.0$d	$2.75\times10^3\pm21.0$d	$1.70\times10^3\pm16.0$d
100	$2.51\times10^3\pm23.0$e	$2.09\times10^3\pm18.0$e	$1.78\times10^3\pm19.0$e	$1.38\times10^3\pm15.0$e
200	$1.51\times10^3\pm16.0$f	$1.26\times10^3\pm24.0$f	$7.41\times10^2\pm19.0$g	$4.27\times10^2\pm20.0$f
400	NGg	NGg	$1.78\times10^3\pm25.0$f	$2.19\times10^2\pm21.0$

NG = no growth
Results are expressed in cfu/g
Values are expressed as mean ± standard deviation. n = 3
Values with different letters within a column indicate significant difference ($p<0.05$)

The pH of the spent carbide contaminated soils were alkaline compared to the acidic pH of the control which is hazardous for the environment. Microbial groups have characteristics pH preferences [23]. Most known bacteria are neutrophiles while most fungi prefer more acidic surroundings of pH of 4 to 6. The pH of the spent carbide contaminated soil reveals that the pH remained strongly alkaline with increase in the concentration of the waste. This is in agreement with [7] research on air-exposed waste which showed that such waste does not change into calcium carbonate for at least a decade and even over 13 years, the pH of such waste remains strongly alkaline. In fact only dilution at a waste to water ratio 1:1000 causes a decrease in pH sufficient for it to be harmless to the environment.

Spent carbide brought about decrease in moisture contents of the contaminated soils as this impact was very prominent in the 400 g/kg contaminated soil. Some workers [24] reported that soil water is the principal factor which affects the availability of moisture to organisms as well as soil aeration status, the nature and amount of soluble materials, the osmotic pressure and the pH of the soil solution.

The nutrient adversely affected was nitrogen. There was notable reduction in the concentration of nitrogen in the spent carbide contaminated soil samples which was more at a high concentration of the spent carbide. Acetylene gas, a product of reaction of calcium carbide with water may have inhibited nitrification process in the soil hence more loss in nitrogen in the soil samples contaminated with higher concentrations of spent carbide. The carbon and phosphorus content of the spent carbide contaminated soil samples were higher than the control soil sample. This may have been influenced by some components of the spent carbide.

Table 5: Microbial Isolates from Control and Spent calcium carbide (SCC) contaminated Soil after 4 weeks Treatment

Microbial Isolates in Control (0 g)	**Microbial Survivors in SCC Soil (800 g)**
Bacteria	
Escherichia coli	*Bacillus* subtilis
Bacillus subtilis	*Acinetobacter* calcoaceticus
Acinetobacter calcoaceticus	*Pseudomonas putida*
Pseudomonas putida	*Bacillus pumilis*
Bacillus pumilis	*Micrococcus luteus*
Micrococcus luteus	
Streptococcus faecalis	
Klebsiella aerogenes	
Arthrobacter spp	
Fungi	
Penicillium chrysogenum	*Penicillium chrysogenum*
Aspergillus niger	*Aspergillus niger*
Rhizopus stolonifer	*Rhizopus stolonifer*
Trichoderma spp	
Fusarium oxysporum	
Mucor mucedo	

The chemical composition of the spent carbide reveal that the waste sample is composed mainly of calcium compound in the form of calcium oxide (CaO) which comprised 44.99% of the waste. This is responsible for the strong alkaline pH of the contaminated soil sample. The alkaline pH is hazardous for the environment. It was reported that soil pH is a measure of the acidity of the soil and is a primary factor in plant growth, most plants prefer a soil pH between 5.5 and 7.5 and the majority do best in the middle part of this range [24]. The pH values recorded for the contaminated soil samples were above this range. When soil pH is maintained at the proper level for a given crop, plants nutrients are at maximum availability and beneficial soil organisms are most active.

The heavy metals concentrations associated with the spent carbide were high with respect to the Nigerian Federal Environmental Protection Agency [25] and World Health Organization (WHO) [26] standards. This has serious implications for public health.

Conclusion: It is clear from the observed results that spent calcium carbide had adverse effect on soil microbiota at varying concentration of the waste as there was decrease in the microbial populations of the spent carbide contaminated soils and the decrease was more in the 400 g/kg contaminated soil. Although there were variations in the physiochemical parameters of the spent carbide contaminated soils, all the soils had alkaline pH.

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References

1. Dominati E, Patterson M and MacKay A: A framework for classifying and quantifying natural capital and ecosystem services for soils. J. Ecol. Econs 69:1858-1868. 2010.
2. Doran JW and Zeiss MR: Soil health and sustainability: Managing the biotic component of soil quality. J. Appl. Soil Ecol 15:3-11. 2000.
3. Ranjard L and Richaume AS: Quantitative and qualitative microscale distribution of bacteria in soil. J. Res. Microbiol 152:707-716. 2001.
4. Dunbabin JS and Bowmer KH: Potential use of constructed wetlands for treatment of industrial wastewater containing metals. J. Sci. Total Environ 111:151-168. 1992.
5. Eliers KG, Debenport S, Anderson S and Fierer N: Digging deeper to find unique microbial communities: the strong effect of depth on the structure of bacteria and archaeal communities in soil. J. Soil Biol. Biochem 50:58-65. 2012.
6. Patnaik P: Handbook of Inorganic chemical compounds. McGraw-Hill, New York, 235pp. 2003.
7. Semikolennykh AA, Rahleeva AA and Poputnikova TB: Spent carbide waste retain toxicity long-term after disposal in caves and mines. Acta Carsologica 41(1): 129-137. 2012.
8. William R: Discovery and Identification of calcium carbide in the U.S.A. J. Clin. Endocrinol. Metabiol 43:64-67. 2000.
9. Zhang H, Voroney RP, Price GW: Effects of biochar amendments on soil microbial biomass and activity. J. Environ Qual 43 (6): 2104-2114. 2014

10. Skroc K, Hoffman C, Morris C, Ulvestad L. And Gelderman R: Soil testing procedures in use at South Dakota State soil testing and plant analysis laboratory. Plant Science Department, South Dakota State University Agriculture Experiment Station. 80pp. 2006.
11. Walkley A : A critical examination of a rapid method for determining organic carbon in soils: effect of variations in digestion conditions and inorganic soil constituent. J. Soil Sci 63:251-263. 1947.
12. Bremmer IM and Mulvaney CS*:* Total Nitrogen. In: American Society of Agronomy and Soil Science of America (eds). Methods of Soil analysis Agronomy Monograph 9. Madison, Wisconsin. pp 595 - 627. 1982.
13. Onyeonwu RO: Manual for wastewater, soil/sediment, plant and fish analysis. Mac Gill Environmental Research Laboratory Manual. Benin City, 81pp. 2000.
14. Kalra YP and Marynard DG: Methods manual for forest soil and plant analysis. Edmonton, Canada: Ministry of supply and services. 125pp. 1991.
15. Skoog DMW and Donald M: Fundamental analytical chemistry. Saunders college publishing, Philadephia. 990p. 1992.
16. AOAC: Official methods of analysis of AOAC International 18th ed. Washington DC, USA. 2010.
17. Sharma, P. (2009). Manual of Microbiology, tools and techniques. New Delhi: Ane Books, Pvt. Limited. 405pp. 2009.
18. Holt JG, Krieg NR and Sneath PHA: *Bergey's* Manual of Determinative Bacteriology (Volume 4). London: Cambridge University Press. 2493pp. 1989.
19. Barnett HL and Hunter BB: Illustrated Genera of Imperfect Fungi. Third Edition. New York: Burgess, 225pp. 1972.
20. Bennett, P.M: Plasmid encoded antibiotic resistance: acquisition and Transfer of antibiotic resistance genes in bacteria. Brit. J. Pharmacol 153: 347-357. 2008.
21. Wang YL, Dong SJ, Liu LL, and Cui SP: Using calcium carbide slag as one of calcium-containing raw materials to produce cement clinker. J. Energy Environ Material.171:743-744. 2013.
22. Lavoie KH :Toxicity of carbide waste to hetrotrophic microorganisms in caves. Microbial Ecol 6 (2): 173-179. 1980.
23. Willey J, Sherwood L and Woolverton C: Prescott's Microbiology. Influences of environmental factors on growth. Nineth edition. MacGraw Hill, New York. 751pp. 2013.
24. Price GW, Voroney RP: Papermill biosolids effect on soil physical and chemical properties. J. Environ Qual 36 (6): 1704-1714. 2007
25. Federal Environmental Protection Agency (FEPA): Guidelines and Standards for environmental pollution control in Nigeria. Decree 58 of 1988.238pp. 1991.
26. World Health Organization (WHO): Environmental Health Criteria 27: Guidelines on studies in Environmental Epidemiology. WHO, Geneva. 351pp. 1984.