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RESEARCH PAPER

Techniques in chitosan extraction from indigenous crab and utilization in bio-filtration of crude oil polluted water

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Abstract. The techniques for chitosan extraction from indigenous crabs were assessed using physicochemical properties of the chitosan, and its utilization in the treatment of crude oil polluted water was investigated. Physicochemical properties, heavy metal analytic method (AAS), and hydrocarbon analysis (GC-MS method) of the water samples were employed for this investigation. Results obtained from the physical and chemical properties of the chitosan extracted from indigenous crabs (*Scylla* species) showed a comparable percentage yield (53% and 50%) compared to synthetic chitosan (72%). The pH values obtained from the chitosan ranged from 6.9 – 13.0, while the percentage degree of deacetylation (DDA) ranged from 60 – 75%. The water quality obtained from Ochani River, filtered with chitosan, yielded the best result with chitosan C compared to chitosan B. The heavy metal content in the polluted Ochani River was totally removed with the control (synthetic chitosan A) compared to chitosan C, which showed a comparably reduction. However, the bio-filtration with chitosan B also revealed better reduction compared to chitosan A. The study established that irrespective of the crude method applied in the extraction of the chitosan from indigenous crabs, its efficacy in treating crude oil-polluted Ochani River was highly impactful.

Keywords: Chitosan; Crude oil; Pollution; Ochani River; Heavy metal; Bio-filtration; Polyromantic hydrocarbon

1. Introduction

The necessity for water treatment due to increasing environmental pollution has contributed to the utilization of different mechanisms, including chemical processes, in availing drinkable water. Consequently, chemical substances involved have its limitations as they can confer a level of toxicity when not managed properly. Chitosan a biological biopolymer known for properties such as antimicrobial, biodegradable, biocompatible, non-toxicity, and effectiveness as a coagulant and flocculant with beneficial bonding agents, has influenced its extensive industrial applications, including wastewater treatment plants (Rizeq et al., 2019). Chitosan, as a derivative of chitin, a natural linear biopolymer which is one of the most abundant polysaccharide except for cellulose,

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commonly found in seafood shells of crustaceans, arachnids, and insects (Sudha et al., 2017). Some organisms, such as *Rhizopus* species and *Mucor*, contain elevated quantities generally obtained from their chitin. The chemical structure of chitin β – 1 → 4 N - acetyl – D – glucosamine on conversion to chitosan, is deacetylated to form (β – (1→ 4) glucosamine).

The addition of proton to the amino group of chitosan affords its characteristic of dissolving in acid medium at pH (≤ 6). The exploitation of hydroxyl and amine functional groups provides tractability in forming different structural products with enhanced properties and application (Rizeq et al., 2019). The formation of complex electrolytes from several anionic and cationic reactions demonstrate its film-forming ability, nanoparticle preparation, hydrogel formation, and proficiency in fiber making (Croisier & Jérôme, 2013). The aforementioned properties of chitosan suggest its potentials in treating polluted water; however, the efficiency of chitosan in performance is related to proficiency in the extraction methods. Various extraction methods are involved in chitosan formation; nonetheless, it depends on the needed quality and source of the shells. However, the most utilized extraction methods are chemical processes, considering their efficiency in achieving a high degree of deacetylation (DDA). Moreover, demineralization and deprotonation of the shells in various extraction methods still utilize chemical processes (Lee et al., 2016).

Different alkaline have been reported as beneficial chemicals in removal of protein from chitin during processing, with variations in the concentrations, reaction time, and temperature levels involved in the determining DDA. Furthermore, achieving 100% deacetylated chitosan is nearly impossible. Studies have reported deacetylation of chitosan within 10% or less when the process is augmented (Lertwattanaseri et al., 2009). In view of the extraction techniques employed, the intent is to investigate the chemical processes of chitosan extraction procedures and its application to crude-oil contaminated water as a treatment product. The bio-filtration efficiency of the processed chitosan will determine the proficiency of the methods utilized, considering the parameter measured such as the DDA, removal efficiency, and functional groups.

2. Material and method

2.1. Collection and identification of crab (*Scylla spp*)

The crab (*Scylla spp*) exploited for this study was obtained from the swampy area of Ibii stream in Ihugbe, Okigwe Local Government Area of Imo State, Nigeria. They were collected between 8 a.m. and 10 a.m. in June 2020. Subsequently, the crabs were stored in an ice-packed container, identified and transported to the laboratory, where they were stored at 5°C.

2.2. Preparation of crab shell for chitosan extraction

According to the modified method by Pambudi et al. (2018), the stored crabs were carefully dissected. The fleshy part of the crabs was removed, washed, and dried in a hot air oven at temperature of 70°C for 24 hours. The resulting dried shells were ground, sieved using a tiny mesh filter, and the fine particles were collected for further processing, while the coarse particles were set aside.

2.3. Chitosan extraction methods

The extraction of the chitosan was carried out using acid and alkaline treatments, by changing process protocols and reagents at different temperatures and exposure times. The study employed two different chitosan extraction methods, as reported by Gaikwad et al. (2015) but followed the procedure of extraction reported by Ali et al. (2019). The two extraction methods of chitosan are Deproteination, Demineralization, Decoloration and Deacetylation (DPMCA) and Demineralization, Deproteination, Decoloration and Deacetylation (DMPCA). The procedures for these methods are presented in Figure 1.

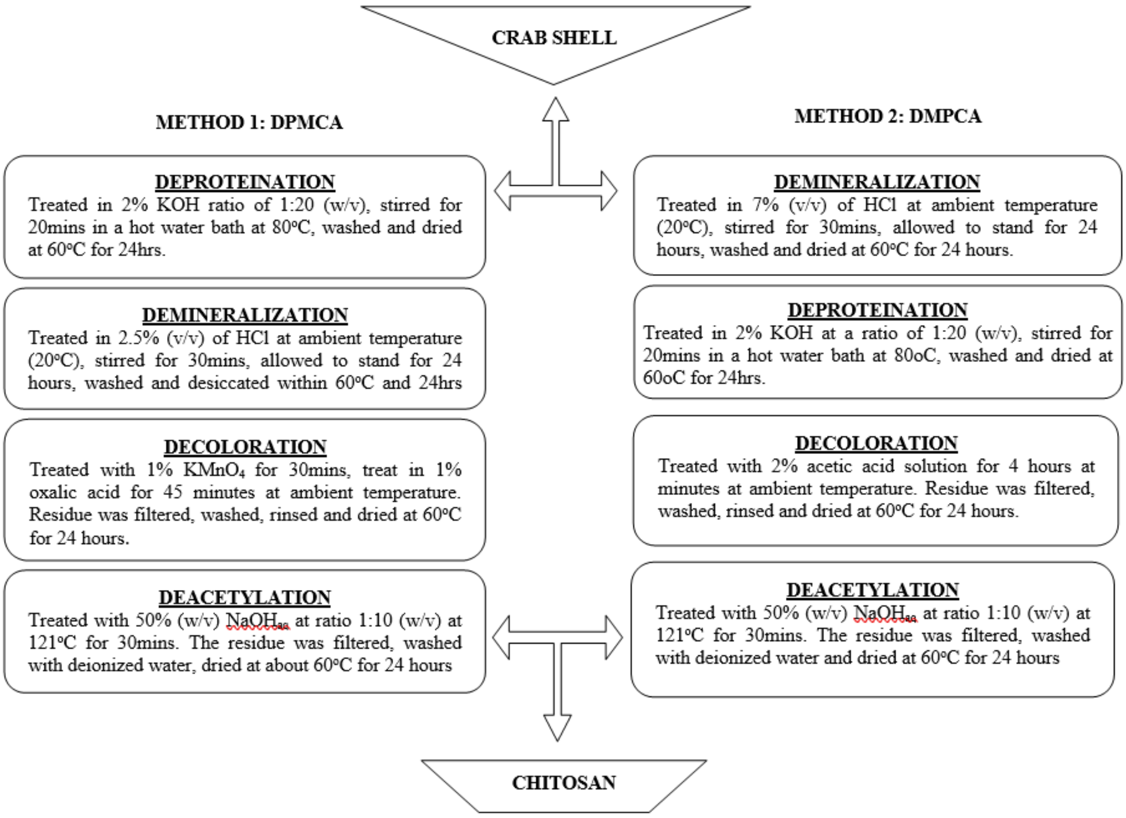

Figure 1. Schematic representation of chitosan extraction from crab shell

2.4. Collection of water sample

The crude oil polluted water sample was obtained from Ochani River situated in the Ejamah-Ebubu community of Ogoniland, River State. The sampling was carried out during the rainy season to ensure total mixing of activities within the water body, including tidal currents during run-off. The containers were thoroughly washed, sterilized with 70% ethanol, rinsed multiple times with distilled water, and finally with the sample solution before collecting the sample for analysis. The collection of the crude oil-polluted water sample from Ochani River followed the procedure reported by Nwogu et al. (2022). The water sample was collected with a 2-liter plastic cup at a depth of 8 – 10 cm and then transferred into a 25-liter container with screw cap, labeled appropriately. The samples were conveyed to the test center where they were kept in ice box at 12°C temperature range and were analyzed within 48 hours of arrival.

2.5. Physicochemical analysis

The physicochemical properties as pH, temperature, color, conductivity, turbidity, total solids, total suspended solids (TSS), total dissolved solids (TDS), total hardness, dissolved oxygen, biochemical oxygen demand (BOD), and chemical oxygen demand (COD) of the water samples were analyzed using the method described by Ukachukwu et al. (2022).

2.6. Heavy metal analysis

Aliquot containing elements such as Iron, zinc, copper, chromium, cadmium, arsenic, and lead were concentrated and analyzed using the Agilent FS240AA Atomic Adsorption Spectrometer, according to the method described by Ugwu et al. (2021).

2.7. Bio-filtration of crude oil and its related components from water sample using chitosan

This research uses three type of chitosan samples: Chitosan sample A, synthetic chitosan commercially procured. Chitosan sample B, locally extracted chitosan using DPMCA extraction method. Chitosan sample C, locally extracted chitosan using DMPCA extraction method. The chitosan solution was prepared by the homogenously mixing of chitosan powder, water, and acetic acid. Five hundred milliliters of the crude-oil polluted water samples from Ochani River were taken and treated with varying concentrations (10%, 30%, 50%, 70%, and 90%) of the chitosan solution. The mixture was stirred, allowed to stand for 10 minutes, the turbidity measured, and then left for 12 hours. This modified application method of application was reported by García et al. (2016).

2.8. Statistical test

The data analysis was performed using Minitab software. The variance was obtained to be a significant at a level of $p < 0.05$. The means and standard deviations of the triplicates were calculated, and Dunnett's multiple comparison was applied to the data.

3. Result and discussion

3.1. Quantification and characterization of synthetic and extracted *Scylla spp* (crab) chitosan

The results obtained from the quantification and characterization of synthetic and locally extracted chitosan are presented on Table 1. The physical and chemical properties analyzed include yield, pH, moisture content, ash content, turbidity, solubility, viscosity, and the DDA of the chitosan.

The results showed variations in the properties between chitosan B and chitosan C (locally extracted). Chitosan B and C had different yield values, with chitosan B at 53% and chitosan C at 50%. The pH value of chitosan C was 13, proving higher alkaline of chitosan C compared to chitosan B, which had a pH of 8. The percentage moisture content was 20% for chitosan B and 30% for chitosan C. Both chitosan B and C had ash content of 10%. The turbidity values for chitosan B and C were 7.5 NTU and 7.6 NTU, respectively.

Regarding solubility and viscosity, chitosan C showed higher values with 50% and 3.9 mg/L viscosity, whereas chitosan B exhibited 30% solubility and 3.0 mg/L viscosity. The DDA for chitosan B was 60%, lower than the 65% value obtained in chitosan C.

Comparing with synthetic chitosan A, chitosan A had a pH of 6.9, lower than both chitosan B and C, indicating greater neutrality. The moisture content of chitosan A was 20%, similar to chitosan B but different from chitosan C. Chitosan A had an ash content of 40%, notably higher than chitosan B and C. The turbidity of chitosan A was not significantly different from that of chitosan B and C. The solubility value of Chitosan A was 70%, higher than both chitosan B and C. Additionally, the DDA for chitosan A was 75%, higher than that of chitosan B and C.

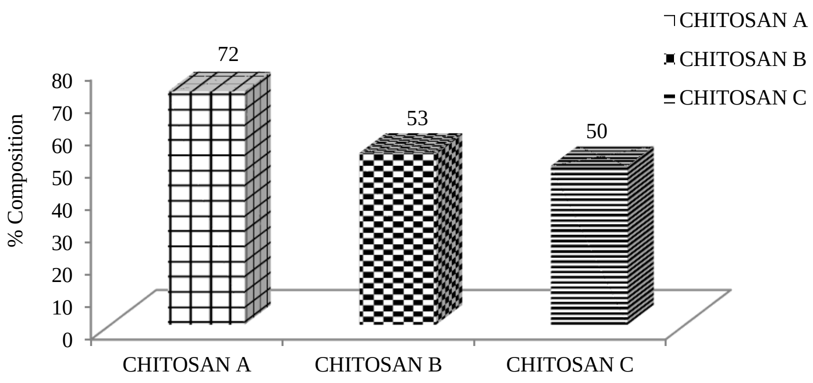

Figure 1a. The yield of synthetic and extracted chitosan

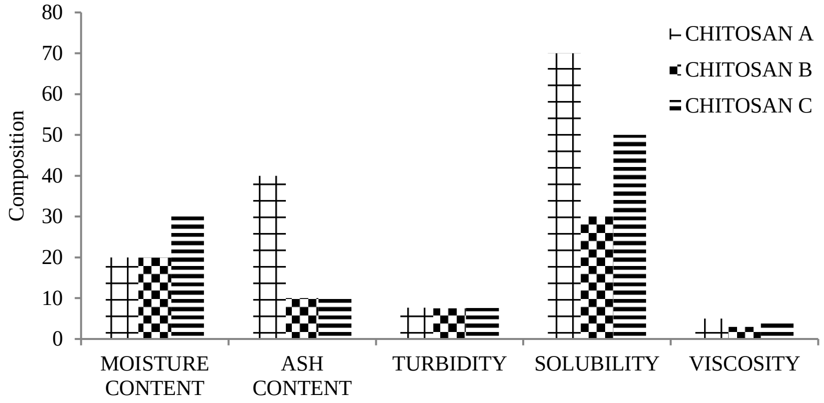

Figure 1b. Physical properties of synthetic and extracted chitosan

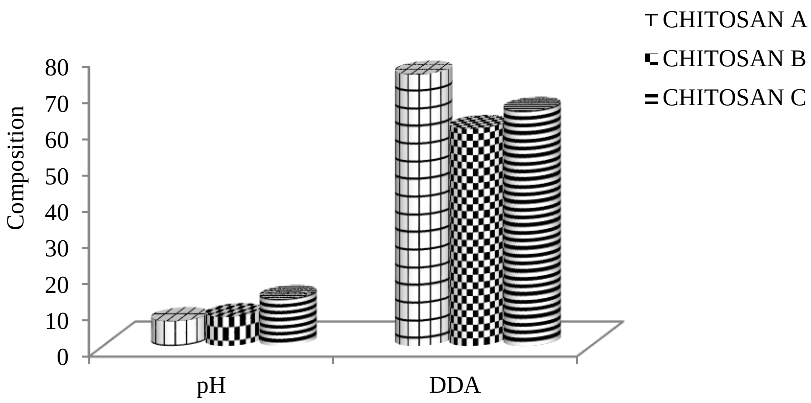

Figure 1c. Chemical properties of synthetic and extracted chitosan

3.2. The structural composition of synthetic and extracted chitosan

The FTIR structural compositions of synthetic and extracted chitosan was presented in Table 1. The obtained values showed that the presence of fundamental compounds in chitosan, including Nitro- compounds, Nitrites, Hydroxyl acid (O-H acids), Amines (N-H 2° and N-H 1° amines), Amide (N-H 1° aminde), Amines stretching, and O-H alcohol stretching in the locally extracted chitosan.

Chitosan A (control) contains all the characteristic compounds except nitrites and amine stretching. Chitosan B contains the compounds except nitro compound, nitrites, and amides, while chitosan C contains nitro compounds, nitrites, O-H acids, amine stretching, and OH alcohol stretching but does not contain N-H 2° amines and amide.

These results shows that nitro compound was present in chitosan A and C but absent in chitosan B. Nitrites was found in chitosan C but were absent in chitosan A and B. O-H acids was found in chitosan A, B and C. N-H 2° amine was found in chitosan B and C but absent in chitosan A. O-H alcohol stretching was present in chitosan A, B and C. N-H 1° amide was found in chitosan A and was absent in chitosan B and C.

3.3. The physical properties of crude oil polluted Ochani River after treatment with chitosan.

The results of the physical properties of the crude oil-polluted Ochani River bio-filtered with chitosan are shown on Table 1. The parameters analyzed, consistent with the prior analysis, include temperature, color, turbidity, solubility, total solids, TDSs and TSS.

The values obtained from the physical properties of the polluted water sample, bio-filtered with chitosan, showed that the temperature of the different concentrations A1 – A5, B1 – B5 and

C1 – C5 were in the range of 25.99 – 29.78°C, 27.34 – 29.23°C, and 27.23 – 30.12°C respectively (Figure 2a). It presents no significant differences among them. Post-treatment, the water color changed from dark brown to the natural colorless nature of water.

Table 1. FTIR composition of the synthetic and extracted chitosan from crab shells

Functional groups	Chitosan A (cm^{-1})	Chitosan B(cm^{-1})	Chitosan C (cm^{-1})
O=N-O Nitro compounds	1384.61	-	1304.036
O=N-O Nitro compounds	-	-	1377.802
C=H Alkenes	1618.79	1618.67	1461.179
C=O Esther anhydrides	-	-	1762.084
C=H bond aromatic	-	-	1852.44
N=C=S Isothiocyanate	2086.48	-	1962.84
C=N Nitrites	-	-	2288.94
O-H Acid or Aldehyde	-	2683.74	-
S-H Thiol	-	-	2593.76
O-H Acid	2446.0	2420.44	2397.93
C-H Alkane stretch	-	2990.07	2956.16
C-H Aromatic stretch	-	3158.72	3194.935
N-H 2^0 Amine stretching	-	3316.11	3379.607
O-H Alcohol	3429.525	3605.33	3603.34
O-H Alcohol stretching (free)	3822	3685.27	3833.63
C-O Acyl, alkyl	1176.327	1138.09	-
C-O acyl, alkyl	-	1299.48	-
N=C=S Isothiocyanate stretching	-	2018.78	-
CΞC Alkyne stretching	-	2139.54	-
C=O Aldehyde	-	2852.80	-
N-H 1^0 Amine	3429.525	3459.58	-
N-H 1^0 Amide or O-H acid	3281.14	-	-
O-H Acid broad	3162.67	-	-
C-H stretching Alkene sharp	3039.566	-	-
O-H Acid broad	2678.061	-	-
N=C=O Isocyanate	2273.3	-	-
C-N	1019.6	-	-
C=H bending Alkene	826.849	809.95	854.82
C=H bending Alkene	698.706	-	-

(-) = Absent; cm^{-1} = per centimeters.

Turbidity results showed values for treated water samples: A (6.1 – 7.3 NTU), B (4.9 – 9.0 NTU), and C (4.9 – 7.0 NTU) (see Figure 2b). B4 exhibited the highest turbidity (9.0 NTU) compared to B3, C1, A5 (7.0 NTU), while B1 and C3 had the least values of 4.90 NTU.

The total solid values increased in B1 and B4 (5.0 and 12.0 mg/L), C3 and C5 (10.0 and 16.0 mg/L), while chitosan A treatment reduced between A2 and A5 (15.0 and 9.0mg/L) (see Figure 2c). Also, TDS ranged from A (12.0 – 19.0 mg/L), B (9.0 – 15.0 mg/L), and C (12.0 – 18.0 mg/L) (see Figure 2d). The highest treatment was at A2 (19.0 mg/L) while B2 had the lowest value (9.0 mg/L). TDS values of C3 and C5 increased from 12.0 – 18.0 mg/L while A1 and A5 decreased from 17.0 – 12.0mg/L. TSS values increased from B1 and B4 (2.5 – 8.0 mg/L) but decreased between C1 and C4 (10.0 – 6.0 mg/L), while chitosan A1 to A5 showed unstable values (see Figure 2e).

The values obtained from the physical properties of the crude oil-polluted water sample from Ochani River are lower in the treated samples compared to the untreated ones. However, the treated water samples also fall below the standard permissible limit set by WHO (2004) for water quality. Table 2 displays the analysis of variance for chitosan treatments A, B and C. The physical properties of treated water samples showed that none of the treatments completely eliminated the effects of the contaminants. Therefore, they were compared based on their mean effects, using

A as control to determine the relative effectiveness of treatments B and C in comparison to A. This comparison was conducted using Dunnett's Comparison Test, and differences were considered significant at a 95% confidence level.

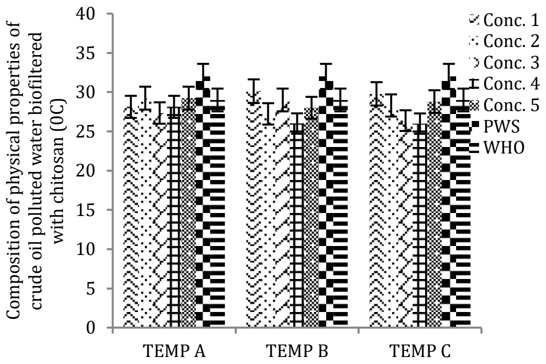

Figure 2a. The temperature of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

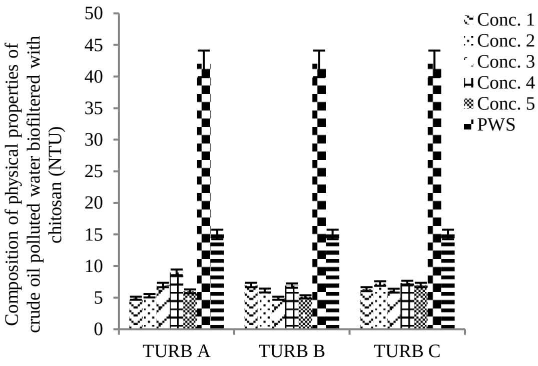

Figure 2b. The turbidity of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

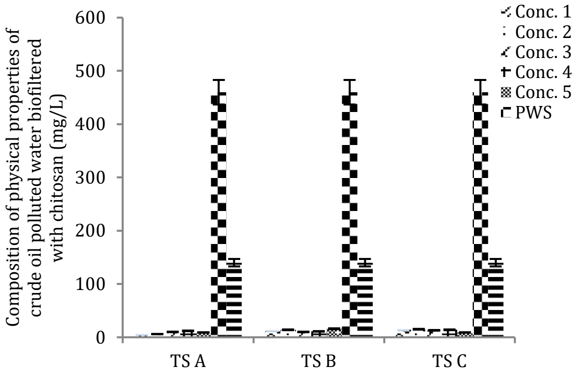

Figure 2c. The total solid of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

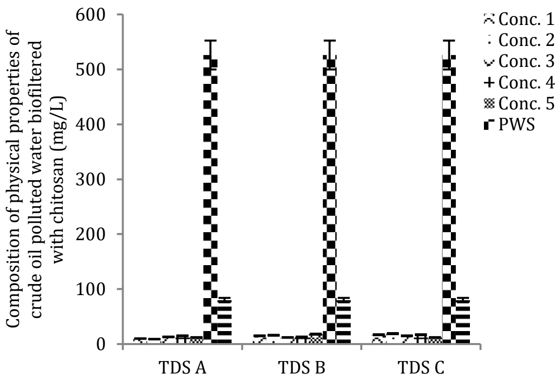

Figure 2d. The TDS of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

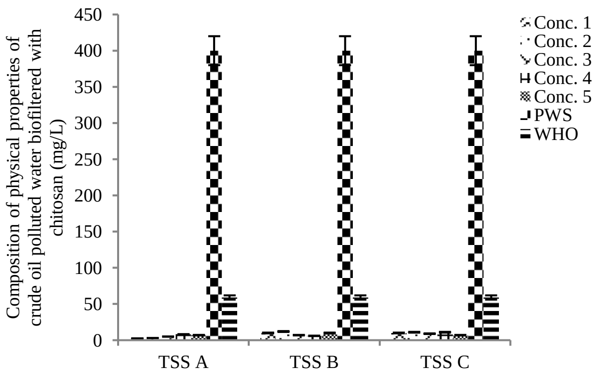

Figure 2e. The TSS of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

Table 2. Comparison of physical properties of bio-filtered water samples

Chitosan Treatment	Temperature	Turbidity	TS	TDS	TSS
Chitosan A	28.4 ± 0.8	6.4 ± 1.6	*8.4 ± 2.7	*11.8 ± 2.3	*5.1 ± 2.2
Chitosan B	28.1 ± 1.5	6.0 ± 0.9	12.6 ± 2.2	14.8 ± 2.2	9 ± 2.3
Chitosan C	27.9 ± 1.5	6.8 ± 0.7	13 ± 2.2	16 ± 2.5	9.6 ± 1.6

*= significant difference at 95% confidence level, TS = Total solid, TDS = Total dissolved solid, TSS = Total suspended solid

3.4. The chemical properties of crude oil polluted Ochani River bio-filtered with chitosan

The results of the chemical properties of the crude oil - polluted Ochani River bio-filtered with chitosan are shown on Table 3. The parameters analyzed include pH, electrical conductivity (EC), total hardness, dissolved oxygen, BOD, COD, nitrates, phosphates, sulphate and exchangeable base.

The pH levels for concentrations A1 – A5, B1 – B5, and C1 – C5 ranged between 7.0 – 9.0, 7.6 – 9.0 and 7.8 – 8.3, respectively (Figure 3a). Post-treatment pH values notably increased, indicating neutralization and reduced acidity compared to pre-treatment levels.

The values obtained for EC of the bio-filtered water samples decreased for A from A1 – A5 (17.0 µScm – 4.0 µScm), increased for B, reaching the highest at B4 (20.0 µScm) compared with B3 and B5 (18.0 µScm & 14.0 µScm), while C showed a decrease from C1 – C4 (20.0 µScm to 10 µScm) (Figure 3b). Regarding the total hardness of the bio-filtered water sample, it increased from A1 to A5 (13.0 mg/L – 19.0 mg/L), with B4 exhibiting the highest value (25.0mg/L) and B2 the least value of 7.0 mg/L (Figure 3c). However, these results did not significantly differ from C2, C3, C5, A1 & A2.

The dissolved oxygen in the treated water sample decreased with treatment B (8.0 – 6.5 mg/L) and showed no significant different from chitosan C1, C3, C4 and C5 (Figure 3d). However, the values for chitosan A reduced as their concentrations increased. A1 recorded the highest dissolved oxygen value (10.89 mg/L), while C2 exhibited the lowest value of 5.45 mg/L. As for the BOD in the treated water sample, was not significant difference observed across the various concentrations of the chitosan used (see Figure 3e). B5 was the highest value at 5.0 mg/L, while C4 displayed the lowest value of 2.10 mg/L.

The COD of the crude oil-polluted water treated with chitosan ranged from 10.0 – 19.0 mg/L, 9.0 – 12.0 mg/L, and 7.6 – 13.1 mg/L (Figure 3f). The highest COD was observed at A1 (19.0 mg/L) compared to the other treatment concentrations, while the least was noted at C2 with a value of 7.57 mg/L. Values of NO_3 (Figure 3g), PO_4 (Figure 3h), and SO_4 (Figure 3i) exhibited insignificant decreases with varying concentrations of the chitosan treatment, all lower than values obtained before the treatment. Calcium (see Figure 3j) and potassium (Figure 3m) values decreased in treatment A1 and A5 and treatment C1 and C5 but increased in treatment B1 and B5. Magnesium increased in treatment with chitosan B but reduced in treatment with chitosan A and C (Figure 3k). Sodium increased across the different concentrations of the chitosan samples used (Figure 3l). However, those values are obtained are lower than the values before the treatment and also fall below the standard permissible limit set by WHO (2004).

The analysis of variance, presented in table 3 for the chitosan treatments, showed that the treatments were unable to totally eliminate the effects of the chemical contaminants. Hence, a comparison was made between their mean effects using A as control to evaluate the effectiveness of treatments B and C in relation to A. Treatments A, B, and C demonstrated similar effects on Total hardness, Calcium and Magnesium. Treatments A and B have the same effect on EC, whereas treatment C showed significant different, indicated without a superscript "a". Regarding Dissolved oxygen, BOD, COD, Sodium, Chlorine, Nitrates, Phosphate and Sulphate, the effects of chitosan A and C were similar, while chitosan B showed a significant difference, indicated without a superscript "a". Finally, for Potassium, the effects B and C are differed significantly from A, indicated without a superscript "a". This implies that for this treatment process, any of the chitosan types can be applied, but A stands out as the most effective treatment.

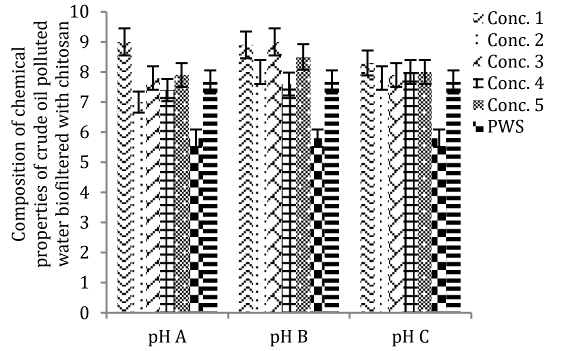

Figure 3a. The pH of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

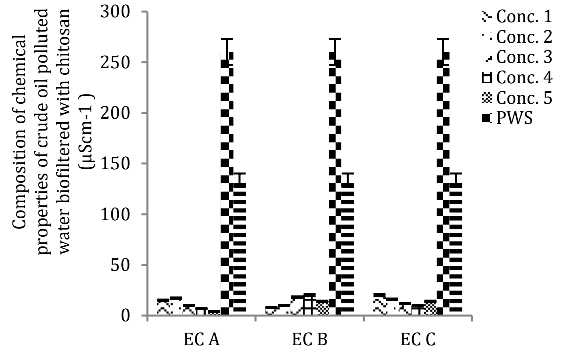

Figure 3b. The EC of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

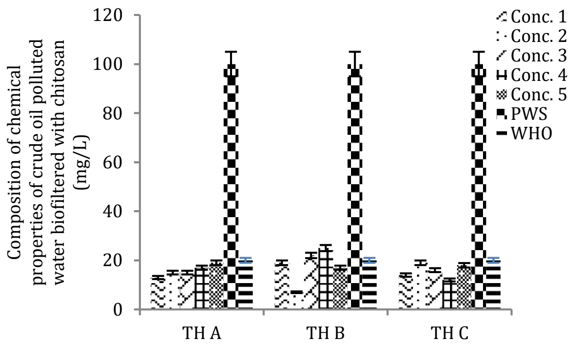

Figure 3c. The total hardness of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

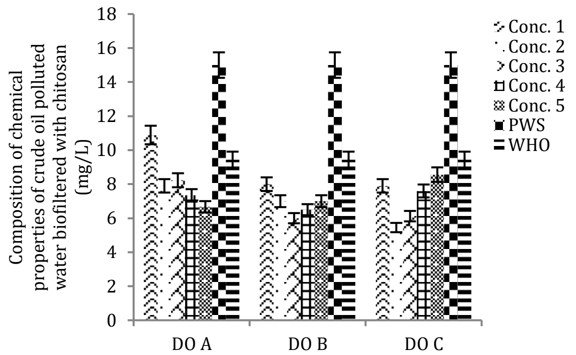

Figure 3d. The dissolved oxygen of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

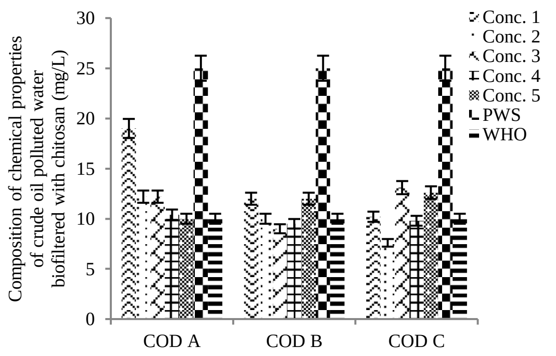

Figure3f. The COD of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

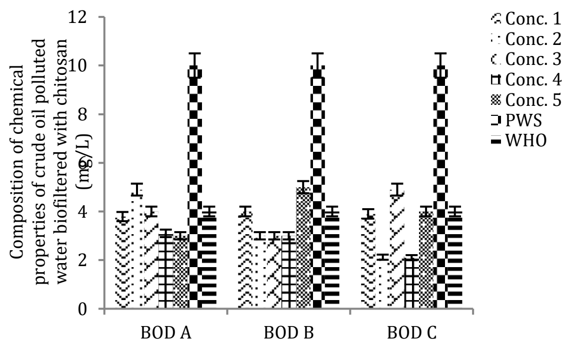

Figure 3e. The BOD of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

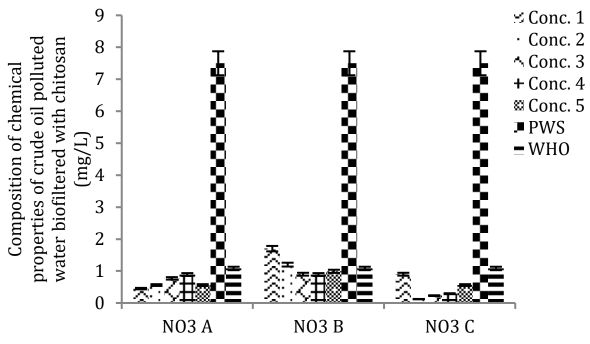

Figure 3g. The nitrate content of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

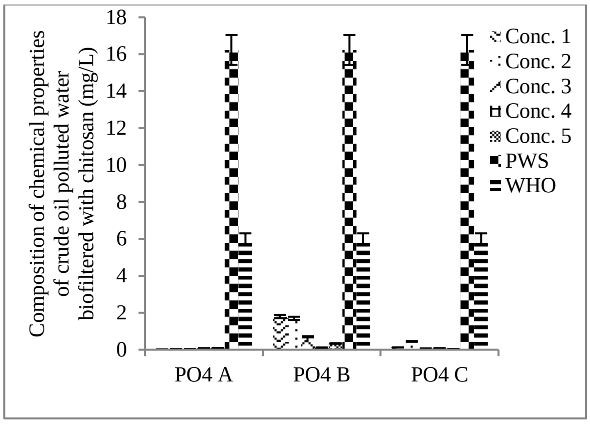

Figure 3h. The phosphate content of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

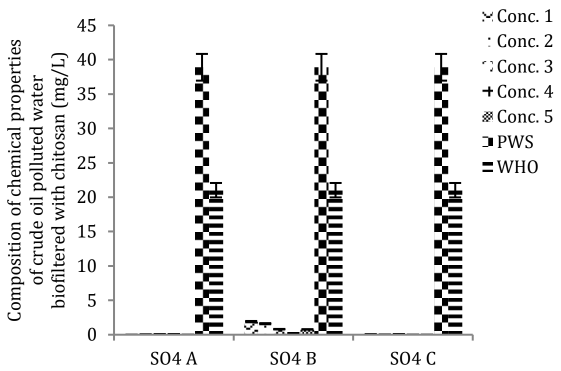

Figure 3i. The sulphate content of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

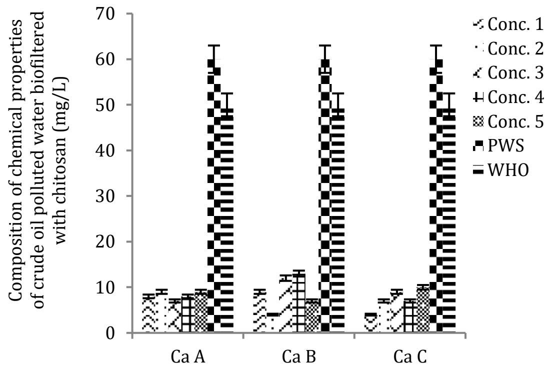

Figure 3j. The calcium content of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

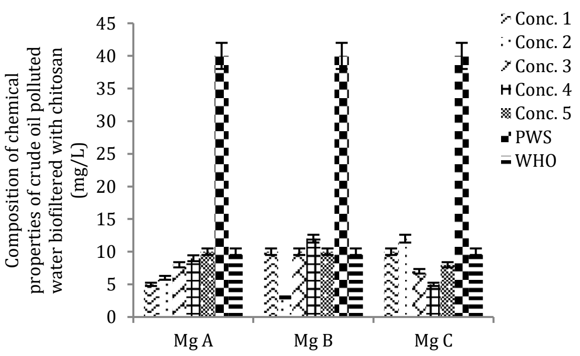

Figure 3k. The magnesium content of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

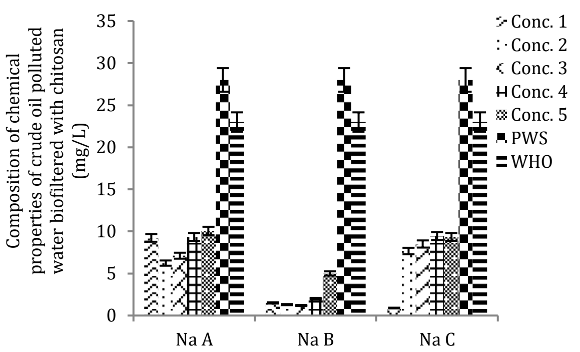

Figure 3l. The sodium content of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

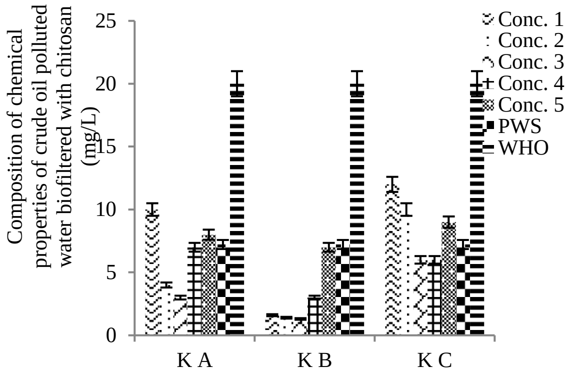

Figure 3m. The potassium content of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

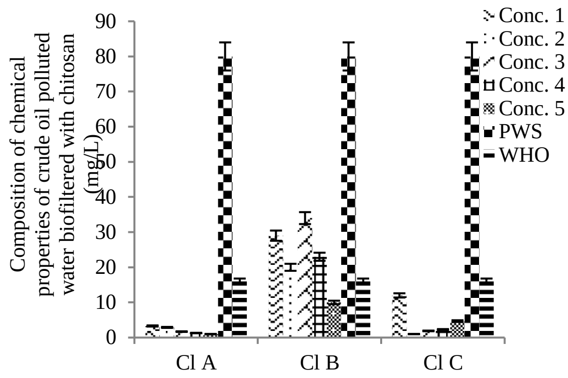

Figure 3n. The chlorine content of crude oil polluted water after biofiltration with chitosan compared with the polluted and the WHO standard

Table 3. Comparison of chemical properties of bio-filtered water sample

Treatment	EC	TH	DO	BOD	COD	NO_3	PO_4
A	10.6 ± 5.0	6.8 ± 0.7	13.0 ± 2.2	16.0 ± 2.5	9.6 ± 1.6	0.6 ± 0.2	0.05 ± 0.03
B	14.0 ± 4.7	6.4 ± 1.6	*8.4 ± 2.7	*11.8 ± 2.3	*5.1 ± 2.2	*1.1 ± 0.3	*0.9 ± 0.7
C	*14.4 ± 3.6	6.1 ± 0.9	12.6 ± 2.2	14.8 ± 2.2	9.0 ± 2.3	0.4 ± 0.3	0.2 ± 0.2

Treatment	SO_4	Ca	Mg	Na	K	Cl
A	9.6 ± 1.6	8.2 ± 0.8	7.6 ± 1.9	8.4 ± 1.5	6.4 ± 2.7	2.0 ± 0.9
B	*1.1 ± 0.6	9.0 ± 3.4	9.0 ± 3.2	*2.2 ± 1.5	*2.9± 2.2	*23.2 ± 8.5
C	9.0 ± 2.3	7.4 ± 2.1	8.4 ± 2.5	7.2 ± 3.3	*8.6 ± 2.4	4.4 ± 4.1

*=significant difference at 95% confidence level, TRT = Treatment, EC = Electrical conductivity, TH = Total hardness, DO = Dissolved oxygen, BOD = Biochemical oxygen demand, COD = Chemical oxygen demand, NO3 = Nitrates, PO4 = Phosphates, SO4 = Sulphates, PWS = Polluted Water Sample.

3.5. The heavy metal content of polluted Ochani River treated with chitosan

The heavy metal content results of the crude oil-polluted Ochani River treated with chitosan are shown in Table 4. The obtained results showed that most of the metals present in the polluted water sample of Ochani River were successfully eliminated during the treatment. Zinc, for instance, was highest in C1 and C2 (0.91 & 0.90 mg/L), while Fe was most prevalent in C1 and B3 (0.89 mg/L), followed by A3 and B5 (0.57 mg/L). The values obtained in C1 and A3 (0.89 mg/L) exceeded the permissible limit set by WHO. Chitosan A effectively removed all the heavy metals except for iron and arsenic. Although Chitosan B might not have completely eliminated all heavy metals, it managed to reduce them below the WHO (2004) permissible limit limit. Chitosan C, on the other hand, completely eliminated some metals and reduced other to a permissible limit. However, copper, cadmium, lead, chromium and arsenic were all reduced lower than the WHO (2004) permissible limit.

3.6. Discussion

The results obtained from the quantification and characterization synthetic and extracted chitosan showed a high yield. However, the percentage yield of chitosans extracted from *Scylla* species was significant different compared to the crude method exploited by Sarbon et al. (2015).

The high yield obtained justifies the potential using of mud crab as an economical method for chitosan production, considering its availability and the low cost of the source.

Table 4. Heavy metal content of crude oil polluted Ochani River bio-filtered with chitosan.

Parameter (Conc.)	Cu (mg/L)	Zn (mg/L)	Fe (mg/L)	Cd (mg/L)	Pb (mg/L)	Cr (mg/L)	As (mg/L)
A1(10%)	-	-	-	-	-	-	0.02 ±0.01
A2(30%)	-	-	-	-	-	-	-
A3(50%)	-	-	0.57 ±0.02	-	-	-	-
A4(70%)	-	-	0.35 ±0.02	-	-	-	-
A5(90%)	-	-	-	-	-	-	-
B1(10%)	0.03 ±0.01	0.23 ±0.02	0.22 ±0.1	0.02 ±0.01	0.01 ±0.01	0.02 ±0.01	0.0 ±0.01
B2(30%)	0.05 ±0.01	0.01 ±0.01	0.45 ±0.1	0.02 ±0.01	0.01 ±0.01	0.03 ±0.01	0.04 ±0.01
B3(50%)	0.01 ±0.01	0.02 ±0.01	0.89 ±0.02	0.04 ±0.01	0.03 ±0.01	0.06 ±0.02	0.01 ±0.01
B4(70%)	0.06 ±0.02	0.04 ±0.01	0.12 ±0.03	0.09 ±0.03	0.07 ±0.02	0.06 ±0.01	0.04 ±0.01
B5(90%)	0.23 ±0.03	0.67 ±0.03	0.57 ±0.02	0.05 ±0.01	0.06 ±0.02	-	-
C1(10%)	0.04 ±0.01	0.91 ±0.02	0.89 ±0.03	0.02 ±0.01	-	0.03 ±0.01	-
C2(30%)	-	0.90 ±0.02	0.09 ±0.03	0.09 ±0.02	-	0.01 ±0.01	0.01 ±0.01
C3(50%)	0.04 ±0.01	0.78 ±0.1	0.23 ±0.1	0.04 ±0.02	-	0.01 ±0.01	0.01 ±0.02
C4(70%)	-	0.57 ±0.02	-	-	-	-	0.04 ±0.01
C5(90%)	-	-	-	-	-	-	0.08 ±0.01
WHO (2004) Standard	1.30	0.10	0.30	0.005	0.015	0.1	0.05
PWS	3.5±0.1	0.45±0.1	10.76±0.02	0.07±0.01	1.00±0.1	0.11±0.02	0.06±0.01

Legend: Conc. = concentrations, WHO = World Health Organization, ± = Standard deviation, ND – Not Detected, Cu = Copper, Zn = Zinc, Fe = Iron, Cd = Cadmium, Pb = Lead, Cr = Chromium, As = Arsenic, WHO = World health Organization, ± = Standard deviation, PWS = Polluted Water Sample

Other researchers, such as Koilparambil et al. (2014), reported a chitosan yield of 46% from shrimp shell waste. Agarwal et al. (2018) synthesized chitosan from *Crustacean's* chitin using different alkaline concentrations, resulting in chitosan yields ranging between 37 and 50%, with degrees of deacetylation (DDA) at 65% to 80%. According to Agarwal et al. (2018), the DDA is an important parameter affecting chitosan's solubility, chemical reactivity, and biodegradability. The DDA values for chitosan B and C in our study were similar, while that of chitosan A was higher. The disparity in values between synthetic and locally extracted chitosan might be attributed to the extraction method. Generally, the DDA of finest quality chitosan can vary from 30% to 95%, depending on the preparation procedure (Rahmi et al., 2017).

The confirmatory test was through the identification of chemical functional groups found in the chitosan (Table 2), which include the O-H alcohol group, N-H amide group, C=N nitrite group, N-H amine group, C=H alkene group, and C-O alcohol group (Vidal et al., 2020). This confirms the presence of chitosan in *Scylla spp* from Ihugbe, Okigwe Area of Imo State Nigeria.

However, the physical properties of the Ochani River, polluted with crude oil, were more when pronounce when compared to the unpolluted water sample (UWS) and the WHO (2011) standard. These findings align with results from studies by Ochekwu and Ezekwe (2020) and Islam et al. (2021). The observed variations in physical properties can be attributed to the degradation, the solubility of dissolved oxygen, and organic and inorganic materials present in the water. High turbidity of the polluted water indicates high microbial pollution (Qureshimatva et al., 2015). The study also showed the water quality surpassed the WHO (2011) standard permissible limit, indicating it is unsuitable for consumption and domestic activities. However, Ochani River sees considerable by rural activity, potentially affecting the local population's health. The observed dark brown color may be attributed to microbial metabolism changing the river's hue, indicating uneven distribution of hydrocarbon contamination along its course (Adoki, 2011).

The chemical properties showed a decrease in pH, suggesting acidity in the crude oil-polluted water sample. This is in consonance with the findings of Adeniji et al. (2017), attributing the river's acidic nature of the biological productivity of microbial metabolism releasing carbonates into the Ochani River. This acidity has altered various ecological niches of organisms. Increased BOD, exchangeable bases, and total hardness were observed, contrasting with the result from UWS and WHO (2011) standard limits. Microbial degradation of the crude oil has led to increased oxygen demand, suffocates aquatic organisms due to oxygen unavailability (Zhong et al., 2021). Exchangeable bases may result from unused bases due to crude oil pollution (Etim, 2018).

Further evaluation of the Ochani River water quality showed highly polluted with crude oil, making the river unsustainable for life. The results of the physiochemical properties after treating Ochani River with chitosan through the bio-filtration method indicate a clear reduction in values compared to untreated polluted water samples. This suggests that the values obtained from the physiochemical properties of the crude oil-polluted water sample from the Ochani River were lower in the treated water samples than in the untreated ones, similar to findings by other researchers (García et al., 2016). Recent studies have shown the utility of chitosan in treating polluted water samples.

Vidal et al. (2020) applied chitosan as a biosorbent to remove crude oil from saline water polluted by crude oil. García et al. (2016) exploited chitosan extracted from *Panulirus argus* (lobster) to depurate wastewater from the fish processing industry. Moreover, while Chitosan B may not have completely eliminated all heavy metals, it reduced their levels below WHO (2011) permissible limit. On the other hand, chitosan C completely eliminated some metals and reduced others to meet the WHO (2011) standards. The heavy metals reduced include Cu, Cd, Pb, Cr and As. This Study have demonstrated the effectiveness of chitosan in filtering polluted water.

4. Conclusion

The treatment of polluted water through utilization of biological materials is encouraged, as it has zero adverse effect on life. Emphasis is often placed on locally sourcing the essential materials and their extraction processes. The properties of chitosan extracted from locally sourced crabs were shown to improve the physiochemical properties of the water after bio-filtration. Though, the efficiency of process were impacted by the production procedure. However, the chitosan extracted from crabs in Ihugbe, Okigwe area of Imo State, was proficient in bio-filtration, effectively eradicating crude oil toxicity in water samples from Ochani River. Furthermore, the chitosan extracted from indigenous crabs reduced the values, but not as as effectively as synthetic chitosan. The impact of chitosan B on heavy metals removal was less than chitosan C and A. Therefore, having shown the proficiency of chitosan obtained from locally sourced crabs, especially in water treatment, a production process that improves yield and efficacy is required.

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