Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2013, 3(3):95-100

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Bacterial and fungal biosorbents of heavy metals associated with hydrocarbon contaminated rainforest soils of the Niger delta region of Nigeria

Akponah E.

Department of Microbiology, Delta State University, Abraka

ABSTRACT

Heavy metal absorption capacities of 43 microbial isolates belonging to seven bacterial and five fungal genera was investigated using initial concentrations of 0.1 and 1.0 (mg/l) of copper, nickel, cobalt, lead and chromium . The bacterial species included Bacillus sp, Norcardia sp, pseudomonas sp, Micrococcus sp, Acinectobacter sp, Alcaligens sp and Serretia sp. The fungal isolates were Aspergillus sp, Rhizopus sp, Penicillium sp, Geotrichum sp, and Fusarium sp. These isolates were obtained from hydrocarbon contaminated soils collected from five mechanic workshops located in Abraka, Warri, Ughelli (Delta State), Kiama (Bayelsa State) and choba (Rivers State) all in the Niger Delta region of Nigeria. Each of the isolates absorbed the various heavy metals though, to varying extents in the order: Aspergillus sp ≥ Rhizopus sp ≥ Fusarium sp > Penicillium sp > Geotrichum sp ≥ Nocardia sp > Bacillus sp > Pseudomonas sp> Micrococcus sp > Acinectobacter sp > Alcaligens sp > Serretia sp. It was observed that uptake of the respective heavy metal by each test isolate at initial concentration of 1.0 mg/l were significantly higher than at 0.1 mg/l (p = 0.05). However, there was no significant difference in heavy metal uptake among organisms of same genus obtained from the five locations (p = 0.05).

INTRODUCTION

Several human activities have resulted in elevated concentration of metals in many terrestrial environments. Chronic and acute metal pollution arises from a number of anthropogenic sources including petroleum industry activities, fossil fuel combustion, industrial fissions, agricultural pesticides and domestic and industrial effluent discharges [1] Heavy metals have a great ecological significance due to their toxicity and accumulative behavior [2]. They constitute a large class of inorganic chemicals that bioaccumulate in food chains where they disrupt biochemical and physiological activities of many organisms. Thus, causing carcinogenesis of some organs, mutagenesis in the genetic material, impairment in reproductive capacity and/or heamorrhage in exposed populations [3].

The threat of heavy metal pollution to public health and the ecosystem has led to an increased interest in developing systems that can remove or neutralize its toxic effects in soils, sediments and wastewaters. It is well recognized that microorganisms have a high affinity for metals and can accumulate heavy metals by a variety of mechanisms. However, in the Niger Delta region of Nigeria, only little information exists on the use of microorganisms in the remediation of heavy metal contamination in terrestrial ecosystems. Therefore, this paper focuses on evaluating the potential ability of the microbiota of heavy metal contaminated soils in the region, in other to use them as metal

bioconcentrators and hence ultimately determining the self-remediation capability of such heavy metal contaminated ecosystems.

MATERIALS AND METHODS

Sample Collection

Surface soil samples were collected from auto-mechanic works located in Choba (Rivers State), Kiama (Bayelsa State), Abraka, Warri and Ughelli (located in Delta State) Nigeria. The samples were collected from areas in the workshops where diesel, petrol and lubricating oil were dumped. Four samples were collected from each mechanic workshop and transported to the laboratory in new, clean black polyethylene bags within six hours of collection for analysis.

Determination of the Physico-chemical parameters of Soil Samples

The four samples obtained from each workshop, were mixed together thoroughly using a sterile spatula and the pH and moisture content of the various samples were determined adapting the methods in AOAC [4].

Determination of Heavy Metals Present in Soil Samples

The heavy metal analysis of each of the soil samples collected was done using atomic absorption spectrophotometer (Unicam 929 AA Spectrometer, UK). The heavy metal present and their respective concentrations determined the choice of heavy metals used in this study.

Isolation and Identification of Test Organisms

The vapour phase technique was adopted for the isolation of bacterial species from the soil samples. This involved the use of mineral salt agar medium with the following composition in g/L: MgSO4.7H2O – 0.4, KCL – 0.28, $K2HPO_4$ – 1.2, KH_2PO_4 – 0.8, NH_4NO_3 – 0.4g, NaCl-20g agar 15g, distilled water 1L. Various dilutions (0.1ml amounts) were inoculated into mineral salt agar using the pour plate technique. Thereafter, filter paper soaked in crude oil was then placed on the lid and incubation followed immediately at room temperature for a duration of 4-7 days.

The method of Fawole and Oso [5] was adapted in the isolation of fungal species. Various dilutions of each sample were inoculated onto the surface of freshly prepared Sabaraud Dextrose agar containing 0.1%v/v crude oil using the spread plate technique. The plates were incubated at ambient temperature for 48hours.

Pure stock cultures of bacterial isolates were characterized and identified using criteria in Holt *et al*. [6]. Fungal identification was done according to Bannet and Hunter [7].

Biosorbent Preparation

Bacterial and fungal isolates from the various stock cultures were inoculated onto the surface of freshly prepared nutrient agar and Sabauraud Dextrose agar plates respectively by streaking in other to obtain a lawn of the of each organism. Plates were incubated at appropriate temperatures at a duration of 24h and 72h for bacteria and fungi respectively. At the end of incubation duration organisms were harvested into clean sterile and pre-weighed crucibles. The respective isolates were dried to constant weight by heating in an oven at 80^oC. Death or non viability of isolates was confirmed by inoculating onto the surface of appropriate medium and absence of growth was indicative of positive result**.**

Heavy Metal Preparation

The heavy metals used in this study include sulphate salts of chromium, lead, nickel, copper and cobalt. The choice of heavy metal used was based on the results of analysis of various soil sample used. One gram equivalent of each of the metals in the different metal salts were weighed and dissolved in 1000ml of sterile deionized water to make stock concentration. Subsequent ten-fold serial dilutions were performed to obtain 100mg/L, 10mg/L, 1mg/L and 0.1mg/L. However, only 0.1 and 1.0 (mg/L) which represented the range of concentration of the various heavy metals present in the soil sample was used.

Biosorption Test Studies

The batch biosorption experimental method was used to determine the sorption of the each heavy metal by the various isolates obtained. Specific weights (0.5g) of the respective biomass were introduced into 10ml of the each

heavy metal concentration contained in a 100ml Erlenmeyer flask for 24h at room temperature on a rotary shaker at 120 rpm. At the end of incubation duration, the biomass was separated by centrifugation at 4000rpm for 30minutes and supernatants were analysed for residual metal concentration using an atomic absorption spectrophotometer. The sorbate or heavy metal uptake [q (mg metal/g dry cells)] was calculated [8] as:

$$q = \frac{V(L) \times (C_i - C_f)(mg/L)}{S(g)}$$

Where,
q = heavy metal uptake
V = volume of metal solution
C_i = initial concentration of metal in solution
C_f = final metal concentration in solution
S = mass of dried cells

RESULTS AND DISCUSSION

Microbial isolates of the hydrocarbon contaminated soil samples consisted of seven bacterial and five mould species as presented in Table 1. The bacterial isolates were *Bacillus sp, Norcardia sp, Pseudomonas sp, Micrococcus sp, Acinectobacter sp, Alcaligenes sp and Serretia sp* while fungal isolates included *Aspergillus sp, Fusarium sp, Geotrichum sp Rhizopus sp* and *Penicillium sp.* The result clearly showed that both bacteria and fungi are associated with hydrocarbon contaminated soils. The isolation of these organisms from hydrocarbon contaminated soil have been attributed to either their ability to utilize the hydrocarbon or an ability to tolerate hydrocarbon toxicity [9]. The occurrence of *Nocardia sp in* soil sample obtained from only one of the six stations is indicative of the fact that it might be unconnected with the utilization of hydrocarbon. Therefore the isolate can be said to be transient.

Results of heavy metal uptake by various isolates obtained Abraka, choba, Kiama, Warri and Ughelli stations are presented in Tables 3, 4, 5, 6 and 7 respectively. Uptake of copper, nickel, cobalt, lead and chromium by *Bacillus* strains isolated across the five stations ranged from 0.45 - 0.8, 0.42- 0.60, 0.18 -38, 0.2- 0.6 and 0.2- 0.0.36 (mg/g dry wt) respectively at initial metal concentration of 0.1mg/l. At initial metal concentration of 1.0mg/l the respective values of uptake ranges were 7.40 -12.00, 4.00 -10.00, 4.00 - 11.42, 5.72 -11.8, 2.4-10.00 (mg/g dry wt). 0.08-0.74, 0.14-0.4, 0.1-0.38, 0.2-0.4 and 0.16-0.46(mg/g dry wt) were the respective ranges of copper, nickel, cobalt, lead and chromium biosorbed at initial metal concentration of 0.1mg/l by *Pseudomonas spp isolated* across the five stations. Also, at initial metal concentration of 1.0mg/l the respective ranges of uptake by *Pseudomonas spp* were 5.70-12.18, 4.22-7.94, 1.40-4.00, 0.56-9.00 and 3,76-8.00 (mg/g dry wt). Similarly, the ranges of the respective heavy metal uptake at initial metal concentration of 0.1mg/l by strains of *Micrococcus Acinectobacter Alcaligens Serratia, Aspergillus, Rhizopus* and *Penicillium* obtained across the five stations were 0.14-0.20, 0.06-0.20, 0.10-0.20, 0.06-0.20 and 0.02-0.2 (mg/g dry wt), 0.00-0.20, 0.04-0.68, 0.04-0.40, 0.02-0.28 and 0.00-0.3 (mg/g dry wt), 0.00-0.04, 0.12-0.20, 0.02-0.2, 0.20-1.02 and 0.00-0.12 (mg/g dry wt), 0.00-0.34, 0.00-0.20, 0.00-0.20, 0.04-0.40 and 0.00-0.34 (mg/g dry wt), 0.80-1.80, 0.80-1.40,0.28-0.64, 1.12-1.92 and 0.6-1.98(mg/g dry wt), 0.82-1.24, 0.10-0.30, 0.66-6.82, 1.30-1.96 and 1.90-2.00(mg/g dry wt), 0.82-1.00, 0.20-0.82, 0.76-0.82, 1.64-1.80 and 1.90-1.98 (mg/g dry wt). Again, the ranges of uptake of copper, nickel, cobalt, lead and chromium by the different strains of *Micrococcus Acinectobacter Alcaligens, Serratia, Aspergillus, Rhizopus* and *Penicillium* obtained across the five stations were 6.0-6.32, 2.0-3.6, 0.20-2.52, 2.0-3.78 and 3.60-3.96 (mg/g dry wt), 0.58-2.00, 0.32-3.26, 0.58-2.98, 0.2-0.28 and 2.4-4.00 (mg/g dry wt), 0.58-2.00, 1.00- 2.00, 0.02-4.00, 0.20-2-00 and 2.40-4.00 (mg/g dry wt), 1.00-2.20, 0.80-2.80, 1.00-2.00, 0.00-2.38 and 1.18-2.00, 13.78-17.00, 5.20-16.00, 12.00-16.00, 9.00-12.14 and 10.00-12.90 (mg/g dry wt), 11.00-14.34, 1.20-14.34, 14.28-17.70, 10.32-13.96 and 13.60-16.62 (mg/g dry wt) and 10.40-11.62, 3.00-11.62, 13.68-16.70, 11.20-11.90 and 11.86-14.00 respectively.

In this experimentation, all the dead bacterial biomass sorbed heavy metals though to varying degrees. The pattern of metal uptake among bacterial isolates varied thus: Cu >Ni ≥ Pb > Co > Cr for *Bacillus sp*, Cu>Ni > Pb > Cr ≥ Co for *Norcadia sp*, Cu > Ni ≥ Pb ≥ Cr > Co for *Pseudomonas sp*, Cu > Ni ≥ Pb ≥ Cr ≥ Co *for Micrococcus sp.* Ni ≥ Cr ≥ Co ≥ Pb ≥ Cu, Cr ≥ Co > Cu ≥ Ni ≥ Pb and Ni ≥ Pb ≥ Cu ≥ Co = Cr were the trends in uptake observed for *Acinectobacter sp, Alcaligenes sp* and *Serretia sp* respectively. Among the fungal sorbents the trend of uptake demonstrated was: Cu > Ni ≥ Co > Cr ≥ Pb (*Aspergillus sp*), Cu = Ni > Co > Cr ≥ Pb (*Fusarium sp*), Co = Cr> Cu = Ni > Pb (*Geotrichum sp*), Co ≥ Cr > Cu ≥ Pb > Ni (*Rhizopus sp*) and Co > Cr ≥ Pb ≥ Cu ≥ Ni (*Penicillium sp*).

Table 1: Occurrence of various bacterial and fungal isolates in study stations

Isolates	Stations				
	Choba	Kiama	Abraka	Warri	Ughelli
Bacteria					
Pseudomonas	+	+	+	+	+
Bacillus	+	+	+	+	+
Nocardia	-	-	+	-	-
Micrococcus	+	-	+	+	-
Acinectobacter	+	+	-	+	+
Alcaligenes	+	-	+	+	+
Serretia	+	+	-	+	+
Fungi					
Aspergillus	+	+	+	+	+
Fusarium	-	-	-	+	+
Geotrichum	-	-	-	+	+
Rhizopus nigricans	+	+	+	+	+
Penicillium notatum	-	+	+	-	+

Key: + = Present
- = Absent

Table 2: Physico-chemical properties of various soil samples

Property	Station				
	Choba	Kiama	Abraka	Warri	Ughelli
Moisture content	21.89	25.10	15.63	17.89	12.44
pH	6.59	6.8	6.4	6.12	6.2
Heavy metals					
Copper	0.09	0.01	1.06	0.08	0.03
Cobalt	0.22	0.06	0.008	0.01	0.48
Chromium	0.13	0.1	0.16	0.1	0.06
Nickel	0.84	1.04	0.07	0.09	0.01
Lead	<0.001	0.01	0.66	1.02	0.01

Table 3: Concentration of various heavy metals sorbed by test isolates obtained from Abraka station at initial metal concentrations of 0.1 and 1.0 (mg/L).

Isolate	Heavy metal uptake (mg/g)									
	Copper		Nickel		Cobalt		Lead		Chromium	
	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$
Bacillus	0.80	10.8	0.42	8.60	0.18	4.00	0.60	5.72	0.20	3.60
Norcardia	1.60	17.94	1.04	13.76	0.30	3.68	1.20	9.60	0.40	4.00
Pseudomonas	0.08	5.70	0.28	4.22	0.10	1.40	0.20	4.00	0.16	3.76
Micrococcus	0.14	6.00	0.06	2.00	0.20	0.20	0.06	2.00	0.02	3.96
Acinectobacter	0.04	1.16	0.04	0.40	0.04	0.58	0.02	0.60	0.00	3.80
Aspergillus	1.18	14.40	1.00	5.20	0.30	14.40	1.80	10.40	1.60	11.60
Rhizopus	1.02	14.00	0.04	5.60	0.76	14.80	1.60	10.00	1.96	15.12
Penicillium	0.96	10.40	0.20	3.00	0.80	16.00	1.64	11.20	1.98	13.86

Key: $I_{0.1}$ = Initial metal concentration of 0.1mg/l
: $I_{1.0}$ = Initial metal concentration of 1.0mg/l

Table 4: Concentration of various heavy metals sorbed by test isolates obtained from Choba station at initial metal concentrations of 0.1 and 1.0 (mg/L).

Isolate	Heavy metal uptake (mg/g)									
	Copper		Nickel		Cobalt		Lead		Chromium	
	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$
Bacillus	0.80	12.00	0.60	10.00	0.32	4.00	0.50	6.00	0.20	3.56
Pseudomonas	0.60	6.00	0.40	6.00	0.28	2.08	0.36	9.00	0.46	4.98
Micrococcus	0.20	6.32	0.20	3.60	0.40	4.00	0.20	3.78	0.20	3.60
Acinectobacter	0.20	2.00	0.18	1.00	0.20	2.52	0.02	1.00	0.10	3.60
Alcaligenes	0.00	2.00	0.12	1.00	0.20	4.00	0.30	2.00	0.02	3.68
Serratia	0.00	1.80	0.00	2.00	0.20	2.00	0.18	1.60	0.22	1.18
Aspergillus	1.20	16.00	0.82	5.80	0.40	16.00	1.84	9.58	1.56	12.00
Rhizopus	1.20	12.96	0.24	2.32	1.96	14.28	1.30	10.32	1.96	13.60

Key: $I_{0.1}$ = Initial metal concentration of 0.1mg/l
: $I_{1.0}$ = Initial metal concentration of 1.0mg/l

The differences noticed in the amounts of heavy metals bioconcentrated by each organism are indicative of the selectivity in metal uptake by the various test isolates. An overview of results obtained as presented in Tables 3 - 7, revealed that metal uptake by the various isolates decreased in the order: *Aspergillus sp ≥ Rhizopus sp ≥ Fusarium sp > Penicillium sp > Geotrichum sp ≥ Nocardia sp > Bacillus sp > Pseudomonas sp> Micrococcus sp > Acinectobacter sp > Alcaligens sp > Serretia sp.* There was a significant difference in the uptake of the various heavy metals between the fungal and bacterial biosorbents. Also, for each heavy metal there was a significant difference at p = 0.05 in its uptake by the respective test isolate. However, among fungal isolates there was no significant difference in uptake of various heavy metals (p = 0.05).

The fungi isolates showed a higher capacity of sorbing all tested metal ions from solution in comparison to bacterial isolates. However, residual concentration of heavy metal in solutions that received *Norcardia* was either equivalent to or a little lower than those of fungal isolates. This may be attributable to its tendency to form mycelia which may have provided a greater surface area for metal sorption. Similarly, the presence of mycelia and hyphae may account for the higher sorption capacity displayed by the dead cells of the fungal isolates.

Table5: Concentration of various heavy metals sorbed by test isolates obtained from Kiama station at initial metal concentrations of 0.1 and 1.0 (mg/L).

Isolate	Heavy metal uptake (mg/g)									
	Copper		Nickel		Cobalt		Lead		Chromium	
	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$
Bacillus	0.64	10.00	0.46	8.26	0.38	7.86	0.20	11.80	0.06	2.40
Pseudomonas	0.74	12.00	0.32	5.18	0.38	2.98	0.40	0.56	0.40	5.56
Acinectobacter	0.00	0.58	0.20	0.32	0.02	1.80	0.20	0.20	0.00	2.40
Serratia	0.20	2.20	0.20	0.80	0.00	1.00	0.00	2.00	0.34	2.00
Aspergillus	1.80	17.00	1.40	15.82	0.60	14.00	1.70	10.00	1.40	11.40
Rhizopus	1.24	14.00	0.38	1.20	0.80	16.40	1.96	12.26	1.98	14.00
Penicillium	1.00	11.18	0.24	4.00	0.82	13.68	1.80	11.78	1.98	11.86

Key: $I_{0.1}$ = Initial metal concentration of 0.1mg/l
: $I_{1.0}$ = Initial metal concentration of 1.0mg/l

Table 6: Concentration of various heavy metals sorbed by test isolates obtained from Warri station at initial metal concentrations of 0.1 and 1.0 (mg/L).

Isolate	Heavy metal uptake (mg/g)									
	Copper		Nickel		Cobalt		Lead		Chromium	
	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$
Bacillus	0.46	7.40	0.44	4.00	0.20	11.92	0.60	6.00	0.36	10.00
Pseudomonas	0.58	12.18	0.14	7.94	0.28	4.00	1.20	7.94	0.32	8.00
Acinectobacter	0.20	2.00	0.68	3.26	0.40	2.98	0.28	2.90	0.30	4.02
Alcaligenes	0.04	1.60	0.18	2.00	0.12	1.64	1.02	1.00	0.12	4.00
Serratia	0.34	1.00	0.20	2.80	0.00	1.64	0.20	1.00	0.40	2.38
Aspergillus	1.02	16.00	1.02	16.00	0.64	15.80	1.92	12.14	1.98	12.9
Fusarium	0.86	12.84	0.86	12.84	0.40	12.18	1.04	9.04	0.80	10.22
Geotrichum	0.34	8.00	0.34	8.00	0.32	12.00	1.20	6.78	0.32	12.00
Rhizopus	1.20	14.34	1.20	14.34	0.82	17.70	1.94	13.96	2.00	16.62

Key: $I_{0.1}$ = Initial metal concentration of 0.1mg/l
: $I_{1.0}$ = Initial metal concentration of 1.0mg/l

The uptake of heavy metals by bacterial sorbents could be as a result of the interaction between the metals and amphoteric groups such as the carboxyl and phosphoryl groups that occur within the constituent polymers of bacterial cell walls which act as if they were an open ion exchange resin[10]. In this study, Gram positive cells displayed a higher sorption capacity than the Gram negative isolates. According to Beveridge [10], teichoic acid in the cell walls of Gram positive bacteria, had very high potentials as chemosorption sites.

Again, it was observed that uptake of the respective heavy metal by each test isolate at initial concentration of 1.0 mg/l were significantly higher than at 0.1 mg/l (p = 0.05). This finding suggest that metal uptake may involve diffusion phenomenon whereby, metal ions move from regions of high concentrations to low concentrations and the fact that the steeper the concentration gradient, the more rapid is the movement of molecules or ions [11].

Furthermore, there was no significant difference in heavy metal uptake among organisms of same genus obtained from the five locations (p = 0.05). This clearly indicates that the performance of these strains across the stations

studied were similar. Therefore, in events of contamination by hydrocarbons, the presence of these organisms would go along way in immobilizing the associated heavy metal contaminants in soils thereby minimizing toxic effects irrespective the location of contamination.

Table 7: Concentration of various heavy metals sorbed by test isolates obtained from Ughelli station at initial metal concentrations of 0.1 and 1.0 (mg/L).

Isolate	Heavy metal uptake (mg/g)									
	Copper		Nickel		Cobalt		Lead		Chromium	
	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$	$I_{0.1}$	$I_{1.0}$
Bacillus	0.80	10.80	0.42	8.60	0.18	4.00	0.60	5.72	0.20	3.60
Pseudomonas	0.08	5.70	0.28	4.22	0.10	1.40	0.20	4.00	0.16	3.76
Micrococcus	0.14	6.00	0.06	2.00	0.20	0.20	0.06	2.00	0.02	3.96
Acinectobacter	0.04	1.16	0.04	0.40	0.04	0.58	0.02	0.60	0.00	3.80
Alcaligenes	0.00	0.58	0.20	1.80	0.02	0.02	0.20	0.20	0.00	2.40
Serratia	0.00	1.00	0.00	2.00	0.00	2.00	0.00	1.20	0.00	1.60
Aspergillus	0.80	13.78	0.80	5.84	0.28	12.00	1.12	9.00	0.60	10.00
Rhizopus	0.82	11.00	0.10	4.00	0.66	15.12	1.76	12.00	1.98	14.00
Penicillium	0.82	11.62	0.82	11.62	0.76	16.70	1.76	11.90	1.90	14.00

Key: $I_{0.1}$ = Initial metal concentration of 0.1mg/l
: $I_{1.0}$ = Initial metal concentration of 1.0mg/l

REFERENCES

[1]Van, L., Iyklema, M.C., Noode, M., Schrae, G. and Zelinder, A.J.B., *App. Environ. Microbiol*.**1987**, 53, 1893-1897.
[2]Numberg, H.W., *Analty Chem*, **1983**, 316,557-565 .
[3]Onwurah, N.E., Ogugua, V.N., Onyike, N. B.,Ochonogor, A.E. and Otitoju, O.F., *S.Environ. Monit. Assess*,**2007**,pp.1-3.
[4]AOAC Association of Official Analytical Chemist. *Method of Analysis*. 13th edn. Washington D.C. USA., **1990**, pp 438.
[5]Fawole, M.O. and Oso, B.A.. *Laboratory manual of Microbiology*. Spectrum books ltd., Ibadan, Nigeria. **1988**. p 56.
[6]Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley And T. Williams**.** Bergey's *Manual of Determinative Bacteriology*, 9th ed., Williams and Wilkins, Baltimore, USA. **1994**.
[7]Barnet, H.L. and B.B. Hunter. *Illustrated genera of imperfect fungi*. 3rd edn, Burges Publishing Company, Minneapolis, **1972**. pp 208.
[8]Volesky, B. And H.A. May-Philips. *Appl. Microbiol. Biotechnol*. **1995**.42, 797 806.
[9]Nwachukwu, S.C.U., *Int. J. Environ.Edu. Info*. **2000**, 19 (1), 55-62
[10]Beveridge, T.J., *Annu. Rev. Microbiol*. **1989**,43, 147-171.
[11]Taylor, D.J., N.P.O Green and G.W. Stout. *Biological Sciences*. 3rd ed. Cambridge University Press. **1997**. pp 983.