RESEARCH ARTICLE

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Assessment of mycological profile and heavy metal concentrations of Romi River, Kaduna - Nigeria

ABSTRACT

Waste water discharged from petrochemical plants and petroleum refinery contains many pollutants including heavy metals. The objective of this study was to assess macro-morphological and microscopic characteristic of fungal isolates and levels of heavy metals from a petrochemical and petroleum refinery industry effluent and water from River Romi in Kaduna State Nigeria using standard methods. Isolation and identification of fungi was carried using standard method, the atomic absorption and spectroscopy method was used for the analysis of heavy metals. The results obtained from this study revealed that the fungal population isolated was predominantly native of the genera *Aspergillus* (30.69%). The mean and ± std. of the total heterotrophic fungal count of the river were found to be Upstream Mean ($4.9x10^{-4}$ ±$3.8x10^{-4}$ CFU/mL) Downstream Mean: ($4.9x10^{-4}$±$4.3x10^{-4}$ CFU/mL). The percentage occurrence of the fungal species in respect to their sampling sites shows that the upstream part of the river had lower count than the downstream part of the river. Majority of the heavy metals assessed also shows that their concentrations were above the recommended value set by World Health Organization as standard for their presence in environmental samples. There was a significant different ($p < 0.05$) between the 1st and 6th month, and also between the 3rd and 6th month respectively. From the values of the analysis of heavy metals, it can be deduced that Romi River is highly contaminated with the aforementioned metals, and as such, immediate respond on the high occurrence of these metals should be addressed.

Key words: Assessment, mycological profile; heavy metals; Romi River; Nigeria

Introduction

The massive development and growth in petroleum hydrocarbon exploitation and refining processes in Nigeria and the world at large, especially to those countries known to be oil producing nations has led to increase in oil pollution in the environment (Israel *et al.,* 2018). Large amount of effluents are produced during petroleum refining. These effluents are very toxic and result in the environmental pollution of water bodies that are at the receiving end, as well as soil and microorganisms which are found to be inhabitants of such environment (Israel *et al.,* 2018). Waste water discharged from petrochemical plants and petroleum refinery contains many pollutants including heavy metals (Rai, 2019). The behavioural pattern of the petroleum hydrocarbons entering the water body is dependent on the type of chemical contaminant, mechanism of entry and the physical characteristics of the receiving water body (Rai, 2019). Fungi are members of a large group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms (Buckley, 2017). Fungi have a profound impact on the global ecosystems. They modify habitats and are essential for many ecosystem functions (Chen and Wang, 2019). Kingdom Fungi (the true fungi) is a monophyletic group of eukaryotic heterotrophs that reproduce with spores and have chitinous cell walls. The most familiar fungi are kitchen molds and mushrooms. The kingdom may include 1.5 million species, of which about 80,000 species have been named and described. Fungi form soil, recycle nutrients, decay wood, enhance plant growth and cull plants from the environment. The kingdom Fungi are divided into five phyla namely; Chytridomycota, Zygomycota, Basidiomycota, Ascomycota and Deuteromycota (Ezeonuegbu *et al.,* 2015; and Buckley, 2017). Biological materials from plants and microbes can bind to all type of heavy metals but only those with sufficiently high binding capacity and selectivity for heavy metals are suitable for use in bioremediation processes (Payne, 2018). A large number of microorganisms belonging to various groups (cyanobacteria, bacteria, yeast and fungi) respond to heavy metals in different ways depending on the nature of the microorganisms and on the concentration of the

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heavy metal in the environment (Payne, 2018). Among these microorganisms, fungal biomass offers the advantage of having a high percentage of cell wall material which shows excellent metal-binding properties, easy cultivation at large scale as it has short multiplication time and easy availability of fungal biomass as industrial waste product.

One of the considerable parameters in determining water quality is temperature (Elen *et al.,* 2018). Temperature of surface waters depends mainly on: Water origin, Climatic zone, Season, Altitude, Degree of riparian coverage, Inflow of industrial and municipal sewage (power plants, industrial cooling). Water temperature can fluctuate diurnally and seasonally (Elen *et al.,* 2018). Flowing waters usually have unique temperature depending on flow velocity. The temperature of water affects some of the important physical properties and characteristics of water: thermal capacity, density, specific weight, viscosity, surface tension, specific conductivity, salinity and solubility of dissolved gases among others (Chen and Wang, 2019). Chemical and biological reaction rates increase with increasing temperature. Temperature can exert great control over aquatic communities, especially influence on biological activity and growth. If the overall water body temperature of a system is altered, an aquatic community shift can be expected (Israel *et al.,* 2018). It is measured in degree Celsius (^{0}C).

Heavy metal pollution is typically associated with mining activities or discharges from some industries including petroleum refinery (Hakeem and Bhatnagar, 2016). Heavy metals associated with petroleum operations include Arsenic, Cadmium, Chromium, Lead, Copper, Iron, and Zinc and so on. Persistent toxicants (heavy metals) in water and sediments affected by heavy metal pollution can have serious effects on the aquatic ecosystem and can make water unsuitable for human consumption (Israel *et al.,* 2018). Some animals can also 'bio-accumulate' metals, making them unsafe to eat. Heavy metals are measured in parts per million (ppm) or milligram per litre (mg/L) (Chen and Wang, 2019). The ability of water to conduct electricity is termed conductivity (Ezeonuegbu *et al.,* 2015). Charged particles called ions that become dissolved in water supply the means for water to conduct electricity. The conductivity of water is dependent on its ionic concentration and temperature (Hemambika *et al.,* 2021). As conductivity measures the dissolved ionic content of water it is also commonly used as a measure of total dissolved solids. Because our lakes and streams contain a lot of soluble minerals (called hardness) and high alkalinity (from carbonate ions), the conductivity is fairly high. Conductivity is an easy and accurate way to measure the level of dissolved substances, but cannot indicate what the substances are. A steady increase of conductivity over a period of years is usually indicative of pollution occurring (Jonathan *et al.,* 2018). It is measured in microsiemens per centimetre. Therefore, this study was carried out with the aim to determine the mycological profile and heavy metal concentrations of River Romi water sample receiving effluent from Kaduna refinery and petrochemical company, Kaduna, Nigeria.

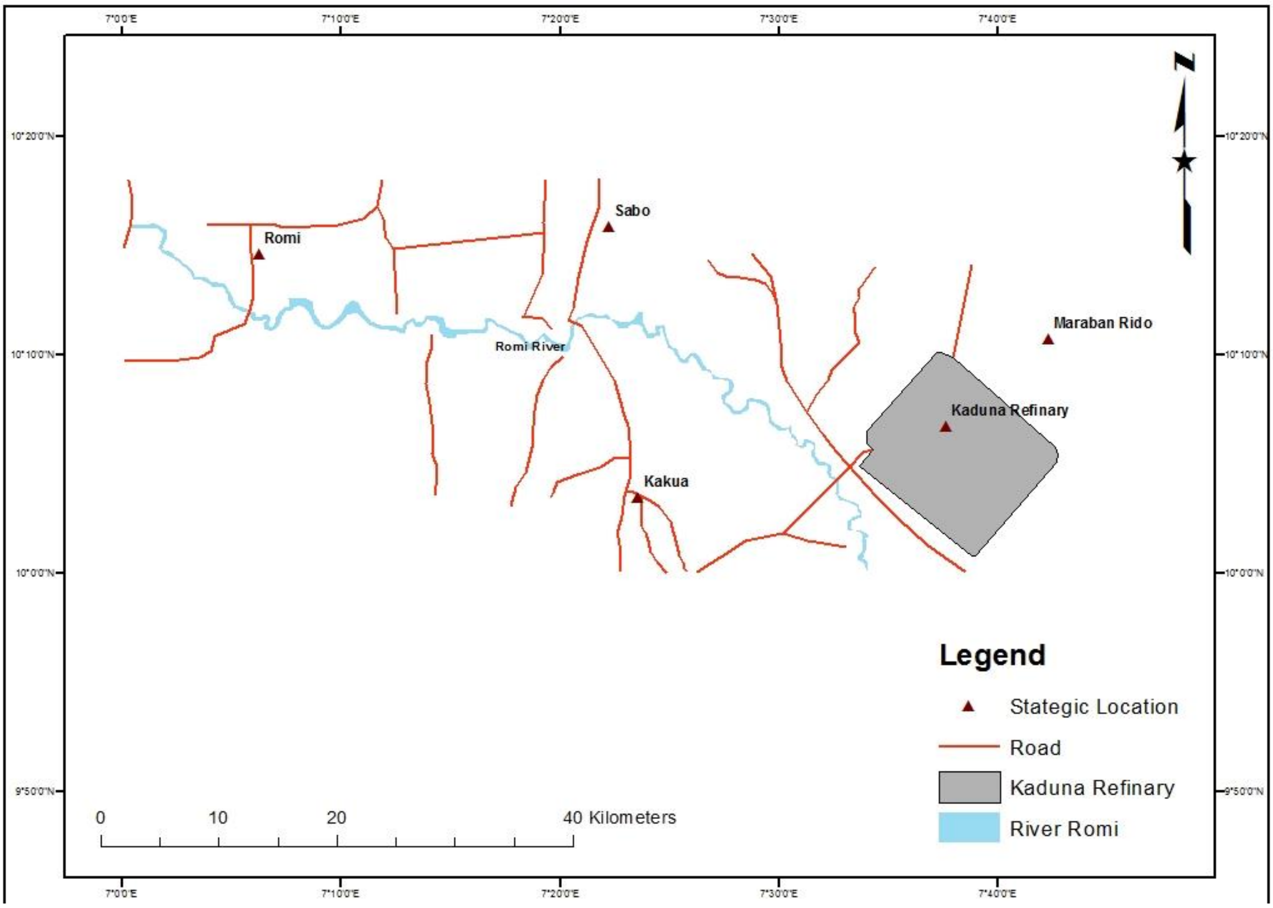

Figure 1. *Map showing the Kaduna Refinery and Petrochemical Company and the Romi River. Source: Kaduna Geographic Information Service, 2020*

Materials and Methods

Effluent Sample Site

Effluent samples were collected from the effluent sites (A, B, C) of a Refinery and Petrochemical Company with latitude 10º 29' 25'' N and longitude 7º 20' 26'' E. Sites A was the point of discharge, Site B the upstream, while Site C was the downstream parts of the river (15Km from the point of discharge).

Collection of Refinery Effluent and Water Samples from Romi River

Sterile sample bottles were used in collecting both water and effluent samples from the industry point of discharge (site A), Upstream (site B), and Downstream (site C). In duplicates, the samples were collected and these were done by lowering the bottles into section of the site that is well mixed (30 cm) deep. Before, the samples were withdrawn, they were allowed to first overflow. The samples were collected for duration of six (6) months at an interval of first month, third month and six month respectively. The rationale behind this sampling frequency was to enable the study to capture the seasons (wet and dry). Vital information about the samples were noted, such as collection code, sample code, as well as sampling time, which was then transferred to the laboratory for fungal isolation, physicochemical and heavy metal analysis.

Physicochemical Analysis of Romi River Water Sample

This was carried out in accordance with the methods described by (Machido *et al.,* 2014). Physicochemical analysis was carried out to know the natural conditions of the samples, extent of pollution of the receiving water body, physical and chemical conditions under which the potential organisms to be isolated exist. The physicochemical parameters analyzed include; pH, temperature, electrical conductivity, dissolved oxygen (DO), biological oxygen demand (BOD), total dissolved solids.

Enumeration of Fungi in Water Sample

Standard method of fungi isolation was followed. Samples were microbiologically analyzed on the same day of the collection in order to eliminate any possible form of microbial spoilage of the samples. Samples were collected, and allowed to stand at ambient temperature (28ºC) on a work bench that is sterile. Nine (9.0 mL) of each sample in duplicates was aseptically dispensed in sterile centrifuge tubes for and centrifuged for 10 minutes at a speed of 250rpm in order to obtain concentrates of the samples. After decanting the supernatant, 0.1 mL of the residue of the each sample was spread-plated on Potato dextrose agar (PDA) and Rose Bengal Chloramphenicol in duplicated, with the aid of bent glass rod 50µg/L of chloramphenicol was dispensed onto the PDA media. At ambient temperature, plates were incubated for 3-5 days.

Isolation and Identification of the Fungal Isolates

Macro- morphological characteristics of the fungal isolates, including color, texture, and color of the reverse side of the fungal isolates were observed and recorded. For the micro-morphological characteristics, small portion of the growing region was mounted on clean grease free slide with a drop of lacto phenol cotton blue, covered with a cover slip and examined by microscopy using 40X objective lens. Characteristics of the sexual reproductive structures, presence or absence of septate, aerial and substrate mycelia, presence of foot cells and chlamydospores were observed and recorded. Taxonomic guide as described by (Cheesbrough, 2018) was used to identify each of the fungal isolate.

Determination of Heavy Metals in Effluent and Water Samples

The concentrations of heavy metals in effluent and water samples were analyzed using the fast sequential atomic absorption spectrophotometer (Model AA240S, Varian technologies, USA). Briefly, the instrument's settings and operational conditions were done in accordance with manufacturer's specifications by calibrating with analytical grade metal standard stock solution. Five milliliters (5.0 mL) of each sample was digested in a beaker by adding 37.5ml of nitric acid and 12.5ml of hydrochloric acid, heated to almost dryness and topped up to 50ml with distilled water. The digested samples were allowed to undergo filtration to remove any insoluble materials that could clog the atomizer. The filtrate was then analyzed for heavy metals using the atomic absorption and spectroscopy (AAS) (Abbas-Alkarkhi *et al.,* 2018).

Statistical Analysis

Statistical analysis of the data in this study was obtained. Means were compared using one way Analysis of Variance (ANOVA) and Duncan multiple range test of post Hoc test (P<0.05).

Results

Table 1 shows the physicochemical properties of the water sample, the pH value was at 6.70 which by nature are relatively neutral. Temperature was at 15.9ºC; Dissolved Oxygen was at 0.40, BOD 0.20, Electric Conductivity 19.10 and that of Total Dissolved Solids 9.55 respectively. Table 2 shows the total heterotrophic count of the fungal isolates for months 1, 3, and 6 respectively. Mean count for the upstream part of the river was $4.9x10^{-4} \pm 3.8x10^{-4}$ Downstream Mean: $4.9x10^{-4} \pm 4.3x10^{-4}$.There was a significant difference ($p < 0.05$) between the groups and significant difference within the groups. A mean difference was equally observed between 1st and 3rd months at 0.007 and 3rd and 6th month at 0.014. While no significant difference was observed for the 1st and 6th month.

Table 1: *Mean Values of the Physicochemical Parameters of Sampling Sites (A, B and C)*

Parameter	Mean Value	WHO/NSDWQ (Mg/L)
pH	6.70±0.82	6.5 - 8.5
Temperature (ºC)	15.90±1.30	Ambient
Dissolved O_2	0.40±0.03*	14
BOD	0.20±0.01*	5
E.C (µS/cm at 25ºC)	19.10±1.60	500
Total dissolve solids (TDS)	9.55±0.94	1000

Legend: WHO= World Health Organization, NSDWQ= National Standard for Drinking Water Quality
Values with asterisk are statistically significant at P-value ≤ 0.05)

Table 2: *Total Heterotrophic Fungal Count of Romi River for Duration of 6 Months*

Months	Upstream (CFU/ml)	Downstream (CFU/mL)	p value
1	$16.0 x 10^{-4}$	$11.5 x 10^{-4}$	0.007*
3	$8.5 x 10^{-4}$	$9.6 x 10^{-4}$	0.014*
6	$2.20 x 10^{-4}$	$4.0 x 10^{-4}$	0.264
Mean:	$4.9x10^{-2} \pm 3.8x10^{-4}$	$4.9x10^{-2} \pm 4.3x10^{-4}$	

Values with asterisk are statistically significant at P-value ≤ 0.05)

Table 3: *Percentage Occurrence of Fungal Species from different Parts of Romi River*

Isolates	Sampling site No (%) A(%)	B(%)	Total (%)
Aspergillus niger	3 (13.64)	7 (23.33)	10 (36.97)
A. fumigatus	6 (27.27)	4 (13.33)	10 (40.60)
A. flavus	4 (18.18)	12 (40.00)	16 (58.18)
A. glaucus	4 (18.18)	2 (6.67)	6 (24.85)
Penicillium sp.	2 (9.09)	0 (0.00)	2 (9.09)
S. apiospermum	0 (0.00)	3 (10.00)	3 (10.00)
Fusarium sp.	1 (4.55)	1(3.33)	2 (7.88)
Microsporum sp.	2 (9.09)	1(3.33)	3 (12.42)
Total	22 (42.31)	30 (57.69)	52 (100)

Legend: A= Upstream of river Romi. B= Downstream of River Romi

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Table 4: *Mean ±SD Concentration of Heavy Metals from the Sampling Sites (A, B and C)*

HM	DP M1 M3 M6	UPS M1 M3 M6	DNS M1 M3 M6	WHO mg/l (µg/L)
Pb	1.1x10-2 ±7.7x10^{-2}	8.5x10-2 ±1.0x10^{-1}	1.0x10-1 ±1.3x10^{-1}	0.01
Fe	1.4x10-3 ±1.6x10^{-1}	1.7x10-1 ±2.0x10^{-5}	3.7x10-1 ±2.3x10^{-1}	0.5
Mn	1.5x10-2 ±8.9x10^{-2}	7.3x10-1 ±1.0x10^{-1}	9.9x10-2 ±1.4x10^{-1}	N
Cu	5.9x10-3 ±5.6x10^{-1}	5.9x10-2 ±8.0x10^{-2}	5.5x10-2 ±7.4x10^{-2}	2
Cd	4.8x10-2 ±1.6x10^{-1}	1.5x10-1 ±2.1x10^{-1}	1.7x10-1 ±2.3x10^{-1}	0.003
Zn	6.1x10-2 ±1.8x10^{-1}	1.7x10-2 ±2.4x10^{-1}	1.4x10-1 ± 1.8x10^{-1}	N

Legend:
H M= Heavy Metal. DP= Discharged Point, UPS = Upstream of the river. DNS = Downstream of the river. WHO = World Health Organization, M= Month N = Nil. There is was no significant different ($p < 0.05$) at the level of heavy metal concentration irrespective of the different sampling sites. Similarly, there was equally no significant different ($p < 0.05$) between the 1st and 3rd month. However, a significant different between the 1st and 6th month, and also between the 3rd and 6th month respectively was seen.

From the table above, the downstream part of the river had the highest percentage occurrence for the fungi (57.69%) while the upstream part had lower (42.31%). In total, the genus *Aspergillus* was the highest occurring fungi amongst other fungal genera that was isolated and identified, with the downstream part recording the highest prevalence of the fungal genera. The least fungi isolated in this study were Fusarium species.

Discussion

Certain physical and chemical aspects of the environment, including temperature and pH, are important for the survival, adaptability, and growth of microorganisms (Abbas-Alkarkhi *et al.,* 2018). In this study, the temperature was 15.90^{o}C. In a similar research conducted by Tariq *et al.* (2016) it was reported that temperature affects the distribution and diversity of filamentous fungi, and that there is greater diversity in tropical areas than in temperate waters. In addition, marine fungi require temperatures between 25°C and 30°C to reproduce. This by implication suggests that the temperature of the river as regards to the present study does not really supports the physicochemical activities of the fungal population present in the water body. The survival of mycobiota and their diversity are influenced by a number of abiotic factors, one of the most important of which is hydrogen concentrations, which influences fungal growth capacity and composition. The fungi isolated, *Aspergillus* (total *Aspergillus* sp) and *Penicillium* was the most commonly observed genera overall (30.69% and 3.85%, respectively) followed by the other genera at lower frequencies. The frequency distribution of the fungi indicated that the genera *Aspergillus* was predominantly found to be abundant amongst other genera of the fungi. This is because; members of the genus *Aspergillus* are cosmopolitan and prevalent components of different ecosystems in a wide range of environmental and climatic zones due to the fact that they can colonize a wide variety of substrates (Ahmed, 2019). However, the result also suggest that the presence of petroleum hydrocarbon as contaminants in the river body which is supposedly to be detrimental to the fungi and other microorganisms inhabiting such environment was utilized as carbon source for their growth. This correspond to the findings of Noor (2017) who stated that the variation in the occurrence of the different fungi species in the various part of the river could be attributed to the fact that the occurrence of fungi in their natural environment are controlled by several factors including microclimate, the availability of substrates, as well as water capacity and complex ecological interactions.

Survival in different environmental and geographical habitats can be related to metabolic diversity, high reproductive capacity, and competitive capabilities of the fungal species. This is in consent with the report conducted by Noor, (2017). Certain fungi species present in the water depth have been reported to cause diseases in fish and other aquatic animals as well as in humans. On the other hand, they have been evidenced to actively participate in the biotransformation of xenobiotics and heavy metals contaminating the aquatic environment, thereby potentially contributing to the alleviation of the effects of anthropogenic stress, and improving water quality (Pietryczuk *et al.,* 2018).

Majority of the heavy metals assessed in this study, their concentrations were above the recommended value set by World Health Organization (WHO) as standard for their presence in environmental samples. The result gotten from this study is similar to that of Ezeonuegbu, (2015) who found out that the concentration of heavy metals in refinery effluent discharge from Kaduna refinery had a greater values as compared to the standard set by U.S.EPA. The persistent of these heavy metals in their high concentrations in the environment could lead to adverse effect on both the environment and to human health (Ahmed, 2019). Anthropogenic activities at regular basis always takes place in the river, these includes but not limited to, swimming, irrigation farming, fishing, disposal of wastes, and domestic usage of the river water for both cooking and drinking by the immediate communal residents of the area, particularly those who resides just within few meters away (2-4 Km) off the river bank.

Similarly, no statistically significant difference observed ($p < 0.05$) between the 1st and 3rd month of the analysis. However, there was a significant difference between the 1st and 6th month, and also between the 3rd and 6th month respectively. The significant high level of heavy metal concentration in the 6th month suggested that, the concentration of heavy metal is seasonal dependent since the samples were collected and analyzed 1st and 3rd month (dry season) at different seasons as against the sample analyzed for month 6th (wet season).

In conclusion, the present study revealed that fungal populations isolated were predominantly native of the genera *Aspergillus*. Heavy metals concentrations were found to be significantly higher. There was no significant difference ($p < 0.05$) at the level of heavy concentration irrespective of the sampling sites, i.e. discharge point, upstream and downstream parts of the river. This suggests that, the concentrations of these heavy metals are sparsely distributed in the entire river. The fungi enumerated from the downstream part of the river were higher than those enumerated from the upstream with a significant difference being observed between and within the groups across the six months' analysis. It can be deduced that Romi River is highly contaminated with the aforementioned metals, and as such, immediate respond on the high occurrence of these metals should be addressed. Biological techniques such as bioremediation/biodegradation of the toxic pollutants (heavy metals) of the river should be encouraged so as to limits or detoxify these toxic pollutants found in the river in other to avert health risk should be employed.

Competing interests disclaimer

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