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Bioremediation of Effluents Samples from Textile Industry in Kano State, Nigeria

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Abstract In the current study, bioremediation potentials of isolated microorganism (bacteria) was assessed. Industrial effluents samples from African Textile Manufacturers in Challawa Industrial estate, Kano State, Nigeria were collected for the period of six months (August 2017 to January 2018) for the experiment. Bacteria were isolated from the effluent, immobilized on agar-agar and biochemical tests were carried out to identify the bacteria. Different masses (5g, 10g, 15g, 20g, and 25g) of the identified bacteria were used in the treatment of 250 ml of the effluents. Temperature, pH, BOD, COD, SS, TDS, Sulphate (SO_4^{2-}), Phosphate (PO_4^{3-}), Nitrate (NO_3^-), Chloride (Cl^-) and some heavy metals (Cr, Fe, Mn, Ni, Pb, Zn, Cd, and Cu) were determined before and after treatment of the effluents with the bacteria. The bacteria were identified to be *Bacillus megaterium*. The results of post-treatment analysis using the different masses of the *Bacillus megaterium* indicates that there is a great decrease in the levels of the physicochemical parameters and the heavy metals as well when compared with the levels observed at the pre-treatment analysis. The decrease could be attributed to, not only due to the increase in the exact mass of the bacteria but also to the multiplicity in the mass of the *Bacillus megaterium* which subsequently increases the surface area for the remediation. The average high percentage reduction (70% to 100%) of these parameters and heavy metals implies that the *Bacillus megaterium* has a higher potential for the treatment of effluents from the textile industries.

Keywords *Bacillus megaterium*, bioremediation, effluent, immobilization, textile

Introduction

Industrial effluents are the most important sources of toxic contaminants in any environment [1]. With rapid industrialization, water pollution has become a major problem. Characteristics of industrial effluent depend upon the type of industrial raw material and the output of the product [2]. Industrial effluents account for several point sources of water pollution, while developed nations adopt stringent water quality requirement to control river pollution from point and non-point sources, the situation is different in most developing countries like Nigeria. Wastewater treatment in Nigeria is not given the necessary priority it deserves and therefore, industrial waste is discharged into receiving water bodies without treatment and the consequences of which include among others, river pollution, loss of aquatic life, uptake of polluted water by plants, diseases burden and shorter life expectancy in developing countries [3].

The textile industry is well known for its high-water consumption and complex wastewater due to the variety of additives and finishing processes. Waste generated in the industry is essentially based on various activities of wet processing of textile. The main cause of generating the effluent is the use of a huge volume of water either in the actual chemical processing or during re-processing in preparatory, dyeing, printing and finishing. With increasing interest in process water recycling and tighter regulations on the disposal of dye-containing wastewaters, segregation and separate treatment schemes are becoming important for textile wastewaters with high dye concentrations. The major chemical pollutant present on the textile dyes containing carcinogenic amines, toxic heavy metals, pentachlorophenol, chlorine bleaching, halogen carriers, free formaldehyde, biocides, fire-resistant, and softeners. The effluent of the textile industry is highly coloured and disposal of this is wastewater into the environment can be extremely deleterious. Their presence in the watercourses is aesthetically unacceptable and may be visible at a concentration as low as 1 ppm. A huge amount of various chemicals such as alum, ferric chloride, and hydrochloric acid are used by the textile industries for waste treatment which creates a problem of solid waste management and also increases the overall cost of the wastewater treatment. Wastewater containing dyes is very difficult to treat since the dyes are recalcitrant molecules resistant to aerobic digestion and stable to light. Effluent from the textile industry is characterized by high values of BOD, COD, TDS, colour and an alkaline pH. The high BOD values can cause oxygen depletion if disposed in an untreated form and the high COD values are toxic to aquatic life [2].

Pollutants pose a substantial risk to public health and the environment. Environmental bioremediation is an emerging technology which is receiving great attention because conventional methods to clean up the environment are cost-intensive sand eco-unfriendly. Bioremediation makes use of micro-organisms and higher plants to treat hazardous organic and metallic residues or by-products which enter into soils and sediments from various processes associated with domestic, municipal, agricultural, industrial and military activities. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. It is based on the idea that all organisms remove substances from the environment to carry out growth and metabolism. The Principles of Bioremediation is that microbe feeds on contaminants, digest it, and this waste is transformed into water and harmless gases which are later expelled out of the microbes. The resultant metabolic wastes that they produce are generally safe and somehow recycled into other product. Although the microorganisms are present in contaminated water, they cannot necessarily be there in the numbers required for bioremediation of the site. Their growth and activity must be stimulated. Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time. However, metallic residues cannot be degraded in composting but may be converted into organic combinations that have less bioavailability than mineral combinations of the heavy metals. Microbial transformation of metals serves various function generally, microbial transformations of metals occur either by redox conversions of inorganic forms or conversions from inorganic to organic forms and vice versa. Bioremediation is amenable to a variety of organic and inorganic compounds and may be applied either *in situ* or *ex-situ*. In addition to this, it is easy to implement and maintain, does not require the use of expensive equipment or highly specialized personnel and is environmentally friendly and aesthetically pleasing to the public [4].

Effluent discharge by industries is one of the major causes of environmental pollution, particularly in developing countries. These industrial effluents, if not treated, pose a serious threat to human life and the environment. The conventional methods used for the treatment of industrial wastewater present some limitations. Bioremediation method is now considered as an alternative technique because of its relatively low-cost, low-technology techniques, which generally have high public acceptance and can often be carried out on site.

This study was carried out in Kano. Kano lies on Latitude 11°30’N and 8°30’N and Longitude 11°5’N and 8°5’E and 8°5’E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery and textile-related activities are the dominating industries and this could be one of the reasons for her high population density [5]. The climate is characterized by well-defined wet and dry seasons. The wet season spreads from May to October, August usually being the wettest whereas the dry season lasts from November to April. River Challawa originates from the Challawa Gorge dam in Challawa village and stretches to River Kano that empties into Lake Chad. The river receives waste from tanneries and textile industries, urban water storm, and agricultural runoffs from farming communities along its course. River Challawa serves as fishing, farming, and water supplies for the communities in the area. The domestic water supply for Challawa, Sharada, and Bompai industrial areas and their surroundings comes from Challawa River [6]. A number of workers have studied bioremediation of textile effluent using microorganism. However, limited literature is available on bioremediation of textile effluent using immobilized bacterial cells in Challawa industrial estate

Materials and Methods

Sample Collection

Effluents were collected from the Textile Industry in Challawa Industrial Estates, Kano, Nigeria. The effluents were collected for a period of six months (August 2017 to January 2018). It was collected from the effluent reservoir in the industries in a sterile 4-litre plastic containers with a unique identification number and was preserved using an ice-box which was transported to Microbiology laboratory, Department of Microbiology, the Bayero University of Kano for analysis.

Sample Preparation and Sample Analysis

Immediately after the collection of the effluent, pH, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Nitrate, Sulphates, Phosphates, Chlorides and Heavy metals (Cr, Fe, Mn, Ni, Pb, Zn, Cd and Cu) concentrations were determined.

pH was determined using Ecotests pH meter and Total Dissolved Solids (TDS) was determined using AQUALYTIC TDS-Salinometer. Biological Oxygen Demand (BOD) was determined as described by standard method [7]. Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), Nitrate (NO_3^-), Sulphates (SO_4^{2-}), Phosphates (PO_4^{3-}) and Chlorides (Cl^-) were determined using DR/2010 HACH portable data logging spectrophotometer [8]. The heavy metals were determined using Atomic Absorption Spectrophotometer (BUCK Scientific ACCUSYS 211) according to manufacturer instruction.

Isolation and Identification of Bacteria from the Textile Effluents

Bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA [9]. The biochemical test such as oxidase, catalase, coagulase, indole, citrate, methyl red (MR), nitrate reduction, Voges-Proskauer (VP), Starch Hydrolysis, Glucose, Maltose, and Lactose Tests was carried out to identify the bacteria present according to Ajao *et al* [10].

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. 50ml of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10g of the powder in distilled water and made up to 500ml mark in an Erlenmeyer flask and was sterilized in an autoclave for 15 minutes and allowed to cool to 40-45 ^oC [10]. Four millilitres (4ml) of the bacterial isolates in the nutrient broth was mixed with 36ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 ml phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The Phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used. Five grams (5g), ten grams (10g), fifteen grams (15g), twenty grams (20g) and twenty-five grams (25g) of the immobilized bacteria were then weighed [11].

Two litres (2 l) of the effluent was supplemented with minimum Basel medium in g/l: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121^oC for 15 minutes. [12].

Two hundred and fifty milliliters (250ml) of the effluents were transferred into different two hundred and fifty milliliters (250ml) conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber [12]. The same procedures were carried out for the ten grams (10g), fifteen grams (15g), twenty grams (20g) and twenty-five grams (25g) of the immobilized bacteria in separate two hundred and fifty millilitres (250ml) effluents in conical flasks and agitated for ten days

The treated effluents samples were taken on the tenth day and analysed for the parameters pH, Total Solids (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Nitrate, Sulphates, Phosphates, Chlorides and heavy metal concentrations (Post-treatment determination) at the different grams of bacteria to evaluate and compare the bioremediation efficiency.

Statistical Analysis

The data were represented as Mean ± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $P \leq 0.05$ was considered statistically significant.

Results and Discussion

Results of the Physico-chemical parameters and Heavy Metals in the Industrial Effluents before the Bioremediation

Results of the Physico-chemical parameters in the industrial effluents before the Bioremediation were shown in table 1. The mean level of temperature (30.33±3.79 ºC) was found below WHO (35ºC) and NESREA (40ºC) recommended standard limits; pH(8.28±3.53) within WHO (7.0-8.5) and NESREA (6-9); BOD(38.75±16.20 mg/l) was below the NESREA (200 mg/l) but above the WHO (10 mg/l).;COD(1941±837 mg/l) above the WHO (40 mg/l) and NESREA (40 mg/l); SS (208±235 mg/l) above the WHO (30 mg/l) and NESREA (10 mg/l); TDS (266±25 mg/l) was above the WHO (250 mg/l) but below the NESREA (500 mg/l); Nitrate (20.58±22.59mg/l) was found below the WHO (50 mg/l) and NESREA (40 mg/l); Sulphates (48.42±37.28mg/l) was below the NESREA (500 mg/l) and WHO (100mg/l); Phosphate (30.77±32.97 mg/l) was found far above WHO (0.1 mg/l) and NESREA (3.5mg/l) and Chlorides (14.82±7.98 mg/l) was below the recommended limits of WHO (250 mg/l) and NESREA (350 mg/l).

Results of the heavy metals in the industrial effluents before the Bioremediation were shown in table 2. The mean level of chromium (0.171±0.12 mg/l) was found above the WHO (0.05mg/l) but below the NESREA (0.5 mg/l) recommended standard limits; iron (0.822±0.63 mg/l) was found within WHO (0.5 mg/l) but below NESREA (1.0 mg/l); manganese(0.217±0.48mg/l) and nickel (0.285±0.06 mg/l) were above the NESREA (0.01 mg/l) and the WHO (0.02 mg/l); lead (0.273±0.20 mg/l) was above the WHO (0.05 mg/l) and NESREA (0.1 mg/l); zinc (0.035±0.03mg/l) was below the WHO (5 mg/l) and the NESREA (0.2 mg/l); cadmium (0.068±0.02 mg/l) was above the NESREA (0.01 mg/l) but below the WHO (0.5 mg/l) and copper (0.172±0.16mg/l) was found below WHO (1.00 mg/l) but above NESREA (0.01mg/l) the recommended limits.

Table 1: Mean Values (mg/l) ± S.D of Physicochemical parameters in effluents from the Industries before and after Treatment of the effluents with the different masses of the bacteria

Parameter	**Before**	**After**				
		5g	**10g**	**15g**	**20g**	**25g**
Temperature(ºC)	30.33a±3.79					
pH	8.28ab ± 3.53					
SS	208±235	27ad±34	32ad±37	18ae±22	32bd±36	40ad±55
TDS	266±253	4ad±2	4de±2	4c±2	4cd±2	4ce±2

BOD	38.75±16.20	1.15c±0.99	0.96b±0.88	0.83b±0.74	0.71c±0.57	0.55b±0.36
COD	1941±837	0.33±0.41a	0	1.00±0.84a	0	0
Nitrate	20.58±22.59	5.21a±4.12	5.78a±12.57	5.64a±3.97	5.87b±4.59	7.38a±6.28
Sulphate	48.42±37.28	17.37a±23.21	14.70a±18.39	12.02b±15.56	8.36b±11.04	7.52b±8.78
Phosphate	30.77±32.97	2.83a±3.01	2.62a±2.88	2.38a±3.06	2.45b±3.02	2.29b±3.93
Chloride	14.82±7.98	0.38a±1.83	0.44b±1.47	0.64a±1.27	0.46b±1.40	0.39a±1.68

Replicate= 6 (months)

Within the columns, means with different alphabets are statistically different (p<0.05).

Table 2: Mean Values (mg/l) ± S.D of Heavy metals in effluents from the Industries before and after Treatment of the effluents with the different masses of the bacteria

Parameter	Before	After				
		5g	**10g**	**15g**	**20g**	**25g**
Cr	0.171a±0.12	0.049a±0.05	0.046a±0.07	0.013a±0.02	ND	0.170a±0.00
Fe	0.822±0.63	0.532c±0.11	0.488b±0.18	0.485b±0.36	0.202a±0.21	0.287ab±0.29
Mn	0.217a±0.48	0.002a±0.00	ND	ND	ND	ND
Ni	0.285±0.06	0.180a±0.07	0.180a±0.09	0.100a±0.10	0.070a±0.11	0.055a±0.11
Pb	0.273±0.20	0.118a±0.05	0.076a±0.04	0.073a±0.04	0.051a±0.05	0.043a±0.05
Zn	0.035±0.03	0.028a±0.02	0.028ac±0.03	0.027a±0.02	0.020ab±0.02	0.013a±0.02
Cd	0.068±0.02	0.058a±0.02	0.058a±0.03	0.050a±0.02	0.058a±0.03	0.063a±0.02
Cu	0.172±0.16	0.125b±0.12	0.068b±0.08	0.122b±0.13	0.100b±0.11	0.078b±0.08

ND= Not Detected

Replicate= 6 (months)

Within the columns, means with different alphabets are statistically different (p<0.05).

Table 3: Preliminary Identification of the Bacterial Isolates

Gram staining	Shape	Spore formation
+	Rod	+

+ = Positive   - = Negative

Table 4: Biochemical Characteristics of the Bacterial Isolates

Citrate	Catalase	Coagulase	Starch	Glucose	Oxidase	Indo	Lactose	Manitol	Maltose	MR	VP	NR
+	+	+	+	+	+	-	+	+	+	+	-	-

MR=Methyl Red; VP= Voges-Proskauer, NR=Nitrate Reduction

Table 5: Correlation coefficient (r) between different masses of bacteria and physicochemical parameters

Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
SS	0.50	25
TDS	0.93*	86
BOD	-1.00	99
COD	-0.24	6

NITRATE	0.85*	72
SULPHATE	-0.99	98
PHOSPHATE	-0.93	86
CHLORIDE	-0.37	14

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

SS=Suspended Solid, TDS=Total Dissolved Solid, BOD=Biochemical Oxygen Demand and COD=Chemical Oxygen Demand

Table 6: Correlation coefficient (r) between different masses of bacteria and heavy metals

Heavy metals	**Correlation coefficient (r)**	**Percent dependence (rxrx100) (%)**
Cr	-0.93	87
Fe	-0.85	72
Mn	-0.71	50
Ni	-0.95	91
Pb	-0.95	90
Zn	-0.92	85
Cd	-0.94	89
Cu	-0.39	15

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Table 7: Percentage reduction of the physicochemical parameters after Treatment of the effluents (250ml) with the different masses (5g, 10g, 15g, 20g, and 25g) of the *Bacillus Megaterium*

Parameter (mg/l)	**Percentage Reduction (%)**				
	5g	**10g**	**15g**	**20g**	**25g**
COD	100	100	100	100	100
BOD	97	98	98	98	99
SS	87	85	91	85	81
TDS	98	98	98	98	98
Nitrate	75	72	73	71	64
Chloride	97	97	96	97	97
Sulphate	64	70	75	83	84
Phosphate	91	91	92	92	93

Table 8: Percentage reduction of the heavy after Treatment of the effluents (250ml) with the different masses (5g, 10g, 15g, 20g, and 25g) of the *Bacillus Megaterium*

Parameter (mg/l)	**Percentage Reduction (%)**				
	5g	**10g**	**15g**	**20g**	**25g**
Cr	71	73	92	100	100
Fe	35	41	41	75	65
Mn	99	100	100	100	100
Ni	37	37	65	75	81
Pb	57	72	73	81	84
Zn	19	19	24	43	62
Cd	15	17	41	41	51
Cu	27	60	29	42	54

Results of the Preliminary Identification and Biochemical Characteristics of the Bacterial Isolates

Results of preliminary identification and biochemical characteristics of bacterial isolates were shown in table 3 and 4 respectively. After 48 hours of incubation, the nutrient agar media plates were checked for bacteria growth.
The results showed that the bacterial isolate was found to be gram-positive, rod-shaped and recorded positive results for spore formation.
The results of the biochemical test indicated that the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose, mannitol, starch hydrolysis. MR and coagulase tests. The bacteria showed negative results for nitrate reduction, VP and Indole tests. Base on the Preliminary Identification and biochemical test results, the bacteria isolated were identified to be *Bacillus megaterium*.

Results of the Physico-chemical parameters and Heavy Metals in the Industrial Effluents after the Bioremediation

Table 1 shows the mean results of the Physico-chemical parameters in the industrial effluents before and after the bioremediation using the different masses (5g, 10g, 15g 20g, and 25g) of the *Bacillus megaterium*. Also, Table 5 shows the results of the correlation coefficient (r) between the different mean masses of bacteria and the physicochemical parameters.
The mean value (mg/l) of the SS after the bioremediation varies between 18±22 and 40±55. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the SS was in the order 25g>10g>20g>5g>15g. A positive and no significant correlation (0.50) exist between the masses of bacteria and the SS. There was a low correlation (25%) between the masses of the bacteria and the SS.
The mean value (mg/l) of the TDS after the bioremediation was 4±2. A positive and significant correlation (0.93*) exist between the masses of bacteria and the TDS. There was a high correlation (86%) between the masses of the bacteria and the TDS.
The mean value (mg/l) of the BOD after the bioremediation varies between 0.55±0.36 and 1.15±0.99. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the BOD was in the order 25g>20g>15g>10g>5g. A negative and no significant correlation (-1.00) exist between the masses of bacteria and the BOD. There was a very high correlation (99%) between the masses of the bacteria and the BOD.
The mean value (mg/l) of the COD after the bioremediation varies between 0 and 1.00±0.84. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the COD was in the order 5g>15g. A negative and no significant correlation (-0.24) exist between the masses of bacteria and the COD. There was a very low correlation (6%) between the masses of the bacteria and the COD.
The mean value (mg/l) of the nitrate after the bioremediation varies between 5.21±4.12 and 7.38±6.28. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the nitrate was in the order 5g>15g>20g>20g>25g. A positive and significant correlation (0.85*) exist between the masses of bacteria and the nitrate. There was a high correlation (72%) between the masses of the bacteria and the nitrate.
The mean value (mg/l) of the sulphate after the bioremediation varies between 7.52±8.78±4.12 and 17.37±23.21. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the sulphate was in the order 25g>20g>15g>10g>5g. A negative and no significant correlation (-0.99) exist between the masses of bacteria and the sulphate. There was a very high correlation (98%) between the masses of the bacteria and the sulphate.
The mean value (mg/l) of the phosphate after the bioremediation varies between 2.29±3.93 and 2.83±3.01. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the phosphate was in the order 25g>15g>20g>10g>5g. A negative and no significant correlation (-0.93) exist between the masses of bacteria and the phosphate. There was a very high correlation (86%) between the masses of the bacteria and the phosphate.
The mean value (mg/l) of the chloride after the bioremediation varies between 0.38±1.83 and 0.64±1.27. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the chloride was in the order 5g>25g>10g>20g>5g. A negative and no significant correlation (-0.37) exist between the masses of

bacteria and the chloride. There was a very low correlation (14%) between the masses of the bacteria and the chloride.

Table 2 shows the mean results of the heavy metals in the industrial effluents before and after the bioremediation using the different masses (5g, 10g, 15g 20g, and 25g) of the *Bacillus megaterium*. Also, Table 6 shows the results of the correlation coefficient (r) between the different mean masses of bacteria and heavy metals.

The mean value (mg/l) of the chromium after the bioremediation varies between not detected and 0.170±0.00. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the chromium was in the order 20g>25g>15g>10g>5g. A negative and no significant correlation (-0.93) exist between the masses of bacteria and the chromium. There was a high correlation (87%) between the masses of the bacteria and the chromium.

The mean value (mg/l) of the iron after the bioremediation varies between 0.202±0.21 and 0.532±0.11. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the iron was in the order 20g>25g>15g>10g>5g. A negative and no significant correlation (-0.85) exist between the masses of bacteria and the iron. There was a high correlation (72%) between the masses of the bacteria and the iron.

The mean value (mg/l) of the manganese after the bioremediation varies between not detected and 0.002±0.00. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the manganese was in the order 25g=20g=15g=10g>5g. A negative and no significant correlation 0.71) exist between the masses of bacteria and the manganese. There was an average correlation (50%) between the masses of the bacteria and the manganese. The mean value (mg/l) of the nickel after the bioremediation varies between 0.055±0.11 and 0.180±0.07. The relative potential or efficiency of the different 0.180±0.07. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the nickel was in the order 25g>20g>15g>10g=5g. A negative and no significant correlation (-0.95) exist between the masses of bacteria and the nickel. There was a very high correlation (91%) between the masses of the bacteria and the nickel.

The mean value (mg/l) of the lead after the bioremediation varies between 0.043±0.05 and 0.118±0.05. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the lead was in the order 25g>20g>15g>10g>5g. A negative and no significant correlation (-0.95) exist between the masses of bacteria and the lead. There was a very high correlation (90%) between the masses of the bacteria and the lead.

The mean value (mg/l) of the zinc after the bioremediation varies between 0.013±0.02 and 0.028±0.02. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the zinc was in the order 25g>20g>15g>10g=5g. A negative and no significant correlation (-0.92) exist between the masses of bacteria and the zinc. There was a very high correlation (85%) between the masses of the bacteria and the zinc.

The mean value (mg/l) of the cadmium after the bioremediation varies between 0.050±0.02 and 0.063±0.02. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the cadmium was in the order 25g>20g=15g>10g>5g. A negative and no significant correlation (-0.94) exist between the masses of bacteria and the cadmium. There was a high correlation (89%) between the masses of the bacteria and the cadmium.

The mean value (mg/l) of the copper after the bioremediation varies between 0.068±0.08 and 0.125b±0.12. The relative potential or efficiency of the different masses of the *Bacillus megaterium* in remediating the copper was in the order 10g>25g>20g>15g>5g. A negative and no significant correlation (-0.39) exist between the masses of bacteria and the copper. There was a high correlation (15%) between the masses of the bacteria and the copper.

Percentage reduction of the physicochemical parameters and the heavy metals by the *Bacillus Megaterium*

Table 7 shows the percentage reduction of the physicochemical Parameters after Treatment of the effluents (250ml) by the different masses (5g, 10g, 15g 20g and 25g) of the *Bacillus Megaterium.* The percentage reduction (%) of SS ranged (81-91); TDS (98); BOD (97-99); COD (100); Nitrate (64-75); Chloride (96-97); Sulphate (64-84); Phosphate (91-93); Cr (71-100); Fe (35-75); Mn (99-100); Ni (37-81); Pb (57-84); Zn (19-62); Cd (15-51) and Cu (27-60).

Table 8 shows the percentage reduction of the heavy metals after Treatment of the effluents (250ml) with the different masses (5g, 10g, 15g 20g, and 25g) of the *Bacillus Megaterium.* The percentage reduction (%) of Cr ranged (71-100); Fe (35-75); Mn (99-100); Ni (37-81); Pb (57-84); Zn (19-62); Cd (15-51) and Cu (27-60).

Physico-chemical parameters and Heavy Metals in the Industrial Effluents before the Bioremediation

Results of the Physico-chemical parameters in the industrial effluents before the Bioremediation were shown in table 1. The mean level of temperature (30.33±3.79 ºC) was found below the WHO (35ºC) and NESREA (40ºC) recommended standard limits. The average value of temperature observed in this present study is less than that observed (56.23 to 74.54ºC) by another researcher [13]. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD. The pH (8.28±3.53) is within the WHO (7.0-8.5) and NESREA (6-9).

The average value of SS observed in this present study is less than that observed (3422±122 to 3700±122 mg/l) by another researcher [13]. SS include all the particles suspended in water which will not pass through a filter. Suspended solids are present in natural water another researcher [13], sanitary wastewater, and many types of industrial wastewaters. It is observed that a suspended solid absorb heat from sunlight, causing an increase in water temperature and subsequently decreases the level of dissolved oxygen another researcher [15].

TDS (266±25 mg/l) was above the WHO (250 mg/l) but below the NESREA (500 mg/l). High values of TDS are one of the common sources of sediments which reduce the light penetration into water and ultimately decreased the photosynthetic activities.

The BOD (38.75±16.20 mg/l) was below the NESREA (200 mg/l) but above the WHO (10 mg/l). BOD is a measure of the content of organic substances in the wastewater which are biologically degradable with consumption of oxygen usually indicated as 5-day biochemical oxygen demand (BOD). This is the amount of oxygen in milligrams per litre (mg/l) that consumed by microorganisms in 5 days at 20^{o}C for the oxidation of the biologically degradable substances contained in the water. The high values of BOD recorded are indicative of the presence of total solids in the effluents which are known to be organic with high oxygen demands for their oxidation under required conditions of temperature and oxidants and time, as a result, will naturally lead to the depletion of dissolved oxygen in the water body. The biochemical oxygen demand of the untreated textile wastewater can cause rapid depletion of dissolved oxygen if it is directly discharged into the surface water sources another researcher [16].

COD (1941±837 mg/l) above the WHO (40 mg/l) and NESREA (40 mg/l); SS (208±235 mg/l) above the WHO (30 mg/l) and NESREA (10 mg/l). The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/l, required for degradation of the compound of wastewater to occur. The higher the COD value of wastewater, the more oxygen demand to discharge water bodies another researcher [13]. Reference [15] reported that a high COD value shows that the effluents have high oxygen demanding wastes which cause the depletion of DO which is a fundamental requirement for aquatic life.

Nitrate (20.58±22.59 mg/l) was found below the WHO (50 mg/l) and NESREA (40 mg/l). Reference [17] have reported the presence of nitrate in receiving water which can lead to extensive undesirable algal growth associated with eutrophication. Sulphates (48.42±37.28mg/l) was below the NESREA (500 mg/l) and WHO (100mg/l). Phosphate (30.77±32.97 mg/l) was found far above WHO (0.1 mg/l) and NESREA (3.5mg/l). The sulphate and high phosphate concentration detected in the effluents are also known to cause eutrophication and algal boom [17]. Chlorides (14.82±7.98 mg/l) was below the recommended limits of WHO (250 mg/l) and NESREA (350 mg/l). Chlorides prevent the plants, bacteria, and fish in surface waters growing. The presence of these anions in the textile effluent of the present study might be due to the nature of the raw materials and processes used in the textile industries as at the time of sampling.

Results of the heavy metals in the industrial effluents before the Bioremediation were shown in table 2. The mean level of chromium (0.171±0.12 mg/l) was found above the WHO (0.05mg/l) but below the NESREA (0.5 mg/l) recommended standard limits. Reference [18] reported that the concentration of chromium in the effluents from textile industries in Kano ranged (2.98 to 2.32) mg/l, which is the same order of magnitude reported in the study by

Naeem [19]. Chroium was found to be 1.202 mg/l higher than the value of 0.255 mg/l reported from textile industries effluent in Lagos metropolis, Nigeria [20]. Effluents of textile industries from Kaduna were found to contain the average concentration of 1.70 and 0.75 mg/l [21]. Chromium can cause allergic reactions in the skin, damage the lungs, and asthma attacks maximum concentration of 0.1 mg/l was set up [22].

Iron (0.822±0.63 mg/l) was found within WHO (0.5 mg/l) but below NESREA (1.0 mg/l). Reference [18] reported that the concentration of iron in the effluents from textile industries in Kano ranged (4.21 to 4.05) mg/l, which is the same order of magnitude reported in the study by Naeem [19]. Another study reveals that effluents containing 0.351 mg/l of irons was discharged from the textile industry in Kano, and tend to increase significantly in the rainy season to about 1.539 mg/l [23]. Higher iron content may produce undesirable effects such as astringent taste, colouration, turbidity, deposits, and growth of iron bacteria in pipes affecting the acceptability of water for domestic use. Iron is an essential element in human nutrition, and health effect of iron in drinking water may include warding off fatigue and anaemia [24].

Manganese (0.217±0.48mg/l) was above the NESREA (0.01 mg/l) and the WHO (0.02 mg/l). In another study, 1.02 mg/l of manganese was reported by [21]. However, it exceeded the [25] maximum permissible limit of 0.5 mg/l for water samples. Depending upon the exposure route, manganese may be among the least toxic of the trace elements if ingested low IQ of children is attributed to high manganese intake and hence at high concentration lead to neurotoxins and has an adverse effect on the brain [25].

Nickel (0.285±0.06 mg/l) was above the NESREA (0.01 mg/l) and the WHO (0.02 mg/l). Reference [18] reported that the concentration of nickel in the effluents from textile industries in Kano ranged (3.11 to 3.4 mg/l) which is the same order of magnitude reported in the study by Naeem [19]. The most obvious anthropogenic source of nickel is scrap metal waste, notably alloyed metals including stainless steel. Nickel is considered an essential trace element at very low concentrations. It does bioaccumulate in aquatic systems, and as such elevations above normal concentrations can result in deleterious aquatic effects [17].

Lead (0.273±0.20 mg/l) was above the WHO (0.05 mg/l) and NESREA (0.1 mg/l). Reference [18] reported that the concentration of lead in the effluents from textile industries in Kano ranged (2.45 to 2.46 mg/l) which is the same order of magnitude reported in the study by Naeem *et al*. [19]. Excess quantities of lead may impact human health, especially affecting small children (Orisikwe, 2009). The value 0.646 mg/l and 0.289 mg/l were reported for lead in wastewater samples from the textile industry in Kaduna [19] [26].

Zinc (0.035±0.03mg/l) was below the WHO (5 mg/l) and the NESREA (0.2 mg/l). Reference [18] reported that the concentration of zinc in the effluents from textile industries in kano ranged from 2.52 to 2.45 mg/l, which is the same order of magnitude reported by Naeem *et al*. [19]. Zinc occurs naturally in air, water, and soil, but zinc concentration is rising unnaturally, due to the addition of zinc through human activities. Most zinc is added during industrial activities, such as mining, coal, and waste or sewage sludge from industrial areas. The levels of Zinc in the water and sediment samples contaminated by textile effluent were found to exceed the WHO guideline value of 3.00 mg/l and 6.00 mg/l [16]. High concentration of 1.57 mg/l and 1.07 mg/l was reported by [13], similarly, Zn was the most abundant metal in the area which ranged from 0.264 to 0.947 mg/l for one textile industry studied in Pakistan [24, 27]. It is a trace element that is essential for human health; the danger of which can be to an unborn child when mothers absorbed large concentration of zinc and other health problem such as stomach cramps, skin irritation, vomiting and anaemia [28].

Cadmium (0.068±0.02 mg/l) was above the NESREA (0.01 mg/l) but below the WHO (0.5 mg/l). Reference [18] reported that the concentration of cadmium in the effluents from textile industries in Kano ranged from 1.23 to 2.06 mg/l, which is the same order of magnitude reported by [19]. The cadmium content of textile industry effluent was reported to be 0.02 mg/l, in dye textile effluent [29]. In another study, the average value of cadmium was found to be 0.04 mg/l lower as compared to the value 0.076 mg/l was observed in Bompai industrial area, Nigeria while lower than 1.05 mg/l observed in Peshawar, Pakistan for textile effluents [30].

Cadmium is a nonessential trace element that enters the environment via anthropogenic activities such as industrial effluent, sewage-sludge, fertilizers, and pesticides. Cadmium adsorbs strongly to sediments and organic matter [31]. It has a range of negative physiological effects on organism, such as decreased growth rates and negative effects on embryonic development and children are likely to be exposed to cadmium when is highly toxic and absorbed in skin and can cause lung damage and irritation with shortness of breath dry throat, headache, vomiting, extreme restlessness or irritability etc [32]. Other potential long-term effects are lung damage and fragile bones [22]. Cadmium can still be assimilated from sediments with high organic matter content [33].

Copper (0.172±0.16mg/l) was found below WHO (1.00 mg/l) but above NESREA (0.01mg/l). Reference [18] reported that the concentration of copper in the effluents from textile industries in Kano ranged from 1.06 to 1.01 mg/l, which is the same order of magnitude reported in the study by [33]. Copper is reddish metal in colour that occurs naturally in rocks, soil, water, industrial activities, and sediment and has some practical uses in our society and are found in pipes, electrical wiring, and coins. The level of copper reported from the textile industry in Lagos ranged from 4.0 mg/l to 5.14 mg/l [21]. Copper is generally remobilized with acid-base ion exchange or oxidation mechanism [26]. Copper is an essential element for living organism, including humans, and in small amounts necessary to our diet to ensure good health. However excess of it can cause an adverse effect on the health of living beings, effects include vomiting, diarrhea, stomach cramps, and nausea. It was also associated with liver damage and kidney disease.

The presence of these metals may be due to the addition of some raw materials containing these ions during the production process. It has been reported that the major problem associated with industrial effluents is the presence of heavy metal ions, which arise from the material used in the production process or in a considerably high amount, from metal-containing raw materials [34]. Heavy metals present as impurities in dye effluents or chelated as part of dye molecules. In metal complex dyes, the metal is coordinated or forms a chemical bond with the organic dye molecules. Thus, it is an indispensable constituent of dye and governs the fastness in absorbing colours. The highest value of heavy metal ions in the effluents severely affects soil fertility and depletes the soil quality and its nutrients. Besides, the variations of heavy metals concentration in the wastewater sample were due to the different types of dyestuff used in a different production of the threads when the samples were taken. The concentration of heavy metals could be a serious environmental nuisance if a large volume of such effluents is released into the environment on a regular basis without proper treatment [18]. In line with the findings by [21], it is reported that heavy metals have been associated with the textile effluents because of the copious use of the chemical in dye processes. Reference [23] analysed heavy metal in Kano from Challawa industrial estate and found high concentrations of metal ions. The presence of these heavy metals in the textile effluent of the present study might be due to the nature of the raw materials and processes used in the textile industries as at the time of sampling.

Preliminary Identification and Biochemical Characteristics of the Bacterial Isolates

Results of preliminary identification and biochemical characteristics of bacterial isolates were shown in table 3 and 4 respectively. After 48 hours of incubation, the nutrient agar media plates were checked for bacteria growth. Base on the Preliminary Identification and biochemical test results, the bacterial isolate were identified to be *Bacillus megaterium*.

Reference [10] isolated and immobilized *Pseudomonas aeruginosa* and *Bacillus subtilis* from textile wastewater. Reference [35] isolated and characterised thirty bacterial isolates and found only three potential degraders of textile effluent belonging to *Bacillus* and *Pseudomonas* spp. while [36] and [37] isolated *Bacillus cereus, Bacillus subtilis* and *Pseudomonas* spp from refinery effluent, diesel oil and textile effluent. However, [38] isolated five bacteria species in textile effluent; *Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia,* and *Escherichia coli.* Reference [18] isolated *Bacillus megaterium* from textile industries effluents in Kano, Nigeria.

Physico-chemical parameters and Heavy Metals in the Industrial Effluents after the Bioremediation

Table 1 shows the mean results of the Physico-chemical parameters in the industrial effluents before and after the bioremediation using the different masses (5g, 10g, 15g, 20g, and 25g) of the *Bacillus megaterium*. There was a reduction in the concentration of all the Physico-chemical parameters after the bioremediation process when compared with the concentration of the raw samples before the bioremediation. This is in agreement with the report of [10] in which the concentration of Physico-chemical parameters decrease in biological (immobilized *Pseudomonas aeruginosa* and *Bacillus subtilis*) treated textile wastewater. The results of this work is partially in line with that of [39] in which the rate constants of the first-order kinetic bioremediation process of food and detergent industrial effluents using 5g and 10g of immobilized *Pseudomonas auroginosa* and *Bacillus subtilis* respectively increased, the rate constants of the first-order kinetic increased and concluded that these could be attributed to the increase in the weight of the microorganisms to 10g from 5g [39]. Also, Table 5 shows the results of the correlation coefficient (r) between the different mean masses of bacteria and the physicochemical parameters. In the present study, the decrease in the concentration of the Physico-chemical parameters after the bioremediation was not only due to the increase in the mass of the bacteria but might be also due to the increase in surface area of the different mass of the bacteria.

Table 2 shows the mean results of the heavy metals in the industrial effluents before and after the bioremediation using the different masses (5g, 10g, 15g 20g, and 25g) of the *Bacillus megaterium*. There was a reduction in the concentration of all the heavy metals after the bioremediation process when compared with the concentration of the raw samples before the bioremediation. This is also in accordance with the report made by [10] and [18]. Also, Table 6 shows the results of the correlation coefficient (r) between the different mean masses of bacteria and heavy metals. In the present study, the decrease in the concentration of the heavy metals after the bioremediation was not only due to the increase in the mass of the bacteria but might be also due to the increase in surface area of the different mass of the bacteria.

Percentage reduction of the physicochemical parameters and the heavy metals by the *Bacillus Megaterium*

Table 7 shows the percentage reduction of the physicochemical Parameters after Treatment of the effluents (250ml) by the different masses (5g, 10g, 15g 20g, and 25g) of the *Bacillus Megaterium.*

Reference [18] studied the bioremediation of textile industries effluents using selected bacterial species (*Bacillus megaterium*, *P. fluorescens*, *P. aeruginosa*, *P. putida*, *P. fluorescent,* and *A. faecalis)* in Kano, Nigeria. The decrease in Biochemical oxygen demand range was 50% to 63%. Total suspended solid reduction efficacy ranged by all isolates was from 48% to 60%, while total dissolve solid reduction ranged was from 50% - 60%. However, the reduction efficacy demonstrated by isolates for COD was 53% to 71% levels suggests the fact that the process of bioremediation is in progress. He reported that the species have the potential of adsorption of heavy metals Cd from 2.00 to 0.05 mg/l (97%), Fe from 4.30 to 1.00 mg/l (76%), Cr was adsorbed 2.0 to 0.03(85%) mg/l and Ni was adsorbed to 0.54 mg/l (54%) in the wastewater from textile industries.

Bioremediation of Textile Industrial Effluent using the mixed culture of *Pseudomonas aeruginosa* and *Bacillus subtilis* immobilized on agar-agar in a Bioreactor was carried out by [10]. The result indicates overall % reduction in COD, BOD, Nitrate, Sulphate, Phosphates as 83%, 97%, 61.3%, 62.8%, 61.2% respectively. Heavy metals were also biosorption. It was concluded that immobilized cells represent a promising application in the bioremediation of textile industrial effluent and possible reusability of the cells for its commercial application can be achieved.

Enzymes often are able to work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed [40]. Reference [41] reported the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which make it more economical. Similar work was done by Reference [42] showing the higher reduction with a permeabilized cell of *Ochrobactrum intermedium* strain SDCr-5. The results revealed the isolation and identification of isolates with the potential for reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit [40]. Although a number of workers described microbial degradation of textile effluent, limited literature is available on bioremediation of textile effluent using immobilized bacterial cells.

Conclusion

The textile industrial effluent samples contained variable levels of the physicochemical parameter and heavy metals, which some of them were higher than the recommended standard limits. The presence of these physicochemical parameters and heavy metals might be due to the nature of the raw materials and processes used in the textile industries as at the time of sampling. Base on the Preliminary Identification and biochemical test results, the bacterial isolate were identified to be *Bacillus megaterium*. There was an overall decrease in the concentration of the physicochemical parameters and the heavy metals after the bioremediation using the different masses of the bacterial isolates. However, the decrease is not only due to the increase in the mass of the bacteria but might be also due to the increase in surface area of the different mass of the bacteria. The average high percentage reduction (70% to 100%) of these parameters and heavy metals implies that the immobilized *Bacillus megaterium* is having a higher potential for the treatment of the textile industrial effluents.

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