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Evaluation of Toxicity and Bioremediation of Contaminated Drinking Water Sources in Delta State, Nigeria

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Authors' contributions

The work was carried out in collaboration among all the authors. Author DO conceived, designed and supervised the work. Authors RU, RO and CO collected the samples and handled laboratory aspect of the experiment. All authors read and approved the final manuscript.

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ABSTRACT

Drinking water samples were collected from boreholes in six locations in Ughelli and environs in Delta State of Nigeria and were treated by filtration through a substrate colonized with mycelium of *Pleurotus tuber-regium*. Water samples were analysed for pH, heavy metal concentration and microbiological content before and after filtration. Results obtained showed that the pH of unfiltered water samples were acidic (5.0 – 5.8) and below the WHO and SON permissible limits for drinking water. The same trend was followed by the concentrations of heavy metals such as lead, iron, zinc and chromium. Water samples from all six locations also had high total bacterial and coliform counts. Filtration through the mycelium colonized substrate showed adjustment of pH to a range within the WHO permissible limits. Reduction in heavy metal concentration ranged from 45.0 – 100%. Total bacterial count of mycofiltered water samples was impressively reduced by

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77.3 – 100% and coliform count was not detected. The results obtained in this study makes mycofiltration a potential cost-effective and efficient technique for the treatment of potable water for domestic use.

Keywords: Water pollution; heavy metals; Pleurotus tuber-regium; mycofiltration; coliform count.

1. INTRODUCTION

Since the advent of industrial revolution, environmental pollution has been on the increase while the health of our environment has simultaneously suffered. Chemical pollution such as that from urban run–off, industrial discharge and pest control agents have been reported to have widespread detrimental and long lasting effects on natural populations. The aquatic environment is under stress as rivers, estuaries, oceans and even underground water, the basic source of water for domestic and industrial supply are often ecological sinks or dumping grounds for these anthropogenic chemicals [1,2].

The Delta region of Nigeria is faced with the problem of the availability of potable water. This is due to environmental pollution and degradation in the area as a result of oil exploration and exploitation. Toxic chemicals, their residues, heavy metals and pathogenic microorganisms as well as other pollutants from the oil industry have been reported to be present in sources of potable water for domestic use such as rivers, streams and underground water (boreholes) [3].

Poor water quality can be as a result of natural processes but is more often associated with human activities that is closely linked to industrial development. The effects of industrial effluents as well as the physicochemical and bacteriological parameters of ground well water within an industrial estate were investigated. High magnesium, calcium and chloride content, a result of the compounds associated with the activities of the industrial estate, as well as high colour and turbidity due to the increase in the type of suspended matter released by the industry were recorded [4]. In another study, the effect of Warri refinery effluents on the water quality of Iffie, Ughoton and Ubeji rivers were found unsuitable for domestic use. The concentrations of heavy metals in these rivers were above the WHO permissible limits [5].

The elimination of wide ranges of pollutants and wastes from the environment is an absolute requirement to promote a healthy society with low environmental impact. One of the basic requirements for sustained development is a reliable source of safe potable water [6]. In spite of the considerable investments of the Nigerian government in water supply programmes, over 52% of her citizens do not have access to potable water [7]. Due to the magnitude of this problem and the lack of any solution currently, a rapid cost-effective and ecologically friendly method of clean-up is urgently needed [8].

The problem of pollution of potable water sources can be lessened by water treatment. Methods of water treatment include sedimentation (removal of suspended solids by gravity), coagulation (use of chemical coagulants), disinfection and filtration. Disinfection may be physical by boiling (requires a large amount of fuel and may give water a flat, unpleasant taste); pasteurization (cannot kill all pathogens); ultraviolet radiation, solar disinfection, chemical disinfection (use of chlorine can lead to serious problems if the right quantity/dose is not used. Other disinfectants include ozone (it is relatively expensive, difficult to produce and not practical for household use), iodine and bromine (relatively expensive), mercury silver and copper (are expensive and toxic to humans). The different types of filtration include: rapid sand filtration, slow sand filtration, bank infiltration, cloth filtration, membrane filtration (requires high or low pressure), nanofiltration and reverse osmosis (creates large volume of waste water i.e. only 10 – 20% of the raw water passes through the membrane, requires electricity and is relatively expensive) and ceramic filtration (it reduces turbidity and lowers bacterial concentrations but ceramic filters are prone to clogging and breakage, and require regular cleaning) [9].

Water purification technology is often complicated and requires sophisticated equipment. It is also expensive to run and maintain. Compared to the other methods of water treatment listed above, the use of biological systems for the treatment of water (bioremediation) is a more promising and less expensive way for cleaning up contaminated water [10].

Bioremediation is the ability of biological systems to degrade, transform or detoxify organic compounds and polluted areas by converting undesirable and harmful substances into non-toxic compounds [11]. It is a biotechnological approach of rehabilitating areas degraded by pollutants or otherwise damaged through mismanagement of ecosystems. Biological agents such as bacteria, fungi, algae and other green plants have been reported to be involved in bioremediation process and this they do by producing enzymes, biosurfactants, biopolymers and other fermentative products [12].

Mycofiltration is the use of mycelia of fungi as biological filter to clean water contaminated by pathogens, chemicals, silt and heavy metals [13]. A mushroom's mycelia is fit for ecological remediation. Since mycoremediation wears many caps, mycelia can be utilized as natural channels, wipes for poisons, and strengtheners of soil. The filtration part of mycelia, or mycofiltration, has been generally contemplated as a technique to sanitize storm water. Mycelia offer an effective, green technique for the expulsion of hurtful chemicals [14]. The mycofiltration technology could prove a simple answer to the problem. It could be used to remove the toxic chemicals, bacteria, viruses, and other hazardous materials from water much more effectively at a lower cost than other conventional methods. There are numerous advantages of using mycoremediation over commercialised technologies like public acceptance, nature and environment friendly, low cost, flexibility, fast, safety, simple, and quiet, low maintenance etc. It is eco-friendly and cost-effective and cheapest technology for the treatment of wastewater. It can be used as a remediating tool to reduce pollutants from wastewater by the action of various extracellular enzymes such as lignin peroxidase, laccase, manganese peroxidase, which have the ability of degrading lignocellulosic biomass. When we use fungi like *Pleurotus ostreatus*, for remediation of water, the fungi create an inhibitory effect of the growth of bacteria that are present in untreated water using the mycofiltration technique [15].

The main reason that white-rot fungi are active to such a wide range of compounds is their release of extracellular lignin-modifying enzymes, with a low substrate specificity, so they can act upon various molecules that are broadly similar to lignin. The enzymes present in the system employed for degrading lignin include lignin-peroxidase (LiP), manganese peroxidase (MnP), various H_2O_2 producing enzymes and laccase, although the three types of enzymatic activity are not present in all lignolytic fungi [16]. Nigeria can harness this technology to reduce pollutants from water sources to make it healthy for human consumption.

The aim of the study is to determine the effectiveness of a mycelium-permeated substrate in removing pollutants from contaminated drinking water sources in some rural communities in Delta State of Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

Fig. 1 shows the map of Ughelli North Local Government Area of Delta State of Nigeria from where the water samples were collected.

2.2 Sample Collection

Before collection, nozzles of the taps connected to the boreholes were swabbed with cotton wool soaked in 70% (v/v) ethanol and flamed for 2 minutes. For each sample, collection was done using washed and 3 sterilized plastic containers after running water to waste for 1 minute and taken aseptically into plastic containers, kept in an ice chest and stored in the refrigerator at 4°C for 24 hours. The pH of the samples were taken *in situ.*

2.3 Determination of Physicochemical and Microbial Properties of Water Samples

The water samples were analysed for twelve metals namely: lead (Pb), copper (Cu), cadmium (Cd), chromium (Cr), iron (Fe), zinc (Zn), nickel (Ni), manganese (Mn), aluminium (Al), cobalt (Co), silver (Ag) and arsenic (As) according to standard analytical methods [17] using an Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer A Analyst 100). The metal standards were prepared to known concentrations, labelled and kept inside glass bottles that were pre-cleansed with concentrated nitric acid and distilled water. Values obtained were compared against the Standard Organization of Nigeria (SON) and World Health Organization (WHO) limits.

For microbial analysis, the techniques employed were estimation of total heterotrophic bacteria (THB) by plate count technique and estimation of coliform bacilli by Most Probable Number (MPN) presumptive test [17].

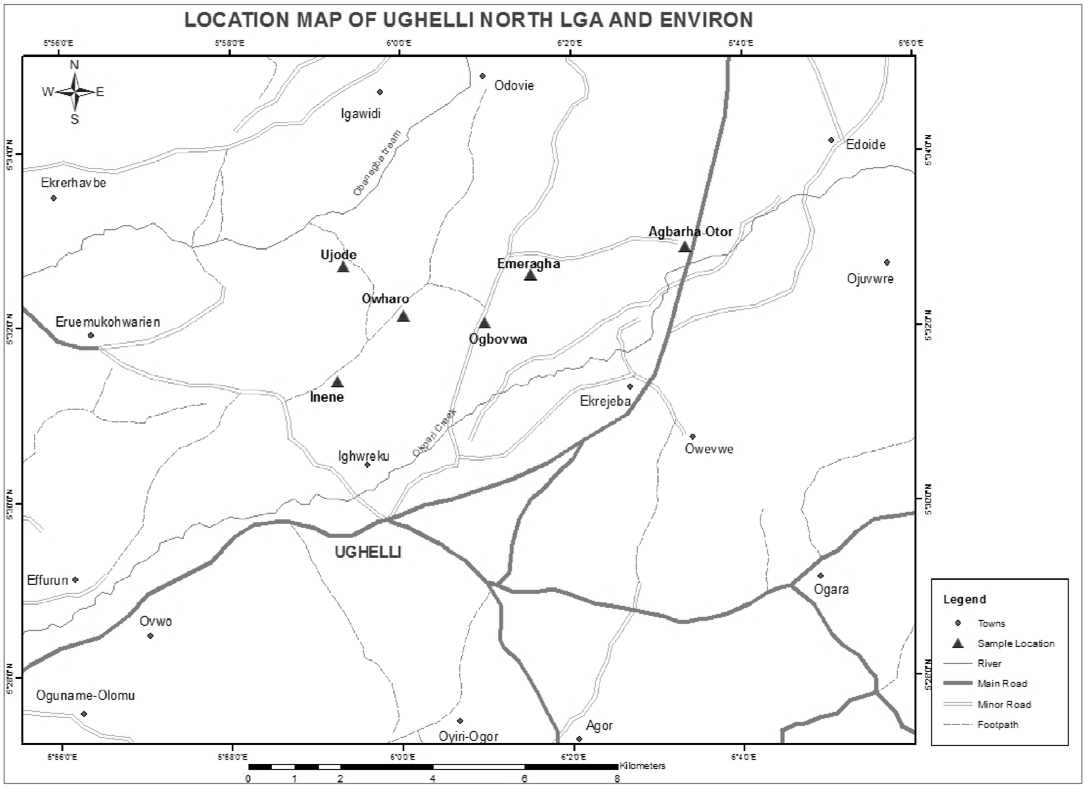

Fig. 1. Map of Ughelli north local Government area and environs showing location of sample collection

2.4 Mycofiltration Procedure

Mycofiltration was done following methods described by Akpaja et al., [18]. Maize cobs were collected from local corn sellers in Iyowa village near Benin City and milled to dust particles and then sundried to constant weight to reduce moisture content. For the preparation of the substrate, 77% of the unfermented maize cob dust was supplemented with 20% (w/v) wheat bran, 1% (w/v) granulated sugar, 1% (w/v) calcium sulphate and 1% (w/v) calcium carbonate. This was mixed properly with water, covered with tarpaulin and allowed to compost for 7 days. To ensure homogenous fermentation, the substrate was mixed by turning every 48 h. The substrate was thereafter loaded into bags (15 x 30 cm) and pasteurized by steaming it for 3 h to reduce contaminants after which it was allowed to cool in a sterile room. After cooling, the substrate was inoculated at the centre with a spawn of *Pleurotus tuber-regium* and allowed to incubate at ambient temperature (26±2 °C) until it was fully colonised by the mycelium of the mushroom. The colonized substrate which has a hollow made in the middle of it was put in a perforated bowl. Filtration was done by pouring the collected drinking water sample into the hollow. Filtrate was collected in a sterile container and analysed for microbial and physicochemical properties.

2.5 Statistical Analysis

Data were analyzed using descriptive statistics as presented in Tables.

3. RESULTS AND DISCUSSION

The study has shown the water purification capacity of the mycelia of *Pleurotus tuber-regium*. Results obtained showed that the heavy metals and microbial content of drinking water can be greatly reduced by filtration using a mycelium colonized substrate.

The pH of the water samples varied with the location of boreholes (Table 1). Most of the pH values recorded for unfiltered water samples were acidic i.e. 5.0 – 5.8, and were below the SON and WHO limits of drinking water pH (6.5 – 8.5), that of UGN 4 was alkaline higher with a pH of 9.7. The pH of unfiltered water samples in UGN 1, UGN 3, UGN 5 and UGN 6 were outside SON and WHO permissible limits of potable water quality probably because these locations host different pharmaceutical companies, chemical plant or paints.

Table 1. pH of borehole water samples

Water sample	Unfiltered water	Myco-filtered water
UGN 1	5.8	6.1
UGN 2	6.4	7.2
UGN 3	5.1	6.8
UGN 4	9.7	8.6
UGN 5	5.0	6.8
UGN 6	5.0	6.7
SON limit	6.5 – 8.5	
WHO limit	6.5 – 9.2	

UGN = Ughelli North

Where obnoxious gases highly concentrated in CO_2, NO_3, SO_2 are emitted, which while in the air form carbonic, nitrous and sulphuric acid respectively fall as weak acid during the rainy season which later infiltrate into groundwater thereby decreasing the pH to acid formation [19]. The effluents from these industrial activities also unregulated hence most of these are discharged into the water formation and soils which during recharge contaminate the groundwater to acidity. The pH values of filtered borehole water samples were within the SON and WHO permissible limits of potable water quality. In a related investigation, slight acidity and high lead content in water samples have also been recorded in Canaanland, Ota in Nigeria [20].

Cadmium, aluminium, cobalt, silver, copper, arsenic and nickel were not detected in the water samples (Table 2). Nickel and copper were detected in water samples from rivers receiving refinery effluents [5].

Except manganese, the quantity of iron, lead, chromium and zinc detected in the river samples were higher than SON and WHO permissible limits for these metals in drinking water. The high concentration of heavy metals in the water samples collected indicates the extent of pollution of underground water. This is as a result of indiscriminate disposal of industrial wastes as well as the seepage of these chemicals and their residues into potable water sources [21]. Similar reports of polluted ground water from other investigators have been reported in Oleh in Isoko South Local Government Area of the State [22], who attributed the presence of lead in some of the water samples to indiscriminate dumping of waste engine oil and spent motorcycle batteries by mechanic workshops in the area. In another report, the high physicochemical parameters with high total bacterial and coliform counts of ground water (well water) in Asa dam industrial estate, Ilorin, Nigeria were reported to be above WHO permissible limits for drinking water quality [4]. The main reason for this were attributed to anthropogenic activities in the area.

Iron and zinc were present in all the unfiltered water samples while chromium was present in UGN 1 and UGN 4 water samples. Manganese was present in all unfiltered water samples except UGN 6 while lead was found to be present only in UGN 2 water samples. The impact of heavy metals in high concentrations on human health is well documented [23], therefore the need to reduce or totally eliminate their presence in drinking water sources cannot be overemphasised. The concentration of heavy metals in the unfiltered water samples in this study was drastically reduced after mycofiltration. The concentrations of iron were significantly reduced after filtration while zinc, chromium and lead were totally eliminated. In a related study, slow sand filtration technique was found to remove 30-90% of iron and manganese present in water [24]. In this study, filtration using mycelium colonised substrate removed 55-100% of iron, manganese and other heavy metals present in the water samples. This makes mycofiltration a more effective technique for the treatment of potable water.

The quantity of coliform count per 100 ml recorded in the water samples tested in this study is within the range stated for boreholes [25]. The values of total bacterial count recorded in unfiltered water samples ranged from 12.2 x 10^3 recorded in UGN 2 water sample to 27.5 x 10^2 recorded in UGN 5 water sample, while that of coliform count ranged from 0.5 x 10^3 recorded in UGN 6 water sample to 9.4 x 10^3 for UGN 1 water sample (Table 3). Lower values of total bacterial and coliform counts were recorded for wells within an industrial estate in Ilorin, Nigeria [4]. The high counts of coliform in unfiltered water samples indicate the presence of contamination of water from human and animal wastes [26].

Any technique for treating water should be designed to remove the vast majority of pathogens [27]. Biofilteration has been suggested as one of the ecosystem biotechnology for catchment scale water quality improvement [28]. When available, water treatment facilities have the potential to reduce the presence of coliform bacteria in samples from very high concentrations to values below levels of analytical detection [29].

Mycofiltration technology uses the vegetative growth of bacteria-predating fungi to transform wood by-products and ligno-cellulosic agricultural wastes into an intricate and dynamic three-dimensional web of tube-like cells, called mycelium, which can strain, adsorb, and digest bacteria as a food source [30]. Some species of fungi consume bacteria and

Table 2. Heavy metal concentration of borehole water samples

Water sample	Treatment	Concentration of metals (mg/L				
		Fe	**Zn**	**Cr**	**Pb**	**Mn**
UGN 1	UF	2.60±0.03	6.90±0.03	0.01±0.00	0±0.00	0.10±0.00
	MF	1.43±0.01	0±0.00	0±0.00	0±0.00	0.02±0.00
	% reduction	45.0	100	100	0	80
UGN 2	UF	4.30±0.00	7.10±0.21	0±0.00	0.03±0.00	0.03±0.00
	MF	0.30±0.00	0±0.00	0±0.00	0±0.00	0±0.00
	% reduction	93.0	100	0	100	100
UGN 3	UF	2.00±0.01	6.40±0.30	0±0.00	0±0.00	0.01±0.00
	MF	0.52±0.00	0±0.00	0±0.00	0±0.00	0±0.00
	% reduction	74.0	100	0	0	100
UGN 4	UF	3.00±0.00	8.00±0.03	0.2±0.00	0±0.00	0.01±0.00
	MF	0.31±0.00	0	0±0.00	0±0.00	0.01±0.00
	% reduction	89.7	100	100	0	0
UGN 5	UF	2.10±0.01	6.60±0.01	0±0.00	0±0.00	0.01±0.00
	MF	0.94±0.00	0±0.00	0±0.00	0±0.00	0±0.00
	% reduction	55.2	100	0	0	100
UGN 6	UF	1.90±0.00	5.10±0.00	0±0.00	0±0.00	0±0.00
	MF	0.61±0.00	0±0.00	0±0.00	0±0.00	0±0.00
	% reduction	67.9	100	0	0	0
SON limit		0.30	3.0	0	0.01	0.20
WHO limit		1.0	3.0	0.05	0.01	0.40

Values are expressed as mean± SEM, n=3, UGN = Ughelli North, UN = Unfiltered, MF = Mycofiltrated, Zero (0) values = undetected in filtered water

Table 3. Microbiological parameters of borehole water samples

Water sample	Treatment	Total bacterial count (cfu/ml)	Coliform count (MPN/100 ml)
UGN 1	UF	24.3×10^3	9.4×10^3
	MF	0.5×10^3	0
UGN 2	% reduction	97.9	100
	UF	12.2×10^3	1.4×10^3
	MF	0	0
UGN 3	% reduction	100	100
	UF	16.3×10^3	2.4×10^3
	MF	3.7×10^3	0
UGN 4	% reduction	77.3	100
	UF	14.5×10^3	1.2×10^3
	MF	0.2×10^3	0
UGN 5	% reduction	98.6	100
	UF	27.5×10^3	5.5×10^3
	MF	4.1×10^3	0
UGN 6	% reduction	85.1	100
	UF	21.6×10^3	0.5×10^3
	MF	2.9×10^3	0
	% reduction	86.6	100

UN = Unfiltered, MF = Mycofiltrated water secrete antimicrobial metabolites. *Pleurotus* sp. produce bioactive compounds and metabolites which are effective against a wide range of microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae*, *Salmonella typhi*, *Bacillus* sp., *Aspergillus flavus* and *A. candidus* [31,32].

A reduction of 77.3 – 100% total bacteria count was observed in myco-filtered water samples. 100% reduction of coliform count was recorded for all myco-filtered water samples (Table 3). The decrease and the total bacteria count recorded and colony forming unit observed in the myco-filtered water samples is as a result of the antimicrobial polysaccharides produced by the mycelium of *Pleurotus tuber-regium* [33]. One of the short comings of cloth filtration is that some bacteria are small enough to pass through the holes in the cloth. The study showed that unfiltered water in the locations tested may cause serious problems when used for drinking. UNICEF recommends that any water used for drinking should be properly treated to ensure physicochemical and microbiological safety [9]. Treatment of water samples by filtration with mycelium colonized substrate substantially removed the microorganisms in the water and also reduced the concentration of heavy \metals in the water samples. As noted by other investigators, for the actual removal of heavy metals, the remediating fungi should also be removed as the fungi tend to accumulate the metals within them. So, it is also imperative to develop strategies to overcome this limitation [34]. Filtration through a mycelium colonized substrate was also effective in the treatment of cassava mill effluent with up to 100% removal of the cyanide content of the effluent [18].

4. CONCLUSION

The results reported in this study showed that the potable water in the six locations tested were contaminated with heavy metals and microorganisms, and therefore not fit for human consumption. However, on treatment by filtration through a *Pleurotus tuber-regium* mycelium colonized substrate, the contamination was sufficiently reduced to make the water relatively safe for drinking.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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