THE EFFECT OF *ALTERNARIA SP., ASPERGILLUS SP., FUSARIUM SP.* ON THE HEAVY METAL POLLUTED RIVER OF MMIRI ELE IN NNEWI, ANAMBRA STATE.

ABSTRACT

The greatest challenge of man today is to deal with metal pollution problem because unlike organic compounds which are decomposed naturally, heavy metals tends to persist on the aquatic environment, hence get accumulated at different sensitive sites. Given the growth in environmental awareness, emphasis is given on this exploration of environment friendly ways for decontamination procedures.

Attention has been drawn to bioremediation which is a good alternative to conventional remediation technologies. The preference for it is based on the fact that it is of low cost, and generates non-toxic by product.

Microorganisms have acquired variety of mechanisms to adopt themselves to the toxicity of heavy metals. The results obtained from bioremediation of cadmium, chromium, copper in both water samples and sediment samples using alternaria sp, Aspergillus sp, Fusarum sp, show that there is a significant decrease in the quantities of cadmium, chromium and copper in both the water samples and sediment samples upon treatments with the aforementioned micro organisms.

UNDER PEER REVIEW

INTRODUCTION

Over the century, nature has been subjected to serious threat due to the introduction of harmful substances into it. The Royal Commission on Environmental pollution in Unity Kingdom in the third report defined pollution as the introduction by man into the environment of substances or energy liable to cause hazards to human health, harm to living resources and ecological systems, damage to structure or amenity or interference with legitimate uses of the environments. According to section 1(3) of the U.K Environment protection Act,1990, the term pollution means the release (into the environment medium) from any process of substances which are capable of causing harm to man or any other living organisms supported by the environment. Reported

sources of heavy metal in the environment through pollution are geogenic, industrial, agricultural, pharmaceutical, domestic effluents and atmospheric sources (Van*et al*., 2005). Environmental contamination can also occur through metal corrosion, and soil erosion.

Heavy metals have been recognized as usual contaminant of the environment especially the aquatic environment because most of the can easily dissolve in water (Kiyani*et al.,*2013). Heavy metals are metallic elements with relatively high density and the heaviness been inter-related to its toxicity (Duffus, 2002). Being metal ions, heavy metals cannot be degraded or destroyed; therefore, their stability makes them persistent toxic substance in the environment (Harrison, 2001). These heavy metals find its way into the aquatic animals and through food chain into higher animals, causing serious health problems hence the need for its bioremediation.

There are various methods of heavy metal remediation but bioremediation has been found more advantageous, some of the reasons being that it does not use dangerous chemicals (Sharma et al., 2012), it is ecofriendly, non-intrinsic, potentially allowing for continued site use (Kumar *et al;*2011). Bioremediation could be defined as the process by which organic wastes are biologically degraded under controlled conditions to an innocuous state, or levels below concentration limits established by regulatory authorities (Endeshians*et al.,* 2017). Some of the techniques in bioremediation includes: bioventing, biospiling, compositing, biopiles, Bioreactors etc. The purpose of bioremediation is to make the environment free from pollution with the help of environmentally friendly microbes (Ito et al., 2012)

MATERIAL AND METHODS

Equipment

Norkan flask

Incubator

Centrifuge machine

Atomic Absorption Spectroscopy (AAS)

Reagents

Distilled water

UNDER PEER REVIEW

Glucose

Potassium phosphate

Potassium hydrogen orthophosphate

Manganese (II) sulphate

Tetratrdiale

Cobalt (II) chloride dehydrate

Thiamine hydro-chloride

Asparagine

EXPERIMENTAL DESIGN:

The samples were collected using a clean container from mmiri-eleriver in Umudim, Nnewi, Anambra state. There were 8 samples collected in general which are 4 water samples and 4 sediment samples labelled sample A,B,C,D

A= point source samples

B= down -stream samples

C= upstream

D= control.

The samples were then transported to the laboratory were all the analysis necessary will be done using the following methods.

UNDER PEER REVIEW

BIOCHEMICAL AND PHYSICOCHEMICAL ANALYSIS

Biochemical oxygen demand (APHA 5210)

The procedure involves dilution of a known volume of the sample with dilution water containing nutrients to the one litre mark and measuring the initial DO of the sample. This follows incubation of a portion of the diluted samples for 5 days at 20^0C. The final DO measurement recorded after the incubation period. The BOD value was calculated by subtracting the final DO from the initial DO and further dividing it by the dilution decimal fraction.

Chemical oxygen demand (APHA 5220C)

Sample was blended and 2.5ml was measured into a culture tube. Digestion solution was then added followed by sulfuric acid reagent; was then transferred into a digestion block at 150^0C for 2hrs. This was cool, and titrated with 0.1M ferrous ammonium sulfate using ferroin indicator.

$$\text{COD as mgO}_2\text{/L} = \frac{(A-B) \times M \times 8000}{\text{ml of sample}}$$

pH, temperature, total dissolved solids, conductivity, dissolved oxygen and turbidity

These were determined in-situ using Hanna multi-parameter meter water checker. About 50ml volume of the water sample was collected into the instrument sample holder and the pH probe inserted into the sample, ensuring that the pH is calibrated prior to use. The most stable reading from the instrument was then taken.

Phosphate

Bray No.1 method was adopted; 2ml of the sample was treated with 2ml of ammonium molybdate solution and 1ml of stannous chloride. Absorbance was measured after 5 minutes at a wavelength of 880nm using UV –VIS spectrophotometer.

Nitrate

1ml aliquot of the sample was transferred to a vial and treated with brucine reagent and conc. Sulphuric acid and mixed with 2ml of distilled water added and contents allowed to set for 15mins and absorbance measured with spectrophotometer at 420nm.

Heavy metals (APHA 3111B)

The concentrations of Cadmium, Copper, Arsenic and Chromium in samples collected were determined (after nitric acid digestion) by means of an Atomic Absorption Spectrophotometer. Specific metal standards in the linear range of the metal were used to calibrate the equipment. The digested samples were then aspirated and the actual concentrations were obtained using the linear equation from the calibration curve auto-generated by the equipment (APHA, 1992).

Total heterotrophic bacteria (THB)

UNDER PEER REVIEW

Nutrient agar media of 28g was dissolved in 1litre of distilled water. The media was sterilized in an autoclave at 121°C and 15Pascal for 15 minutes and allowed to cool to 47°C. The media was poured into sterilized Petri dishes, allowed to solidify, and then dried in the hot air oven at 40°C for 5minutes. The samples were inoculated in duplicates using spread plate method and incubated at 37°C for 24 hours. The colonies in the plate were counted using a colony counter and the total heterotrophic bacteria enumerated using standard formulae.

Total fungi (TF)

Sabouraud dextrose Agar media of 65g was dissolved in 1litre of distilled water. The media was sterilized in an autoclave at 121°C and 15Pascal for 15 minutes, and allowed to cool to 47°C. The media was poured into sterilized Petri dishes. The poured media was allowed to solidify, and then dried in the hot air oven at 40°C for 5minutes. The samples were inoculated in duplicates using spread plate method and incubated at 20°C for 48 hours. The colonies in the plate were counted using a colony counter, and the number of total fungi recorded using standard formulae.

Catalase test

This test was carried out to identify catalase and non-catalase producing bacteria as described. The slide test method was carried out. A drop of hydrogen peroxide (H_2O_2) solution was placed on a sterile glass slide using a sterile wire loop after which a colony of each of the isolates was collected and placed in another sterile glass slid, which served as a cover slip. This was inverted and placed on the hydrogen peroxide solution and observed for 10 seconds. Formation of bubbles showed a catalase positive result whereas non production of bubbles showed a negative result.

Citrate test

This test was carried out to determine the innate ability of the isolates to utilize sodium citrate as sole carbon source. The method described by (MacFaddin, 2000) was adopted for this test. The test bacterial isolates were inoculated under aseptic conditions into the test tubes (covered with cotton wool, as citrate test requires oxygen) containing Simmons citrate agar and incubated for 48 hours at 35°C. The medium contained a pH indicator bromothymol blue. Test tubes that retained the green colour were adjudged negative, where the colour changed to blue indicated

UNDER PEER REVIEW

positive results. The change of the colour from original green colour to blue is as a result of rise in pH above 7.6.

Gram stain

This test was employed to differentiate the bacterial isolates into two groups of gram positive and gram negative microorganisms. Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in a thick layer in gram positive bacteria. The smear of the test bacterial culture was prepared and heat-fixed on grease-free slide. The slide was flooded with crystal violet for one minute and gently washed off with tap water and then drained off. The slide was exposed to gram's iodine for one minute and then washed with 75% alcohol for 30 seconds. The slide was washed with tap water and air dried. Then 0.25% safranin was used to counter-stain the slide for 30 seconds. The slide was washed, drained, and dried and examined under oil-immersion microscope (x100). A purple colouration indicated gram positive organism as a result of the retention of the primary stain (crystal violet) while pink colouration, arising from the colour of the counter stain (safranin) as a result of the inability to retain the primary stain, indicated agram negative organism (Beveridge*et al.,* 2001)

Indole test

This test was carried out to test the ability of bacterial isolates to breakdown tryptophan by using the enzyme tryptophanase and producing indole using Kovac's reagent. The test was carried out as described by (Cheesebrough, 2002). The test organism was incubated in a test tube containing peptone water for 48 hours at 37°C. After incubation, 0.5ml (about 5 drops) of Kovac's reagent was poured into the test tube and observed after agitation for a minute. A positive indole test is indicated by the formation of a pink to red ring in the reagent layer on top of the medium within seconds of adding the reagent while formation of yellow or slightly cloudy colour indicated a negative result (MacFaddin, 2000).

Methyl red (MR) test

This was carried out to evaluate the ability of the test organism to perform mixed acid fermentation. Organisms with such ability produce enough acid to overcome the buffering

UNDER PEER REVIEW

capacity of the broth medium to decrease its pH from 7.5 to about 4.4 or below. Five (5) drops of methyl red solution was then added to one portion of MR-VP broth. Formation of a red colourindicates high acid production and a decrease in the pH of the culture medium to 4.4 is interpreted as a positive result while yellow colour formation indicates a slightly acidic with pH above 6.0 is regarded a negative result, (Cheesebrough, 2002; Madigan and Martinko, 2008).

Vogesproskauer test

This test was carried out to test the ability of bacteria to produce acetyl methyl carboinolacetoin, a product of fermentation. After incubation, 0.6 ml (about 12 drops) of 5% (w/v in ethanol) α – Naphthol and 0.2 ml (about 4 drops) of 40% (w/v in distilled water) KOH solution was added to the test tube, which was vigorously shaken and kept aside in a slanted position to allow for maximum exposure to oxygen for about an hour. Brick red production indicated a positive result whereas yellow colouris regarded a negative result (Cheesebrough, 2002; Madigan and Martinko, 2008).

Motility test

This test was done to identify motile organisms. A semi solid medium was prepared by dissolving 14 g of nutrient agar in one (1) litre of distilled water. 10 ml of the semi-solid nutrient agar was dispensed into each test tube and allowed to pre-heat, sterilize and cool. A sterile inoculating needle was then aseptically used to pick the test organisms and stabbed into the semi-solid agar and incubated for 48 hours at $37^{o}C$ and observed for growth that deviates from the original needle stab. A deviation indicates that the organism is motile whereas a straight line growth indicates non motile organisms (Cheesebrough, 2002).

Oxidase test

This test was carried out to test the ability of the isolates to produce oxidase enzyme. The dry filter paper method as described by MacFaddin, (2000) was employed. A piece of filter paper was soaked with the reagent solution, allowed to dry and further smeared with a colony of the test organism and observed for formation of purple colour. The purple colour within 2 minutes

UNDER PEER REVIEW

indicated a positive result while isolates with no purple colour within 2 minutes were adjudged negative.

Starch hydrolysis test

This test was carried out to differentiate bacteria based on their ability to hydrolyse starch with the enzyme α-amylase or oligo-l,6-glucosidase. It helps in the differentiation of species from the genera *Corynebacterium*, *Clostridium*, *Bacillus*, *Bacteroides*, *Fusobacterium*, and members of *Enterococcus* (Smibert, and Krieg, 1994; MacFaddin, 2000). The test organism was spotted on a sterile starch agar plate; the plate inverted and incubated aerobically at 35°C for 48 hours. After incubation, the plate was brought out, covered with iodine and immediately examined for the presence of a halo around the growth. The presence of halo around the growth not on the growth indicated positive result while absence of halo zone indicated a negative result.

Triple sugar iron (TSI) test

This test was aimed at the differentiation of microorganisms on the basis of dextrose, lactose, and sucrose fermentation, gas and hydrogen sulphide production. When the carbohydrates are fermented, acid production is detected by the Phenol Red pH indicator. Sodium thiosulfate is reduced to hydrogen sulphide, and hydrogen sulphide reacts with an iron salt yielding the typical black iron sulphide. Ferric ammonium citrate is the hydrogen sulphide (H_2S) indicator. Sodium Chloride maintains the osmotic balance of the medium (MacFaddin, 1980). The agar slant method was used. The TSI agar was prepared according to the manufacturer's instruction and the medium was sterilized by autoclaving at 121°C for 15 minutes at 15 psi. The sterile molten medium was slanted, allowed to cool and inoculated with 24 hour culture of the test organism. The medium was incubated at 37°C for 24 hours. After incubation, the result was interpreted by noting the following observations. An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose, lactose and/or sucrose. An alkaline slant-alkaline butt (red/red) indicates dextrose or lactose were not fermented (non-fermenter). Cracks, splits, or bubbles in medium indicate gas production. A black precipitate in butt indicates hydrogen sulphide production (MacFaddin, 1980).

Urease test

UNDER PEER REVIEW

This test was used to differentiate between bacteria that can produce the enzyme urease from those that cannot. Some microorganisms have the ability to produce the enzyme urease. The urease is a hydrolytic enzyme which attacks the carbon and nitrogen bond amide compounds (eg: urea) with liberation of ammonia as shown below test was performed by growing the test organisms on agar medium containing the pH indicator phenol red (pH 6.8). The medium was prepared by sterilizing urea agar base separately without the urea. The urea was sterilized separately by filtration and then mixed with the sterile molten Christensen's agar base. During incubation, microorganisms possessing urease produce ammonia that raises the pH of the medium. As the pH becomes higher, the phenol red (pH 6.8) changes from a yellow colour to a red or deep pink colour (pH 8.4). Failure of the development of a deep pink colour due to no ammonia production is evidence of a lack of urease production by the microorganisms.

Sugar Fermentation Test

Peptone water was prepared according to the manufacturer's instruction, 1% reduce sugar either glucose, sucrose, lactose was added to it and phenol red was added to the medium and a Durham's tube, the medium was sterilized at $37^{o}C$ for 15 minutes at 15psi. The test organism was inoculated at $37^{o}C$ for 48 hours, the change in colour and bubble in the Durham's tube was observed. The phenol red changes colour to yellow and present of bubble in the Durham's tube was recorded as positive while no colour change, no bubble recorded as negative.

PREPARATION OF BIO- SORPTION MEDIUM FOR Cu(II) BIOSORPTION ASSAY.

The medium used assay was Norkrans liquid medium. Norkrans liquid medium was prepared as directed by Nigieghon (2015) .Norkran ingredients and quantity in 1 litre of distilled water were glucose 20,000g; potassium phosphate (KH_2PO_4) 0.6000g; potassium hydrogen orthophosphate(K_2HPO_4) 0.0050g; manganese(11) sulphatetetrahydrate ($MnSO_4.4H_2O$) 0.0044g cobalt (II) chloride dehydrate ($CaCl_2.2H_2O$) 0.1320g thiamine hydro-chloride ($C_{12}H_{17}C_1N_4OS.HCl$) asparagine ($C_4H_8N_2O_3$)1.2000g.fifty ml (50ml) of the medium was dispensed in flasks and sterilized as described above and these flask were used for the Cu (ii) Bio-sorption assay.

UNDER PEER REVIEW

There were 8 samples collected in general which are 4 water samples and 4 sediment samples labelled sample A,B,C,D

A= point source samples

B= down -stream samples

C= upstream

D= control.

Reduction of copper, chromium and cadmium

It was done according to the method employed by Chen *et al*., 2017with little modification. fungal cells were multiplied in 2% malt extract agar with pure fungal isolates. Five Norkrans flasks (each set preparing a treatment) were amended with 16.1, 8.1,4.0 and 2.0mg/dl. Two balls (0.5cm in diameter) from the plates were used to inoculate each of the flask of the Norkrans liquid medium and subjected to incubation in a shaker incubator at 150 rpm (Babalola, 2011) under room temperature (28± 2ºC). Control experiment containing Norkrans liquid medium and Cu (ii) only,Norkran liquid medium and fungal biomes only were similarly set up.Flask samples were collected on day 0,5, 10,and 20 cenfuntrifuged at 3700 rpm for 8 mins and the concentrations of Cu(ii) in the fungal biomass free supernatant were quantified using Atomic Absorption spectroscopy (AAS)(merck,South Africa).

Copper, chromium and cadmiumbioaccumulation assay

This was done following the method of Chen *et al*., 2013. On the 20th day, the remnant fungal biomes were dried in the oven at 70ºC for 2h and the dry weight were taken.The bio-sorption potential of Cu(ii) by the fungal cell was deduced using the formula below.

$$Q = (Ko_ \ Kr)V \div W \quad (1)$$

Where Ko: initial Cu (11) concentration, V: Volume of flask content,W: dry weight of fungal cells. Fungal tolerance assay to different concentration Cu (ii) used for the bio-sorption of fungal isolates experiment was determined by collecting 1ml of Norkrans sample from each experimental flask on 0.5, 10 and 20 centrufuged at 3700rpm.

Fungal tolerance assay concentration of copper, chromium and cadmium

UNDER PEER REVIEW

The tolerance fungal assay to the different concentration of Cu(ii) used for the bio-sorption experiment was determined by collecting 1ml of Norkrans sample from each experiment was flask on days 0,5,10and 20 followed by colonial counts.

Induced –tolerance training of copper, chromium and cadmium

The ability of fungal isolates to adapt to increasing Cu(ii)concentration was the maximum Cu(ii) concentration used for the tolerance assay control 2%malt extract plates were also prepare and inoculated at ambient temperature (28±2ºC)for a week isolates from the growing fungal culture plates were later sub-cultured to fresh 2% malt extract agar containing higher concentration of Cu(ii)(18ml,20 mg/l and 25 mg/l) to induce tolerance training tolerance index was calculated at the experiment.

STATISTICAL ANALYSIS

Each treatment had five replicates. Data were cleaned in Microsoft excel.Normality test was done and imported to statistical package for the social science (SPSS) where they were subject to analysis of variance (ANOVA). Means and standard errors were extracted from the ANOVA output concentration of Cu (ii) to induce tolerance training index was calculated at the end of the experiment.

UNDER PEER REVIEW

RESULTS

PRELIMINARY STUDIES

Water Analysis

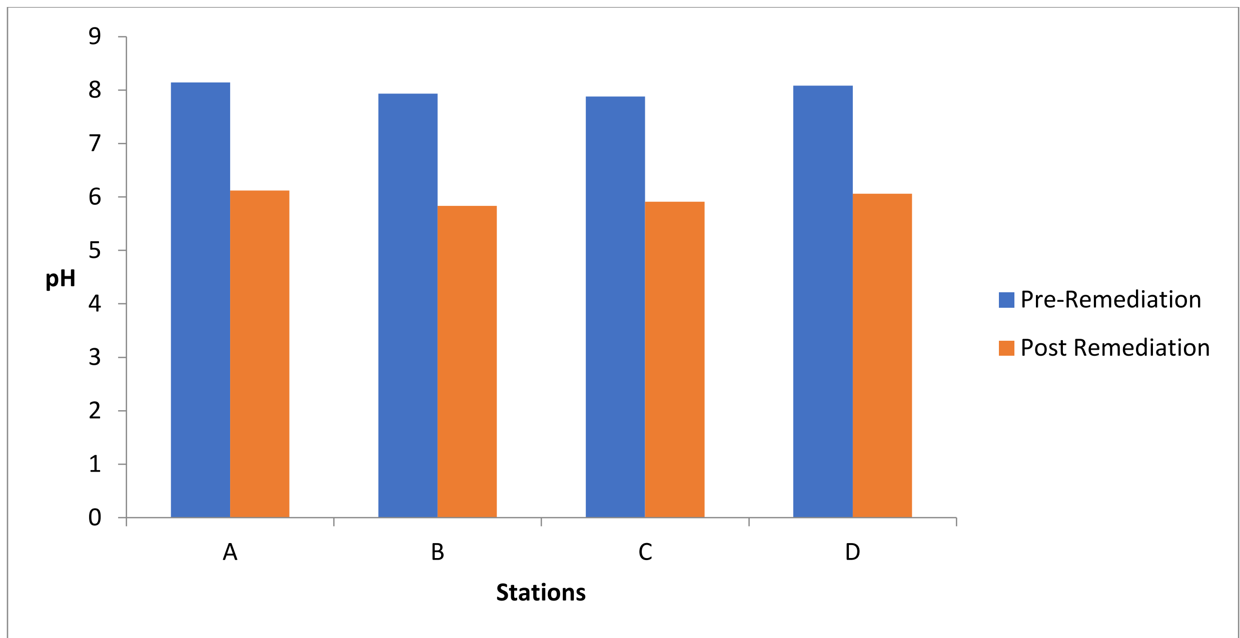

Fig 1.1a: pH of water samples

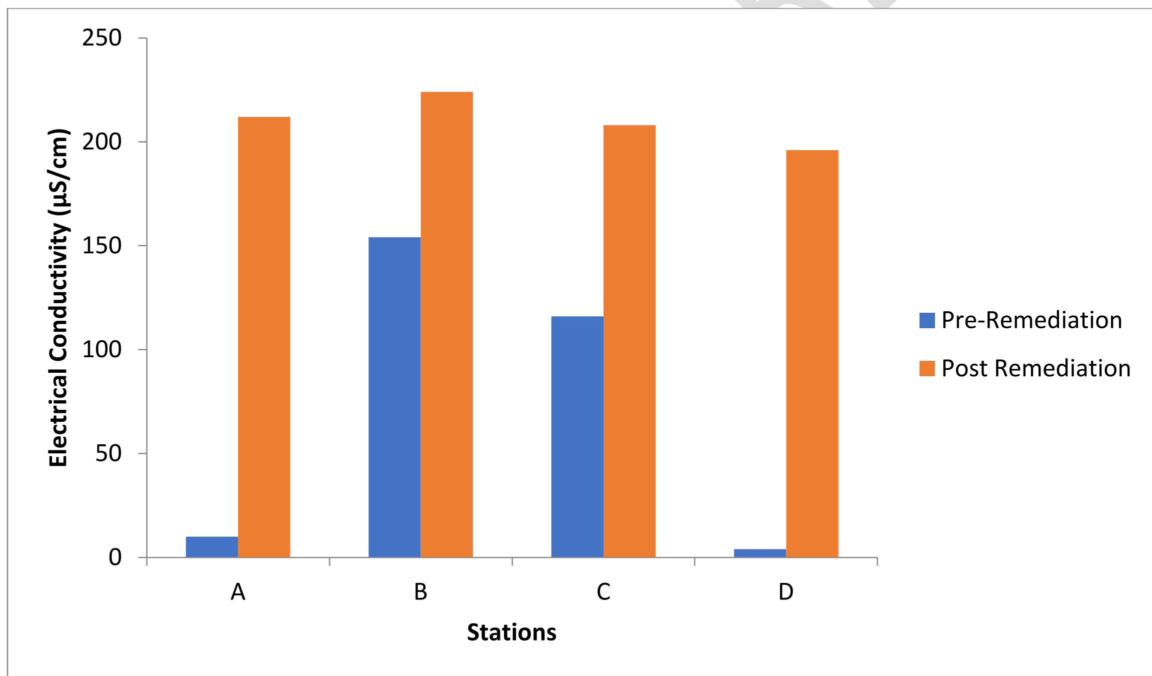

Fig 1.1b: Electrical Conductivity of water samples

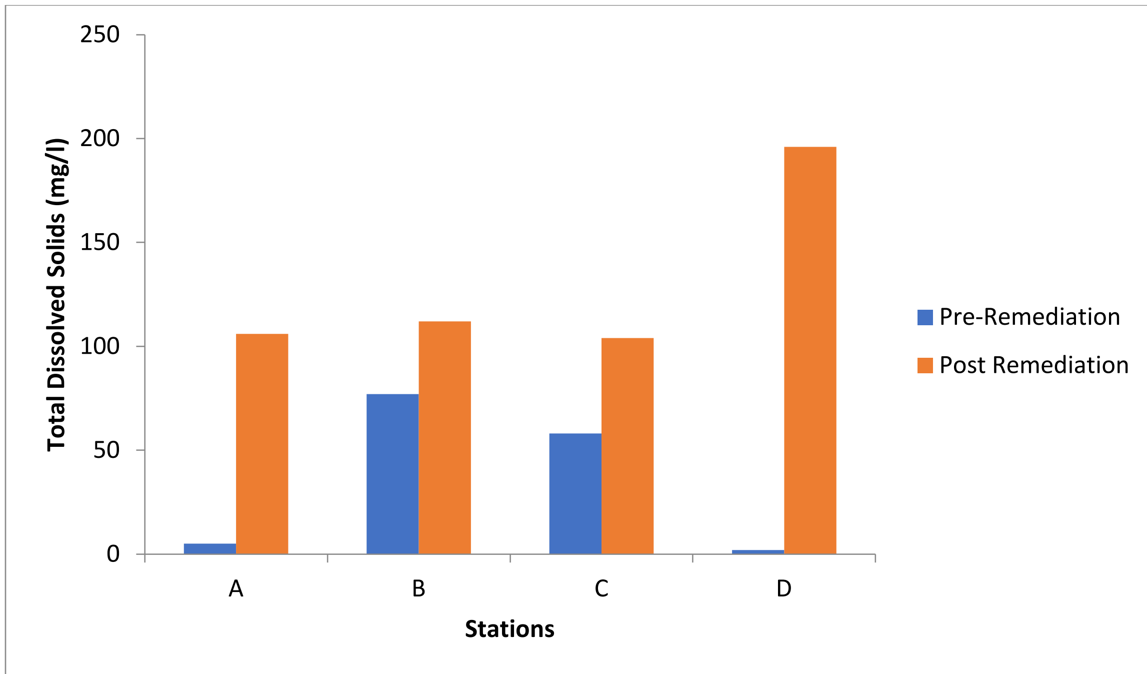

Fig 1.1c: Total Dissolved Solids in the water samples

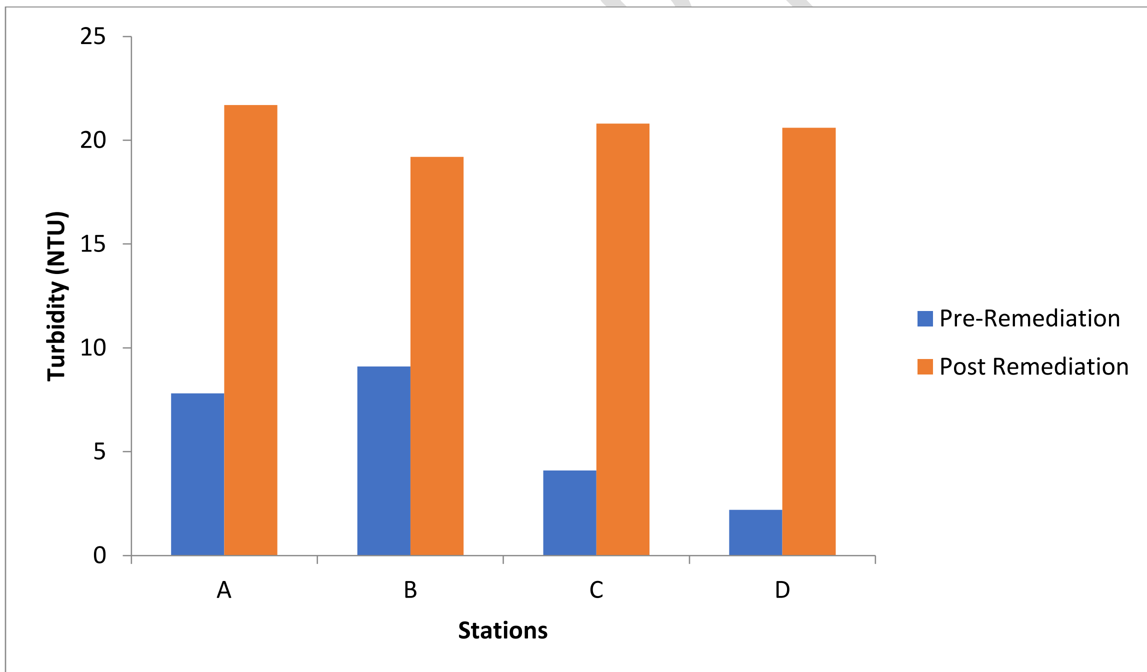

Fig 1.1d: Turbidity of the water samples

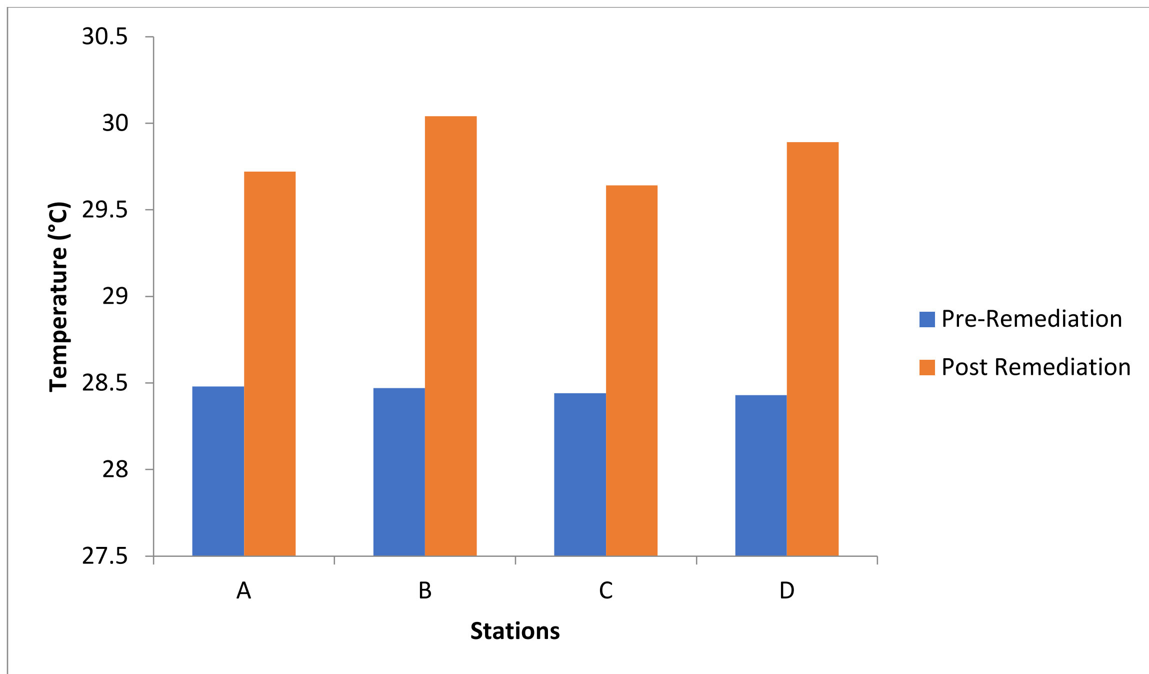

Fig 1.1e: Temperature of the water samples

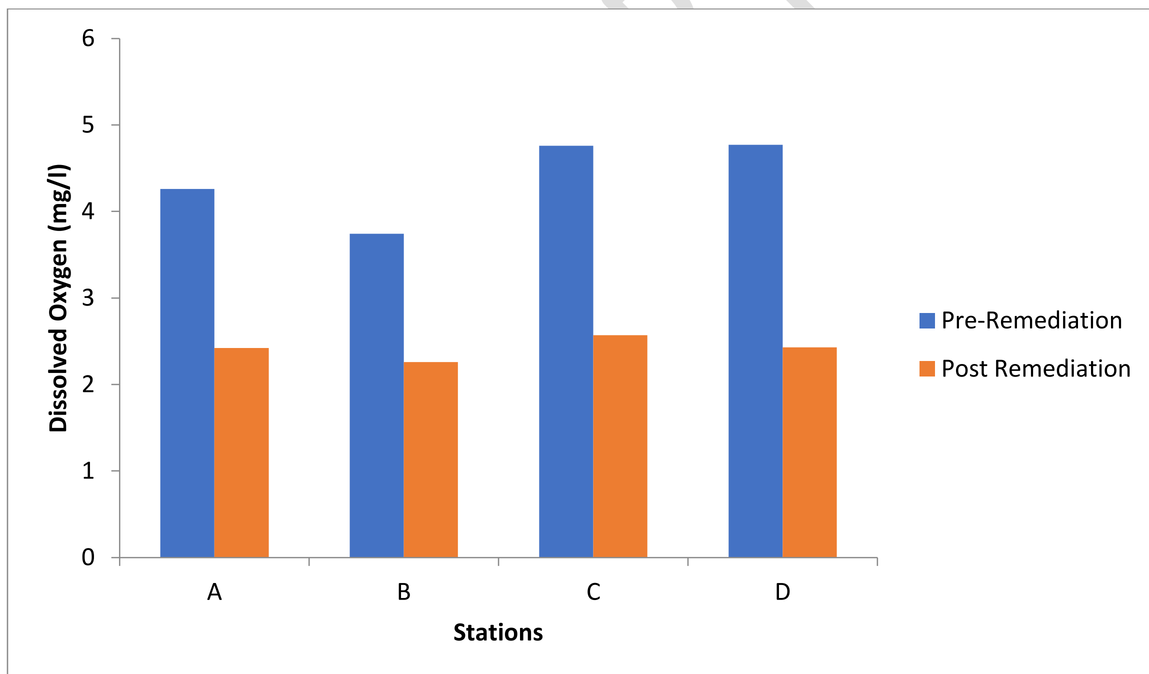

Fig 1.1f: Dissolved Oxygen in the water samples

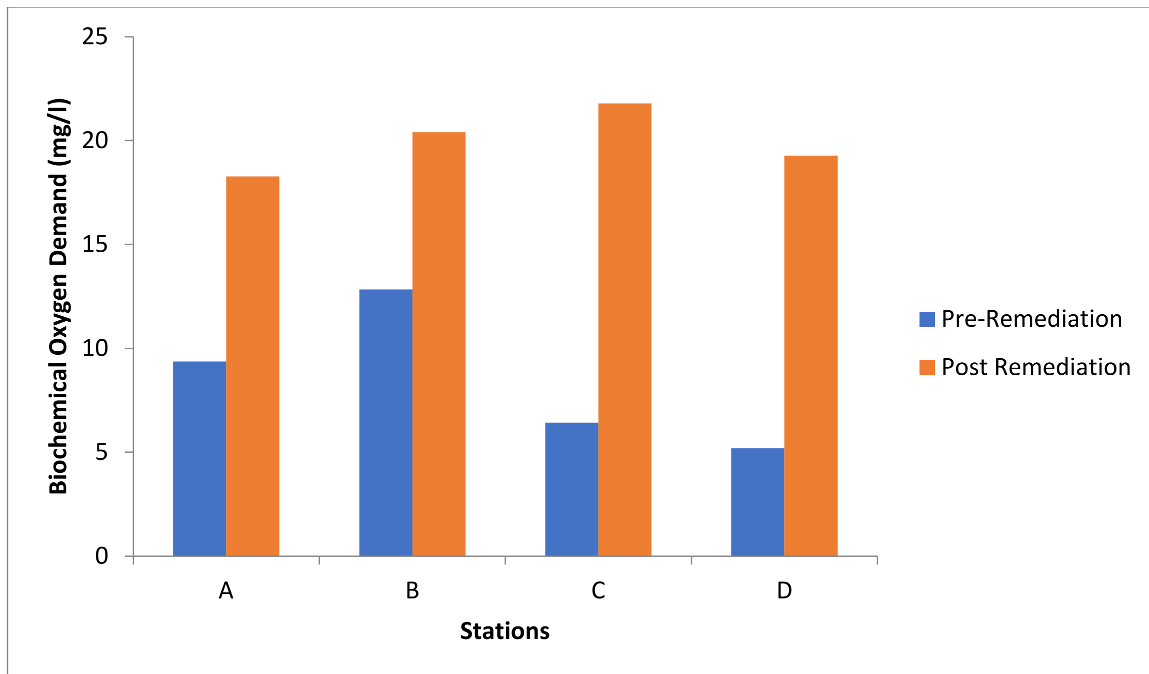

Fig 1.1g: Biochemical oxygen Demand of the water samples

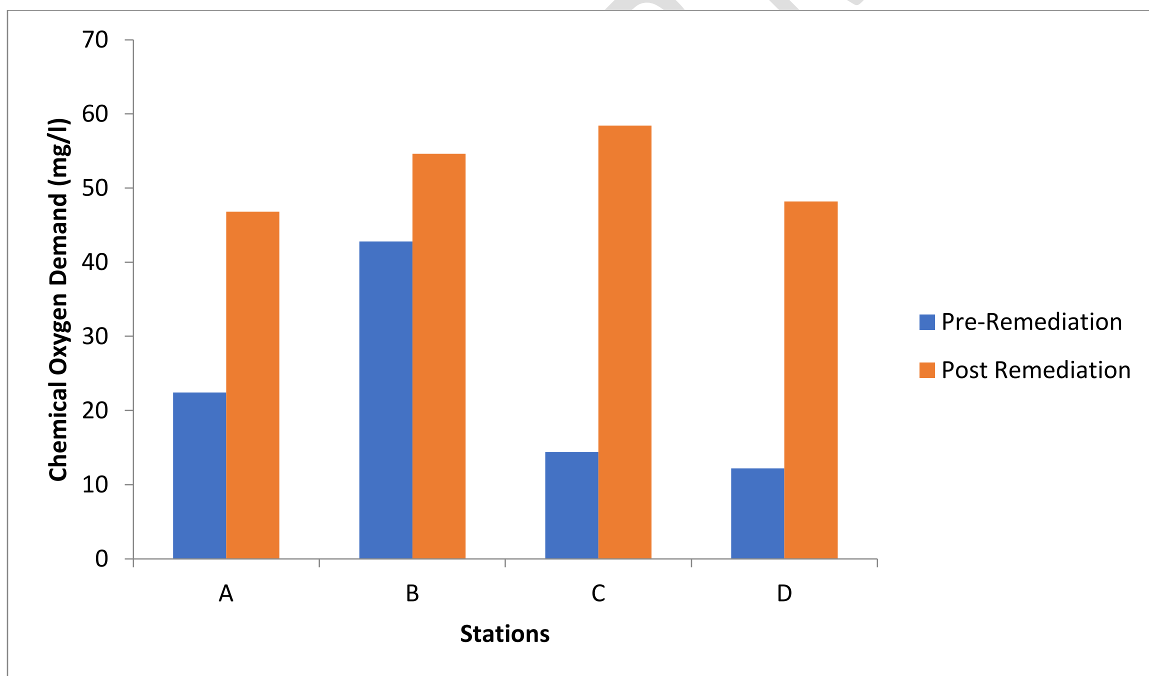

Fig 1.1h: Chemical Oxygen Demand of the water samples

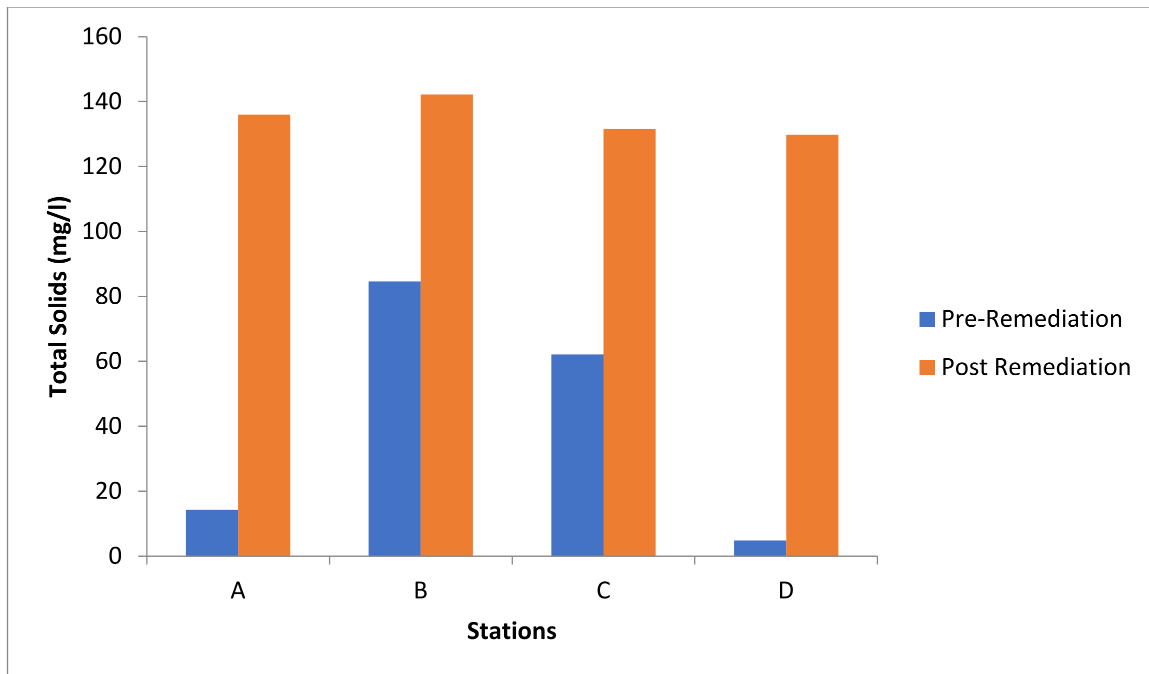

Fig 1.1i: Total Solids in the water samples

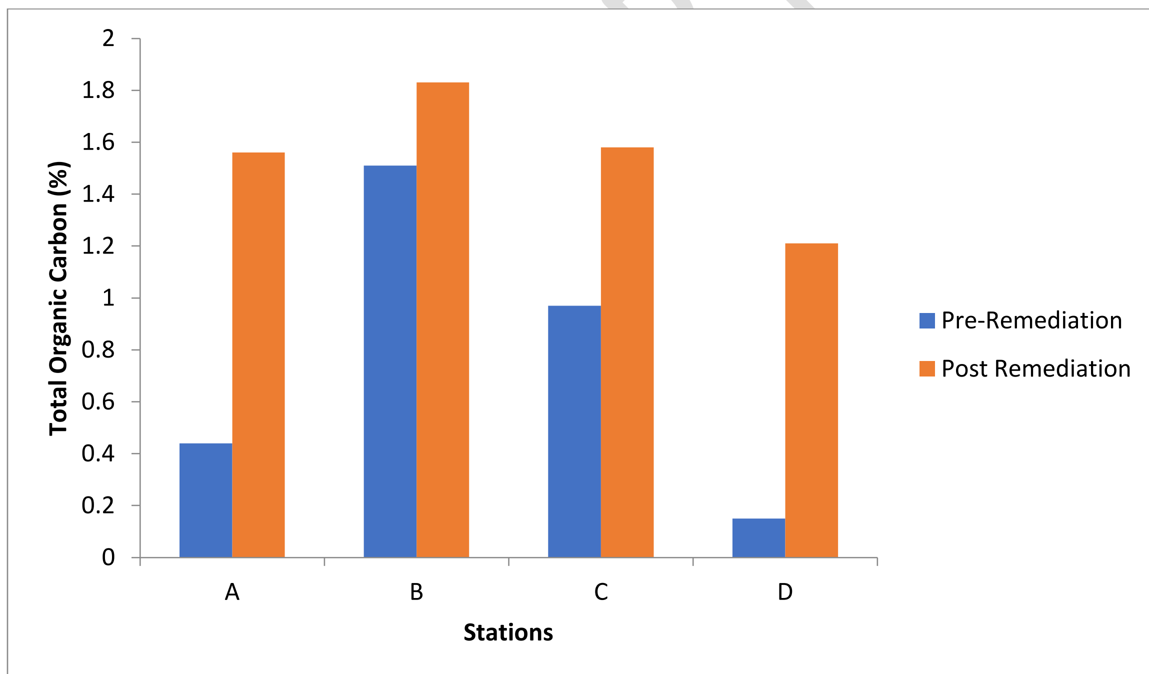

Fig 1.1j: Total Organic Carbon in the water samples

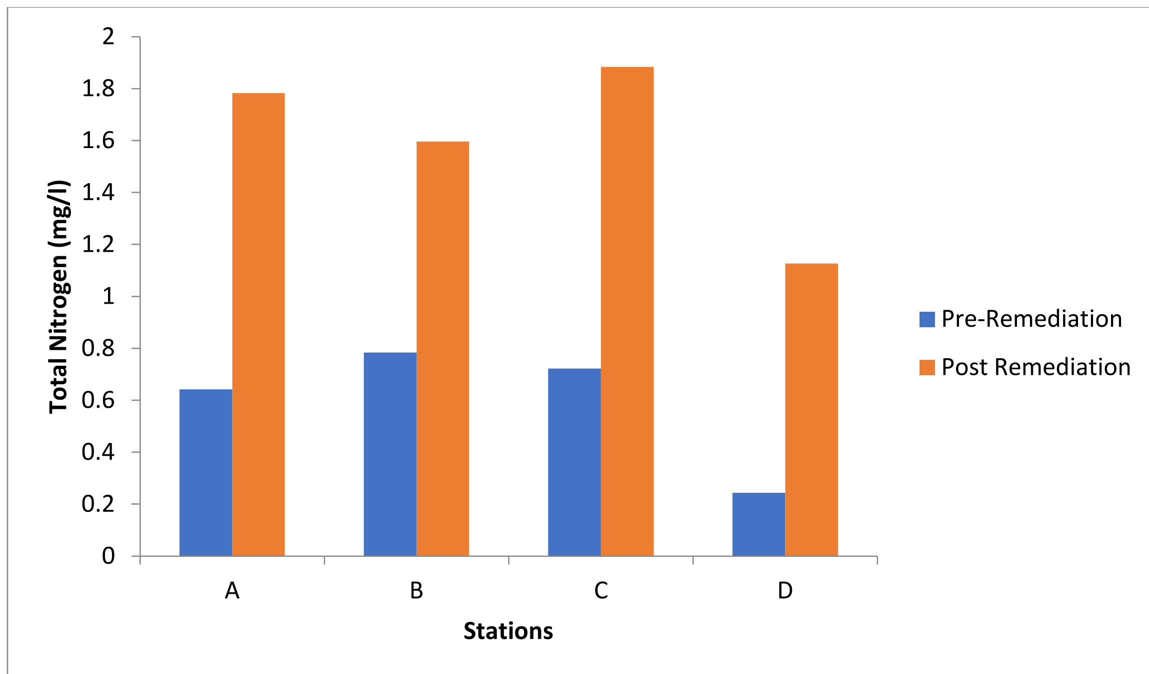

Fig 1.1k: Total Nitrogen in the water samples

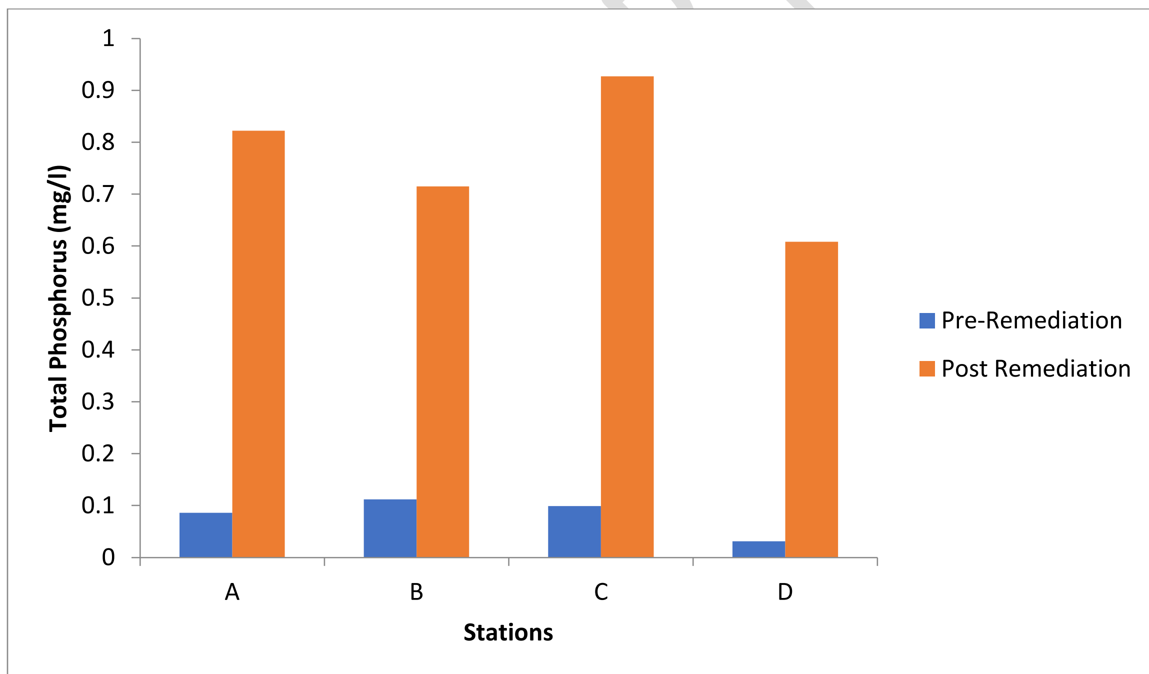

Fig 1.1l: Total Phosphorus in the water samples

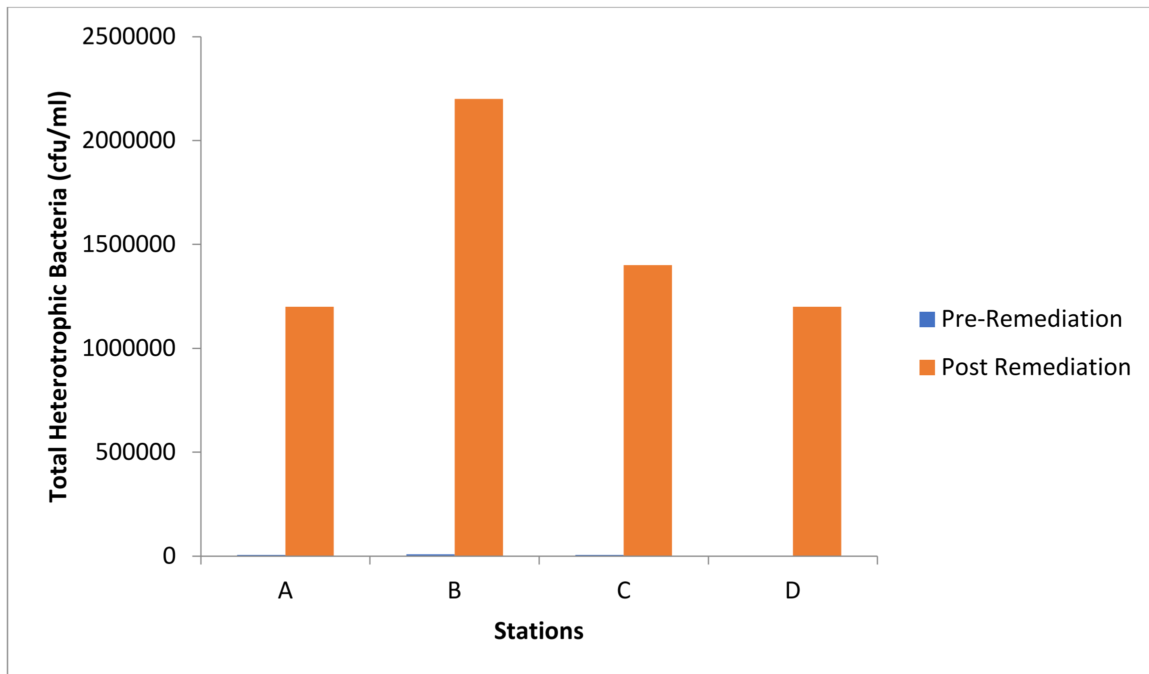

Fig 1.1m: Total Heterotrophic Bacteria in the water samples

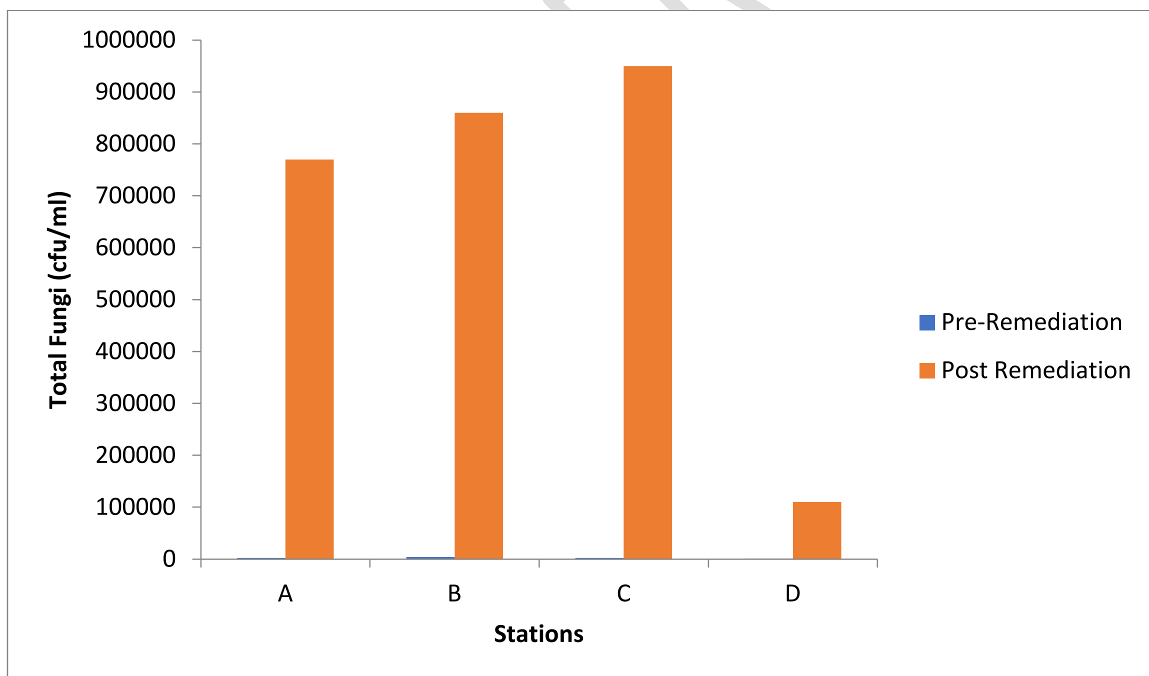

Fig 1.1n: Total Fungi in the water samples

BIOREMEDIATION OF CADMIUM

The result of the bioremediation of cadmium present in a given samples of a polluted water collected from a polluted aquatic environment shows a significant decrease in the amount of cadmium present in the water sample. This is shown by the significant drop in the mean value of the post water treatment, approaching the mean value of the control. This is very close to the value of the control.

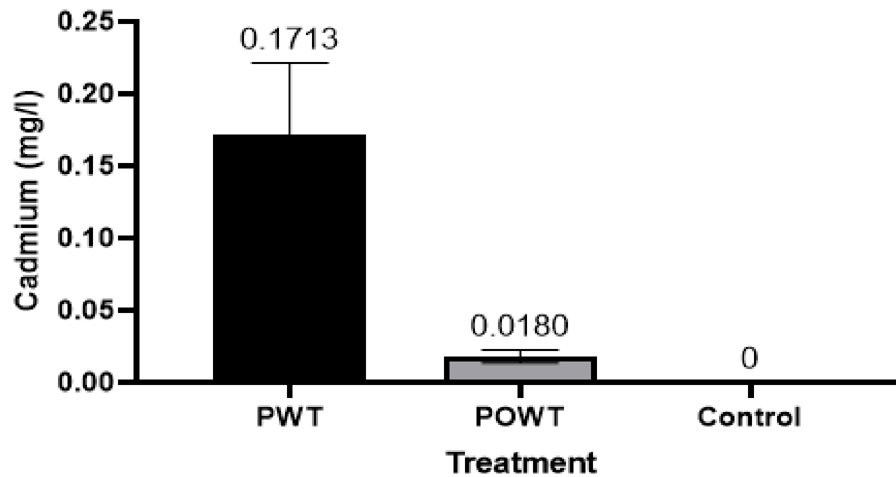

Fig 1.2a: Bioremediation of cadmium in a water sample

UNDER PEER

BIOREMEDIATION OF CHROMIUM

The result of the bioremediation of chromium present in a given sample of a polluted water collected from the polluted aquatic environment as indicated by figure 1.2b shows a significant decrease in the amount of chromium in the sample from the mean value of 0.423 for pre-remediation treatment to 0.08 for post remediation treatment. This is very close to the value of the control.

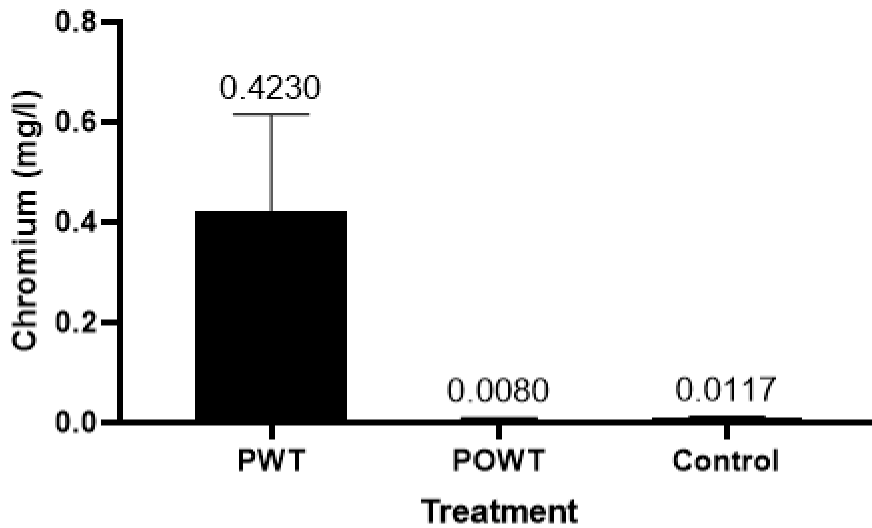

Fig 1.2b: Bioremediation of chromium in a water sample

UNDER R...W

BIOREMEDIATION OF COPPER

Figure 1.2c shows that after the bioremediation process, there was a reasonable decrease in the amount of copper metal in the water sample (from 0.193 to 0.092). This is very close to the mean value of the control.

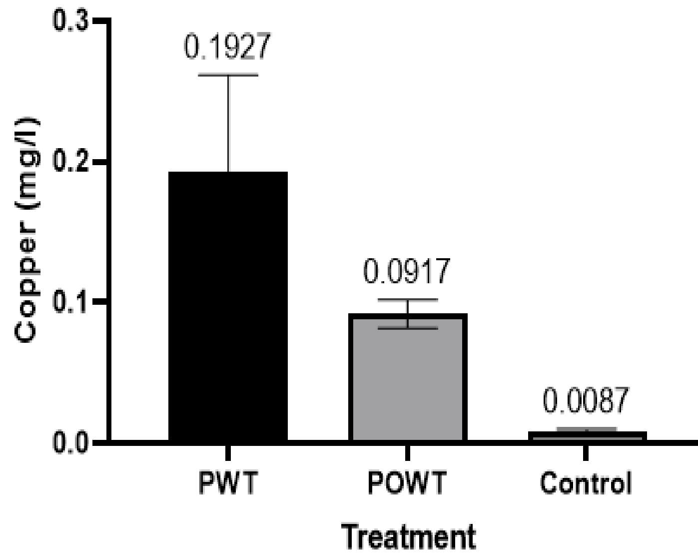

Fig 1.2c: Bioremediation of copper in a water sample

UNDER PEER REVIEW

BIOREMEDIATION OF CADMIUM IN SEDIMENT SAMPLE

Figure 1.2d shows the result for the post bioremediation of cadmium in sediment sample as indicated by the bar chart shows a significant decrease in the amount of cadmium from 0.2430 to 0.1880, tending towards the value of the control.

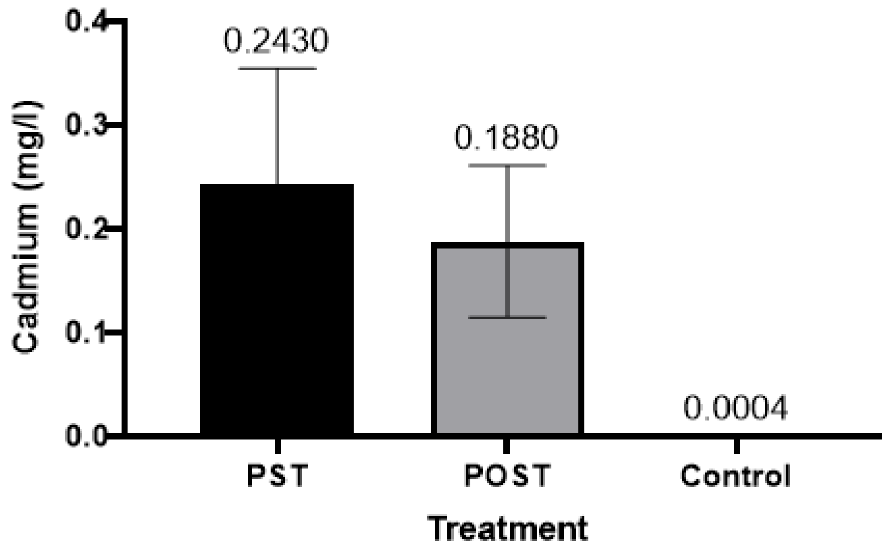

Fig 1.2d: Bioremediation of cadmium in sediment sample

UNDER PEER REVIEW

BIOREMEDIATION OF CHROMIUM IN SEDIMENT SAMPLE

Figure 1.2e shows the post bioremediation of chromium in sediment sample shows a drastic decrease in the amount of chromium in the sediment sample. This decrease tends very close to the control.

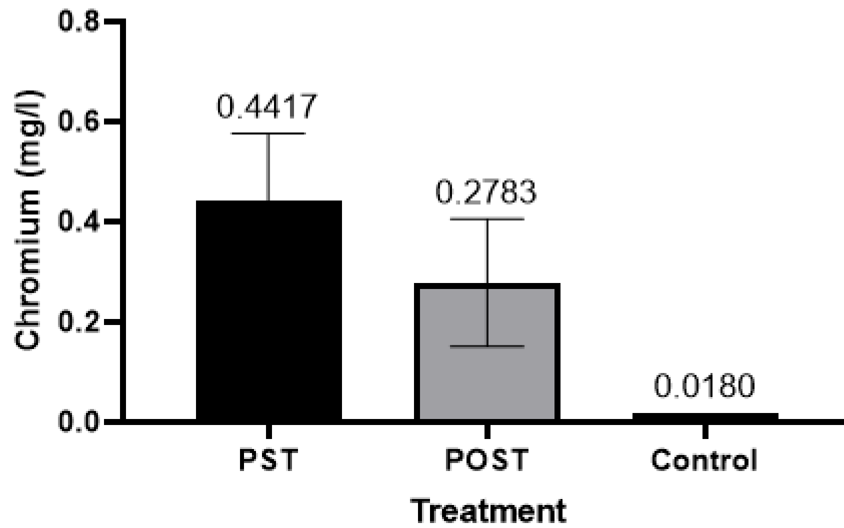

Fig 1.2e: Bioremediation of chromium in sediment sample

UNDER PEER REVIEW

BIOREMEDIATION OF COPPER IN SEDIMENT SAMPLE

Figure 1.2f shows that there was a notable decrease in the amount of copper in the sediment sample after the post bioremediation of the copper present in a water sample.

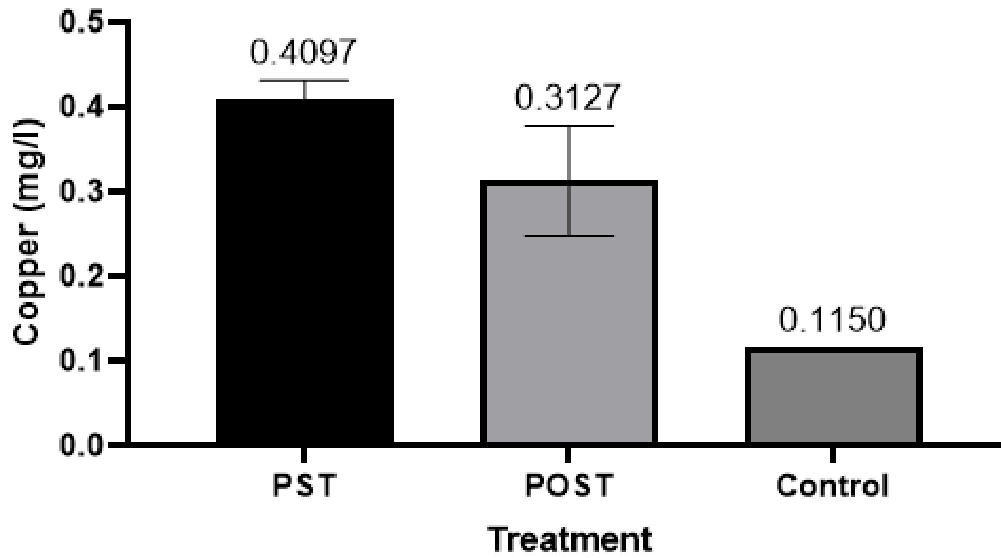

Fig 1.2f: Bioremediation of copper in sediment sample

UNDER PEER

BIOACCUMULATION IN FISH

Table 1.1: Bioaccumulation (μgg^{-4} wet weight) of cadmium and chromium in muscle, liver and spleen of *Tilapia*sp..
Data expressed in mean ± standard deviation

	Muscle			Liver			Spleen		
	Section A	**Section B**		**Section A**	**Section B**		**Section A**	**Section B**	
Cd	0.07 ± 0.06	0.19 ± 0.15	s	0.65 ± 0.61	0.97 ± 1.09	ns	0.08 ± 0.05	0.19 ± 0.18	ns
Cr	0.54 ± 0.42	0.42 ± 0.32	ns	0.72 ± 0.23	0.59 ± 0.26	ns	1.04 ± 0.53	0.66 ± 0.28	s

ns = not significant; s = significant

Bioaccumulation of cadmium in fishes

Figure 1.3a shows the result of the bioaccumulation of cadmium in *Tilapia*fishes collected from the various sites of the polluted aquatic environment.

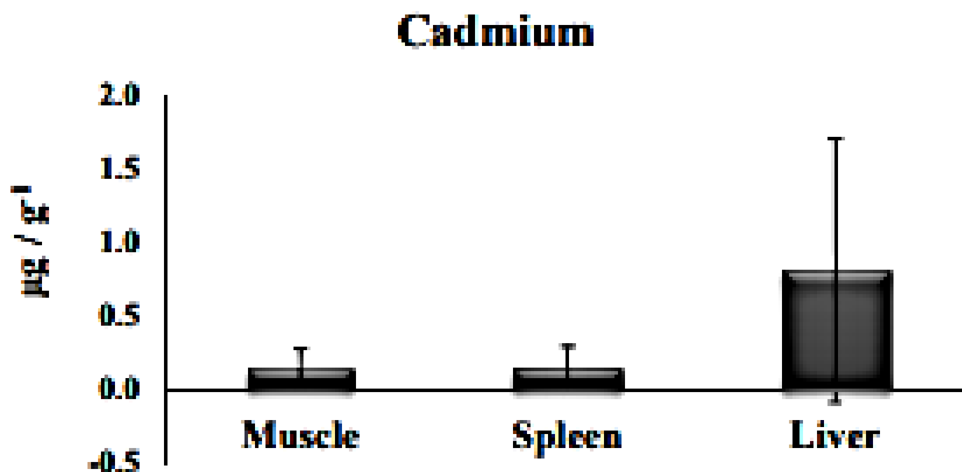

Fig 1.3a: Bioaccumulation of cadmium in *Tilapia*fishes

Bioaccumulation of chromium in *Tilapia*fishes

Figure 1.3b shows the result of the bioaccumulation of chromium in *Tilapia*fishes collected from the sites of the polluted environment.

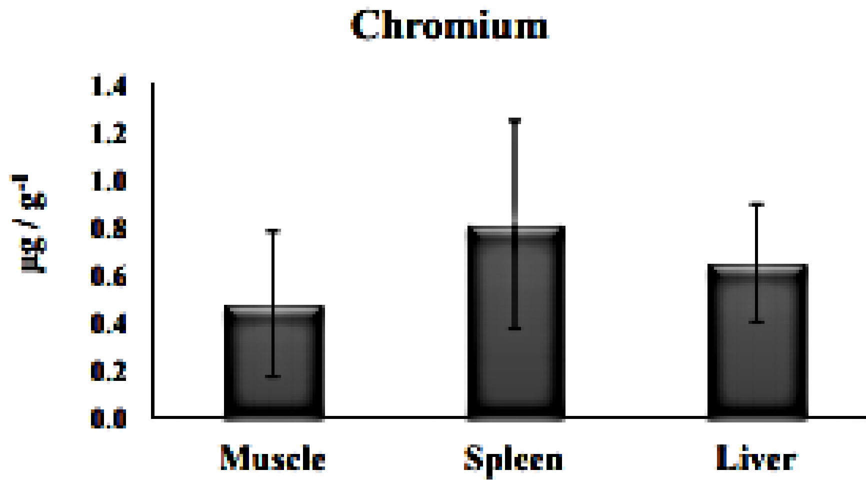

Fig 1.3b: Bioaccumulation of chromium in *Tilapia*fishes

UNDER PEER REVIEW

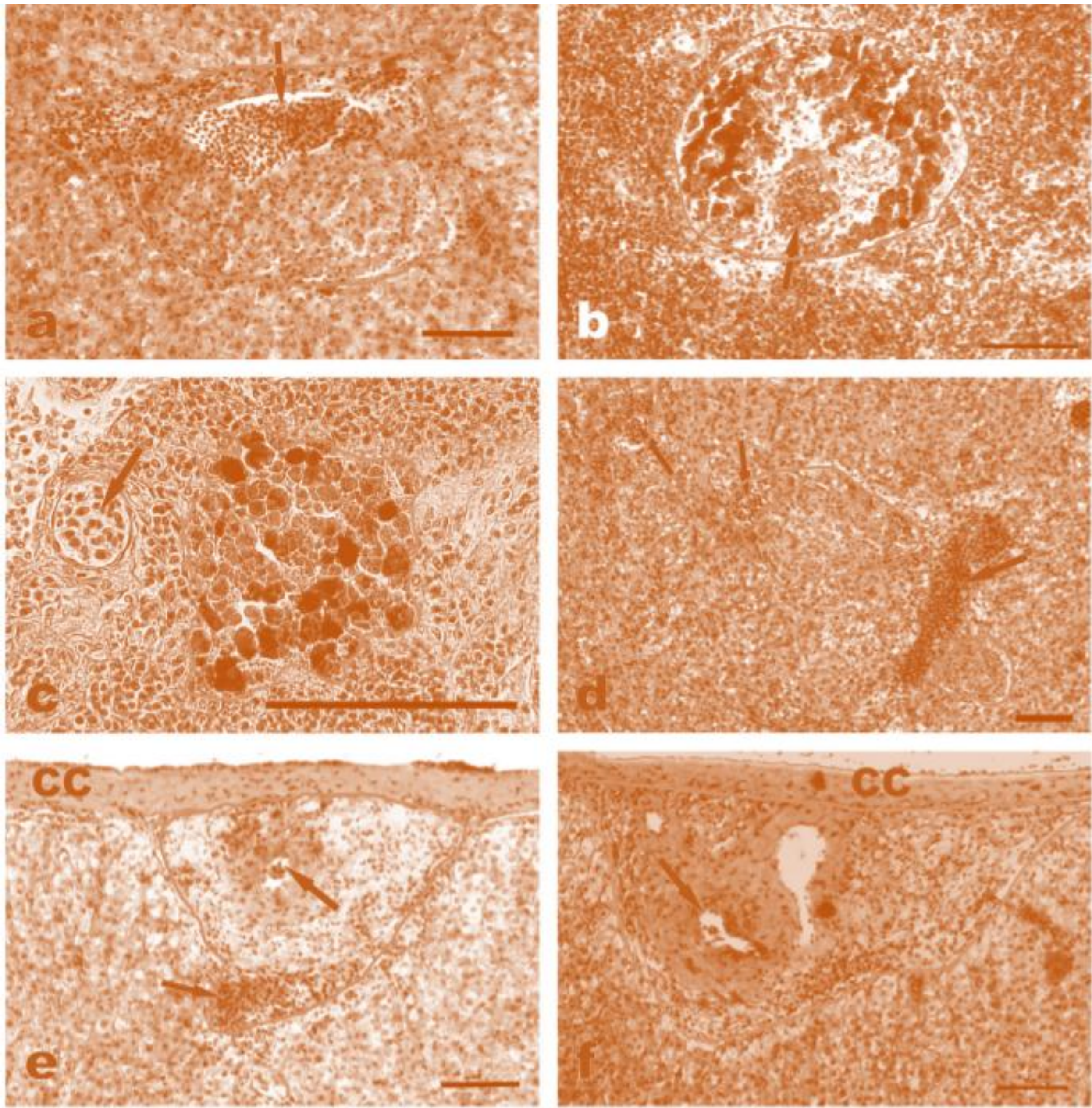

Fig 1.3c: Histological section of the liver and spleen of *Tilapia*fish on chromium, cadmium and copper

DISCUSSION

Heavy metals have been known for ages as metals with heavy weight with the ability to accumulate in the aquatic environment and the body of living organisms (bioaccumulation). For example, the gill of fish has been shown to be an important site for the entry of the heavy metals and is the first target organ for exposure in fish (Romeod, *et al.,* 1999). The higher concentration of the metal in the gill of *P. fluvidraco* and *C. carpio* is due to the metal complexation with the mucus, which is difficult to be removed completely from the tissue before the analysis (Rao, 2000). It has also been shown that heavy metals (Cd, Cr, Cu) can also gain entry from the aquatic environment into some other organs of the fish like liver, spleen, and muscles (Taweel*et al.,* 2013).

In this study, heavy metals (Chromium, Cadmium and Copper) detected upon analysis conducted in the samples collected from river of mmiri-ele in Nnewi, Anambra State was subjected to bioremediation using the fungi (*Aspergillus* sp., *Fusarium*sp and *Alternaria*sp.. These microorganisms were isolated from the natural environment that has been polluted with the heavy metals (Copper, Cadmium and Chromium). The microorganisms were closely studied. The various biochemical tests were also conducted in the samples to ascertain the biochemical conditions of the samples.

Other studies have shown the rise of other microorganisms (Bacteria). For instance, Zaki and Faray isolated *Enterobacter* sp., *Chryseobacterium*sp and *Stenotriphomonas* sp. which were shown to be resistant strain to heavy metals (Xie, *et al.,* 2010). Also, *Ralstoniapicketii* and *Splingomona* species have been isolated and shown to be resistant to Irish concentration Zn, Ni, Pb and copper from contaminated water (Staniland*et al,* 2010).

Bioremediation takes place when the microorganisms can biodegrade the given contaminant and the necessary nutrients such as nitrogen, phosphorus, electron acceptance and trace element. Microorganisms have the ability to reduce the heavy metals depending on the factors like time and concentration of inoculum (Jina and Nahdis, 2017). More reduction was observed with the increase in the incubation time. Also, sulphate reducing bacterium produces H_2S, which reduces

UNDER PEER REVIEW

heavy metals to the sulphide with very low water solubility to deposit, such as Cds and Cus (Barnes, 1991).

From the result, *Aspergillussp*, *Fusariumsp* and *Alternariasp* were shown to degrade Cadmium detected in the polluted sample of the amount very close to the control. Acute effects of exposure to cadmium result primarily from local irritation. After injection, the main effects are nausea, vomiting and abdominal pain. Inhalation results in pulmonary edema and chemical pneumonitis (Goyer, 2001). From the result of the bioremediation using the named fungi, chromium was degraded to the amount very close to the value of the control, suggesting that the process is very effective in the removal of the heavy metal. Chromium a known human carcinogen and induces lung cancer among exposed workers (Goyer, 2001).

Copper is known to be toxic to cells through the redox properties of copper, resulting in lethal oxidative damage to cells (Nandakumar, 2011). Although recent work has revealed that the main toxic action of copper is the replacement of the man cofactor in iron-sulphur clusterproteins. In this work, copper in both the water samples and sediment sample was reduced to a significant level. The results obtained from the sediment samples shows that cadmium, chromium and copper was degraded by the fungi isolates and the new value shown to be very near to the control. Hence, the bioremediation process was a success in that it really degraded the heavy metals (Copper, chromium and cadmium) present in both the water sample and the sediment samples.

UNDER PEER REVIEW

CONCLUSION

The bioremediation of the heavy metals (Copper, Chromium and Cadmium) significantly reduced the amount of these heavy metals in the polluted environment. The bioremediation abilities of the various microorganisms used are enhanced by providing the best environments, necessary for the growth of the microorganisms, e.g. adequate nutrients required, pH, reducing the electrical conductivity of the water etc. Hence, it can be said that under suitable environmental and biochemical conditions, microorganisms can be used in the remediation of the heavy metals present in a heavy metal polluted environment. This process has ultimately resulted to the significant reduction in the amount of heavy metals present.

UNDER PEER REVIEW

References

Kumar A, Škoro N, Gernjak W, Puač N. Cold atmospheric plasma technology for removal of organic micropollutants from wastewater—a review. The European Physical Journal D. 2021 Nov;75(11):1-26.

Kiyani V, Hosynzadeh M, Ebrahimpour M. Investigation acute toxicity some of heavy metals at different water hardness. International Journal of Advanced Biological and Biomedical Research. 2013;1(2):134-42.

Duffus JH. " Heavy metals" a meaningless term?(IUPAC Technical Report). Pure and applied chemistry. 2002 Jan 1;74(5):793-807.

Harrison JE. Synaesthesia: The strangest thing. Oxford University Press, USA; 2001.

Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of botany. 2012;2012.

Kumar R, Varandani D, Mehta BR, Singh VN, Wen Z, Feng X, Müllen K. Fast response and recovery of hydrogen sensing in Pd–Pt nanoparticle–graphene composite layers. Nanotechnology. 2011 May 26;22(27):275719.

Ito F, Ukari T, Takasaki M, Yamaguchi K. New applications of multiply charged ionic probes as cleavable cross-linker and polymerization reagent. Tetrahedron Letters. 2012 Jun 27;53(26):3378-81.

APHA, Compendium of methods for the microbiological examination (1992)

MacFaddin JF. Biochemical tests for identification of medical bacteria, williams and wilkins. Philadelphia, PA. 2000;113.

Madigan MT, Martinko JM, Dunlap PV, Clark DP. Brock biology of microorganisms 12th edn. Int. Microbiol. 2008;11:65-73.

UNDER PEER REVIEW

UNDER PEER REVIEW