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Ecological Risk Evaluation of Inorganic Contaminants and Microbial Activities in Obunagha River, Bayelsa State, Nigeria

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Abstract
The study was carried out to evaluate the Ecological Risk Assessment of Inorganic Contaminants and Microbial activities of Obunagha River in Bayelsa State. Eight water samples were collected from four sampling points in the Gbarain Clan community. The analysis included heavy metal samples of Zinc, Manganese, Cadmium, Chromium, Nickel, Lead, and Copper, and some Microbial assays. Pollution Indices such as Contamination Factor, Pollution Load Index, Heavy Metal Potential Ecological Risk Coefficient, Potential Contamination Index, and Quantitative Microbial contamination were assessed. The Pollution Load Index (PLI) indicated a moderate pollution level for all metals, with Zn (1.3685), Mn (1.3685), Cd (1.26425), Cr (0.9455), Ni (1.291), Pb (1.01875), and Cu (0.99325). The Pollution Contamination Index (PCI) values of Obunagha River reveal significant contamination, especially for Nickel and Lead. The ecological risk landscape is complex, with moderate levels of zinc, manganese, and Cadmium, and varying degrees of contamination for Chromium, Nickel, Lead, and Copper. Microbial activity assessment in water samples indicated potential health risks associated with Vibrio (2.25×10^2 cfu/mL), E. coli (1×10^1 cfu/mL), Salmonella (3.825×10^2 cfu/mL), and Shigella (6×10^1 cfu/mL). This study becomes evident that the samples collected exhibit significantly higher bacterial concentrations, particularly for Salmonella and Shigella, which indicates a higher potential risk to both humans, animals, and aquatic biota. It is therefore, recommended that further study should be carried out to determine the tidal flow, geological features and its effect on aquatic biota such as fish.

Keywords: Evaluation, Ecological Risk, Inorganic Contaminants, Microbial Activities, Bacteria

Introduction

Wastes are usually discharged into the rivers. Discharge of sewage, garbage, oil spills and industrial wastes are great threats to the diluting capacity of rivers (Sanda & Ibrahim, 2020). Hence constant assessments of the water quality conditions of receiving water bodies are very critical to effective planning and management (Sanda & Ibrahim, 2020). Heavy metals exist within the earth‘s geological structure which makes up the background levels for heavy metals. However, the concentrations of these metals may be increased through the introduction of heavy metals into the environment. There are two major ways through which heavy metals are introduced into the environment; natural sources and anthropogenic sources (Singh et al., 2018). The natural sources of heavy metals include weathering of heavy metal bearing rocks, windblown dust, volcanic eruptions, acid rain and dew (Masindi & Muedi, 2018). The major contributor to elevated levels of heavy metal concentration beyond the background levels has been anthropogenic sources which include waste from industries and municipalities, erosion of metals through oxidation and leached agricultural chemicals (Keshavarzi et al., 2021; Reis et al., 2019). Agricultural activities also produce heavy metals that can also find their way to the aquatic system through farm inputs like fertilizers, herbicides, fungicides and pesticides which form part of agricultural drainage that are washed as surface runoffs to water bodies (Buzzi & Marcovecchio, 2018). Sewage and industrial effluents are some of the high contributors of heavy metal contamination to rivers and lakes (Mokarram et al., 2020). These heavy metals once introduced into the environment eventually find their way into the aquatic ecosystem through being washed by rain water and runoffs where they are deposited in the surface water and eventually settle to the bottom sediments (Mokarram et al., 2020). The potential hazard of pollutants in any ecosystem is a factor of their concentration and their ability to last long in the system (Saaristo et al., 2018; Onyegeme-Okerenta., 2023). Microbial aspect of this assessment includes Vibrio which is a genus of bacteria characterized by its comma-shaped or slightly curved rod structure. Species within the genus include Vibrio cholerae, which causes cholera. The primary mode of transmission for Vibrio is through contaminated water and food. Infections with Vibrio cholerae can lead to cholera, a severe diarrheal disease. The effects include profuse watery diarrhea, vomiting, and dehydration. Cholera outbreaks often occur in areas with inadequate sanitation and access to clean water, emphasizing the importance of monitoring Vibrio levels in water sources (Baker-Austin et al., 2020). Escherichia coli, commonly known as E. coli, is a rod-shaped bacterium found in the intestines of humans and animals. While most strains are harmless, certain pathogenic strains, such as E. coli O157:H7, can cause foodborne illnesses. The bacteria's rod-shaped structure is typical of the Enterobacteriaceae family. Infections with pathogenic E. coli can result in symptoms ranging from mild gastrointestinal discomfort to severe cases of hemolytic uremic syndrome. Salmonella bacteria have a rod-shaped structure and are known for causing salmonellosis, a common foodborne infection. The bacteria are versatile and can adapt to various environments (Ngogo et al., 2020). Shigella bacteria are rod-shaped and cause shigellosis, a highly contagious gastrointestinal infection (Liu et al., 2018). Shigella`s structure facilitates its transmission through the fecal-oral route. Shigellosis is often associated with poor hygiene and sanitation, as the bacteria can spread easily in crowded or unsanitary conditions.

Materials and Methods

The study was carried out in Obunagha river in Gbarain Clan, Bayelsa State, South-South geopolitical zone of Nigeria. Obunagha is situated in-between Okolobiri and Korama community. The community is located at Latitude 5º 1’ 56’’ N and Longitude 6º 1’ 37’’ E. The Obunagha River flows through Okolobiri down to Korama. The major occupation of the people is fishing and farming. Other occupations include palm oil milling, local gin making, trading and carving. Surface water samples were collected at each selected sampling station using appropriate sampling techniques. Multiple replicate samples were collected along the river at each sampling station to account for variability. Sample location, date of collection, time, and other analytical data for each sample were recorded to maintain data integrity. 3 ml of 1:1 nitric acid (HNO_3) was added to make up 1L water sample to reduce the pH to <2 to stop or slow down the chemical, physical and biological changes in the sample and preserve the metals in the sample. The heavy metal concentration in the different water samples were determined using Atomic Absorption Spectrophotometer with a multi-element hollow cathode lamp system. In this study, pollution load index, heavy metal potential ecological risk coefficient, geo-accumulation index, pollution contamination index, and Quantitative microbial analysis were used to assess the metal pollution levels in the water samples. Reference samples of the studied metals which were used as background values were taken from different locations where no activities are taking place. The contamination factor is an expression of the level of metal contamination in the surface water. It is the quotient attained by dividing the concentration of each metal in water samples by the reference value. It is given by the formula (Mirzaei et al., 2020).

$$CF = \frac{C_{metal}}{C_{\text{background}}} \quad \ldots\ldots \quad (1)$$

Where C_{metal} is the concentration of a given metal in water and $C_{\text{background}}$ is the metal concentration of a control sample.

Pollution load index for a site is evaluated using the formula;

$$PLI = (CF1 \times CF2 \times CF3 \times \ldots \ldots CFn)^{1/n} \quad \ldots\ldots\ldots \quad (2)$$

Where; n is the number of metals and CF is the contamination factor. PLI is a potent tool in heavy metal pollution evaluation. PLI value higher than 1 indicates the sample have been polluted, while the PLI value less than 1 indicates no pollution occurred (Ogoko et al., 2021; Ubuono et al., 2023).

Potential ecological risk index is an approach to evaluate the heavy metal contamination from the perspective of sedimentology (Ngah et al., 2021). It does not only consider heavy metals in the soil, but also associates ecological and environmental effects with toxicology and evaluates pollution using comparable and equivalent property index grading method. The potential ecological risk is related to individual pollution coefficient, heavy metal toxicity response coefficient, and its formula is as follows:

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$$RI = \sum E_r^i = T_r^i \times C_f^i \qquad \text{……….} \qquad (3)$$

$$C_f^i = \frac{C_{Surface}}{C_n^i} \qquad \text{…………} \qquad (4)$$

Where E^r is potential ecological risk of individual coefficient, $T_r{}^I$ is toxicity response coefficient of a certain kind of metal toxicity using standard heavy metal toxicity coefficient development as reference, in accordance with the normalized toxic response factor of 30, 5, 5,5, 2 and 1 respectively for Cd, Cu,Pb, Ni, Cr, and Zn. C_f^i is the accumulating coefficient of element I and RI is the potential ecological risk index (Panghal et al., 2021).

The geo- accumulation index (I_{geo}) was utilized to evaluate the degree of element pollution in the samples by balancing the present with original concentrations; however, it is hard to find original concentrations. The I_{geo} values of a sample can be evaluated with the following equation:

$$Igeo = log_2 \left[{C_i}/{(1.5Bi)} \right] \qquad \text{………………..} \qquad (5)$$

Where C_i is the current elements concentration in the samples and B_i is the geochemical reference value as defined. The modified coefficient, constant 1.5 was utilized to characterize the effect of accumulation and geological characteristic and determine the consequences of human activities. I_{geo} can be separated into seven classes (Nour et al., 2019).

The potential contamination index (Cp) can be evaluated by the equation.

$$C_p = \frac{M_{Sample}}{M_{reference}} \qquad \text{…………} \qquad (6)$$

Where $M_{sample\ max}$ is the maximum concentration of an element in the sample, and $M_{reference}$ is the value of same element in a reference sample. Cp ≤ 1 indicates low pollution; 1< Cp ≤ 3 is moderate pollution; and Cp > 3 is severe or very severe pollution (Kumar *et al.,* 2019).

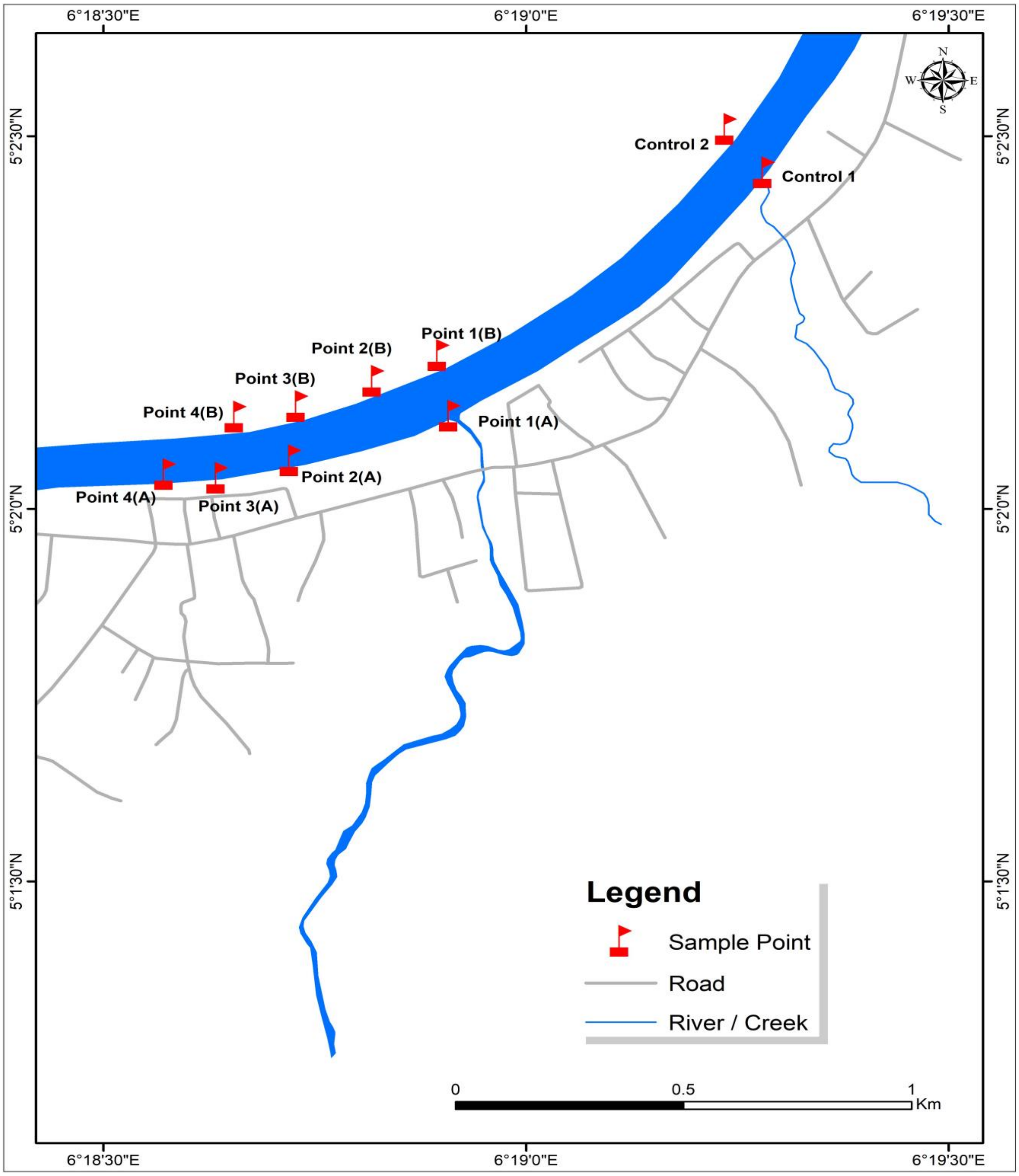

Fig. 1.0: Map Showing Sampling Points (Stations)

Microbial Analysis

Salmonella/Shigella agar (SSA) medium was used for the analysis of Salmonella and Shigella microbial activity (Liu et al., 2018). The medium was prepared according to manufacturer's direction, by weighing and dissolving 63g/L using a conical flask, sterilized by boiling at 100^{0}C, allowed to cool to about 45^{0}C, poured into sterile petri dishes and allowed to solidify. Exactly 0.1mL aliquot of samples were aseptically inoculated using the spread plate technique.

The inoculated plates were incubated at 37^0C for 24hr. Plates were observed for colony development. The colonies with black pigment were counted as Salmonella to obtain colony forming unit per mL (cfu/mL) while colonies with red pigment were counted as Shigelle.

Eosine Methylene Blue (EMB) medium was used for the analysis of E. coli. The medium was prepared according to manufacturer's direction, by weighing and dissolving 36g/L using a conical flask, sterilized by autoclaving at 121^0C for 15 minutes, allowed to cool to about 45^0C, poured into sterile petri dishes and allowed to solidify. Exactly 0.1mL aliquot of samples were aseptically inoculated using the spread plate technique. The inoculated plates were incubated at 37^0C for 24hr. Plates were observed for colony development. The colonies were counted to obtain colony forming unit per mL (cfu/mL) of samples.

Thiosulphate Citrate Bile Sucrose Salt (TCBS) medium was used for the analysis of Vibrio. The medium was prepared according to manufacturer's direction, by weighing and dissolving 89g/L using a conical flask, sterilized by autoclaving at 121^0C for 15 minutes, allowed to cool to about 45^0C, poured into sterile petri dishes and allowed to solidify. Exactly 0.1mL aliquot of samples were aseptically inoculated using the spread plate technique. The inoculated plates were incubated at 37^0C for 24hr. Plates were observed for colony development. The colonies with blue and yellow pigments were counted to obtain colony forming unit per mL (cfu/mL) of samples.

Results

The results of the background concentration of heavy metals from station 1 showed mean concentrations ranged from 0.008±0.00 (Zn) to 1.182±0.009 (Cd) mg/l, respectively as shown in Table 1 below.

Table 1: Mean, Standard Deviation and Range Values of Background Concentration of Heavy Metals from water Samples

Station	**Mg/l**	Zn	Mn	Cd	Cr	Ni	Pb	Cu
Station 1	**Mean**	0.008	0.373	1.182	0.0175	0.025	0.092	0.008
	Stdev (±)	0.00	0.186	0.009	0.002	0.003	0.002	0.00
	Range	0.007-0.009	0.143-0.230	1.004-1.364	0.012-0.023	0.019-0.030	0.087-0.096	0.007-0.008

The contamination factor of the water samples are as shown in Fig. 2 below. Mean contamination factor ranged between 0.109±0.07 (Mn) to 490.15±174.69 (Cr).

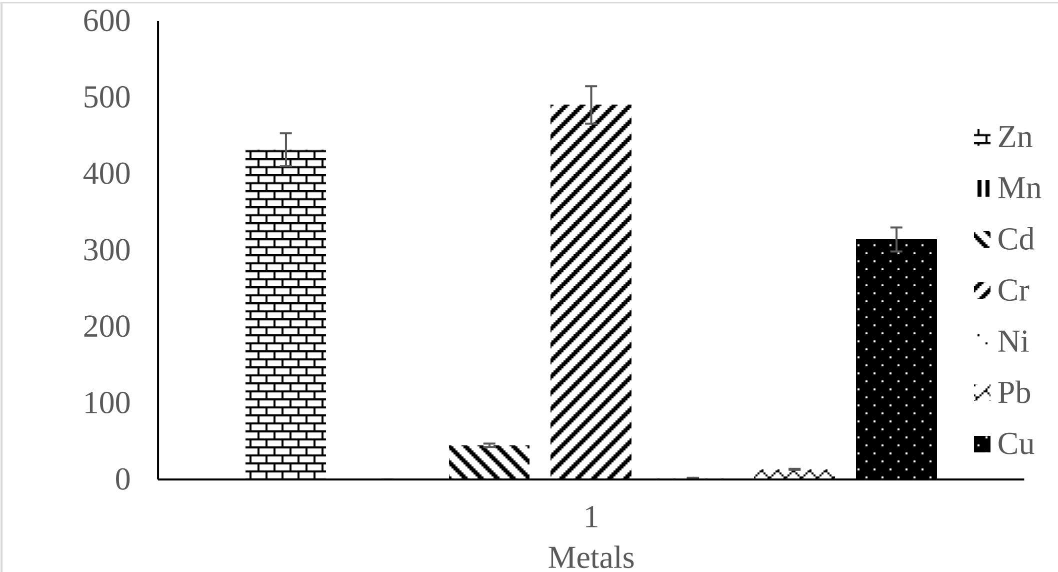

Fig. 2.0: Mean and Standard Deviation of Contamination Factor for the Water Samples

The pollution load index of metals in the water samples is as shown in the bar chart below. Pollution Load Index ranged from 0.109 (Mn) to 431.53 (Zn). See Fig. 3 below for the distribution.

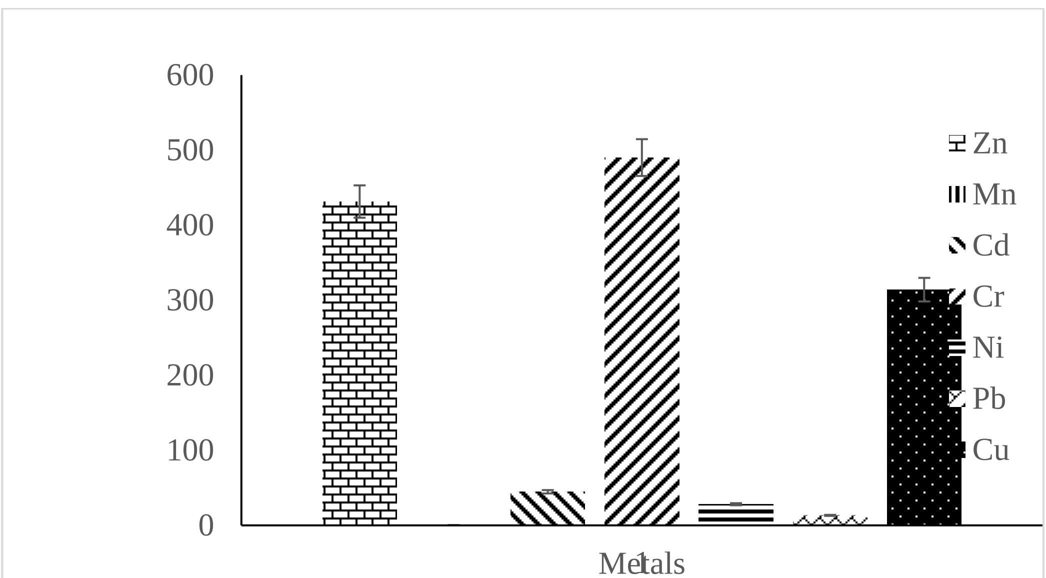

Fig. 3.0: Mean and Standard Deviation of Pollution Load Index (PLI) for the Water Samples.

Mean heavy metal Ecological Risk Coefficient and Potential Ecological Index ranged from 0.100±0.11 (Mn) to 2742.42±40.86 (Zn), while Cadmium was recorded as 147.83 (Fig.4).

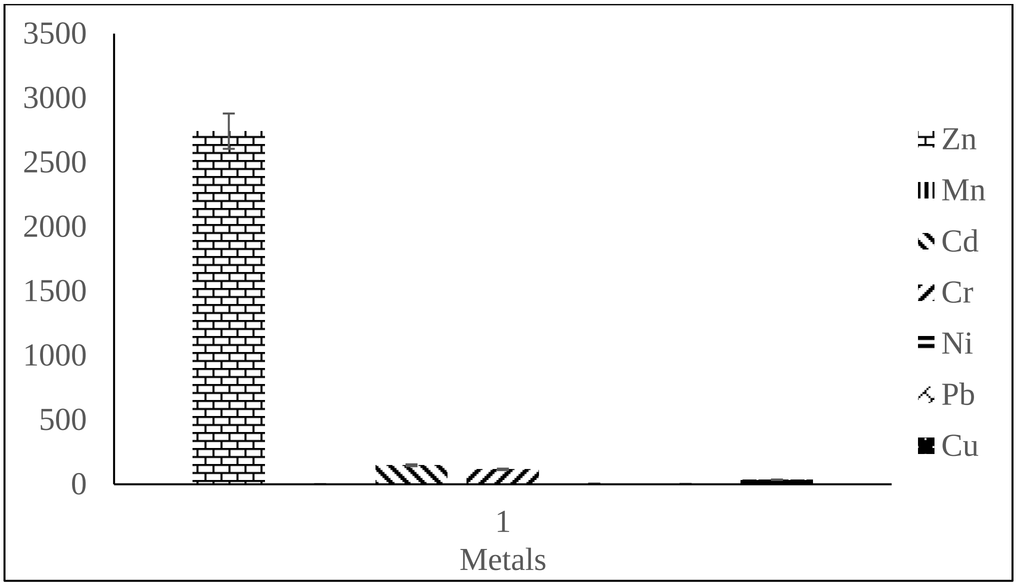

Fig. 4: Mean and Standard Deviation of Heavy Metal Potential Ecological Risk Coefficient and Potential Ecological Index in the Water Samples

The Geo-Accumulation Index of the composition of water samples can be better understood by analyzing the mean concentrations of metals present in them calculated using **Eqn. 5**. Zinc is found to be present in substantial amounts, with a mean concentration of 4.2 mg/L. Mean values of Geo-accumulation Index varies as follows: Zn (4.2), Mn (-2.35), Cd (0.83), Cr (1.99), Ni (0.14), Pb (0.45), and Cu (2.97), respectively.

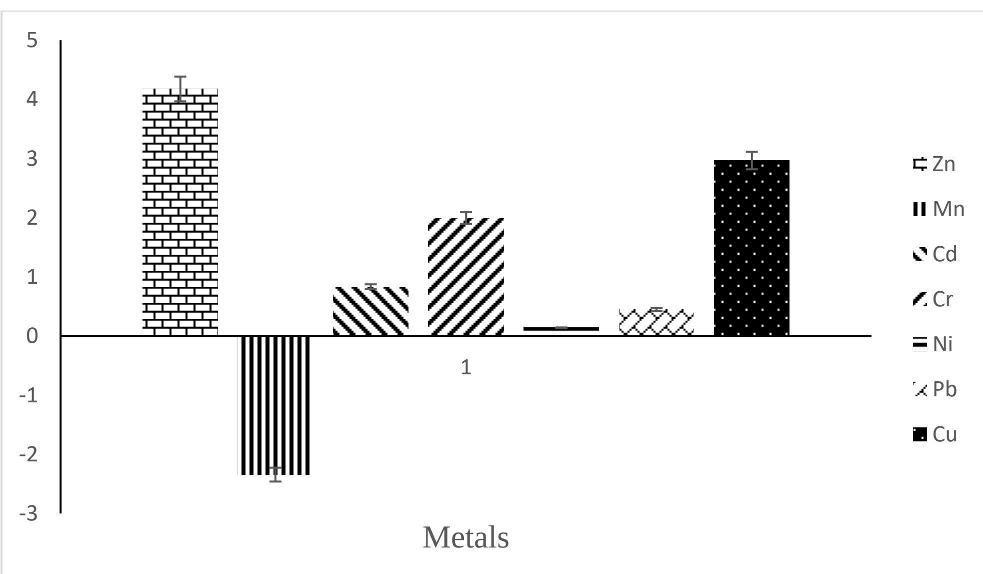

Fig.5: Mean and Standard Deviation of Geo-Accumulation Index for the Water Samples

Mean Pollution Contamination Index ranged from low to very high, with Zn (0.673) being the lowest and Cd (1256.17) recorded as very high. However, Ni and Cu were comparatively recorded as moderate.

Table 2.0: Pollution Contamination Index (PCI) for the Water Samples

Metals	CPI	Pollution Levels
Zn	0.673	Low
Mn	1.670	Low
Cd	1256.167	Very high
Cr	0.759	Low
Ni	3.210	Moderate
Pb	14.276	High
Cu	3.451	Moderate

The mean bacterial concentrations in all the samples are shown in (**Fig. 6**) below. The mean concentration of shigella was recorded highest as 6 x 10^1 cfu/ml followed by salmonella (3.83 x 10^2 cfu/ml), while E. coli and Vibrio have 1.0 x 10^1 and 2.25 x10 cfu/ml, respectively.

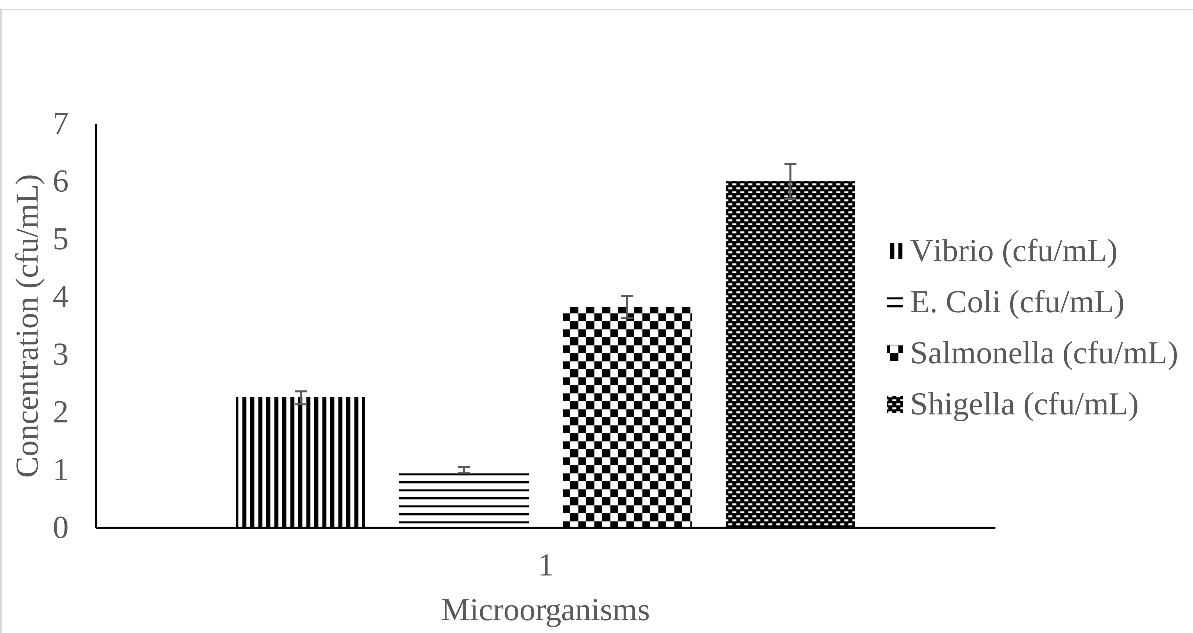

Fig. 6: Mean values of Microbial Activity for the Water Samples

Discussion

Background Concentration of Heavy Metals in Obunagha River (Table 1) shows the concentration of various heavy metals (Zn, Mn, Cd, Cr, Ni, Pb, Cu) in water samples collected from Station 1 in the Obounagha River. The mean concentrations serve as central measures, indicating the average levels of each metal. Zinc displayed a mean concentration of 0.008 mg/L, suggesting a relatively low average presence of this metal which is also below the acceptable limit of WHO, (2022) whose concentration is 5.00 mg/L. Manganese shows a higher mean concentration at 0.373 mg/L, indicating a more substantial average level higher than the acceptable limits of the WHO (2022) of 0.05 mg/L. Cadmium exhibits an average concentration of 1.182 mg/L, which is notably higher compared to the other metals and also the WHO standard of drinking water quality guidelines of 0.01 mg/L. Chromium and Nickel have mean concentrations of 0.0175 mg/L lower than WHO around the respective means.

The low values of chromium and nickel suggests that the concentrations of these metals in the water samples collected from Station 1 in the Obounagha River are relatively consistent. However, for Mn and Cd, the standard deviations are higher, indicating a greater degree of variability in their concentrations. This implies that the concentrations of manganese and cadmium in the water samples can vary more widely compared to the other metals. The range values represent the interval between the highest and lowest concentrations observed for each metal. Notably, for Cd, the range is relatively large (1.004-1.364) mg/L, indicating significant variability in its concentrations. This means that the concentration of cadmium in the water samples collected from Station 1 in the Obunagha River can vary greatly, with some samples having much higher concentrations than others. Conversely, Zn and Cu exhibit very narrow ranges (0.007-0.009 mg/L and 0.007-0.008 mg/L, respectively), suggesting a more consistent presence. This indicates that the concentrations of zinc and copper in the water samples are relatively stable, with little variation between the highest and lowest concentrations observed (Fig.1).

The contamination load analysis of Zinc has a relatively high mean concentration of 431.525 mg/L, indicating a potential source of contamination that requires attention. Manganese, on the other hand, has a very low mean concentration of 0.109mg/L, indicating that it is within acceptable limits and does not pose a significant contamination risk. Cadmium has an elevated mean concentration of 44.787 mg/L, indicating possible contamination and highlighting the need for further investigation and potential remediation measures. Chromium has a remarkably high mean concentration of 490.15 mg/L, indicating a significant source of contamination that requires immediate attention. Both nickel and copper have moderately high mean concentrations of 28.175 mg/L and 314.109 mg/L, respectively, indicating potential concerns for both human health and the environment. Meanwhile, lead has a relatively low mean concentration of 13.345 mg/L, indicating compliance with acceptable limits and a lower risk of contamination (Fig. 2).

The contamination factor of metals in the water samples play a crucial role in understanding the extent of heavy metal contamination (Fig. 3.0**).** Mean concentration values were calculated using Eqn. 2. The mean concentration of Zinc is relatively high at 431.525 mg/L, indicating a significant presence of this metal in the water samples. Similarly, Cadmium exhibits an elevated mean concentration of 44.787 mg/L, highlighting its notable contribution to the overall metal load. Chromium displays an exceptionally high mean concentration of 490.15 mg/L, suggesting a substantial contamination source. Nickel and Copper also demonstrate moderately high mean concentrations of 28.175 mg/L and 314.1092 mg/L, respectively, emphasizing their considerable contributions to the pollution load. On the other hand, Manganese and Lead exhibit much lower mean concentrations of 0.109 and 13.345 mg/L, respectively, indicating relatively lower contamination levels.

The Pollution Load Index (PLI) takes into account the mean concentrations of these metals, providing a comprehensive assessment of overall pollution levels. With Zinc, Cadmium, Chromium, Nickel, and Copper contributing significantly to the PLI, the index suggests higher pollution loads in the water samples. In contrast, the lower contributions of Manganese and Lead to the PLI suggest that their concentrations are within acceptable limits, contributing less to the overall pollution load.

Heavy Metal Potential Ecological Risk Coefficient and Potential Ecological Index in the water samples indicates that the concentration of metals in water samples provide crucial information regarding potential contamination levels. Elevated levels of Zinc and Cadmium indicate a significant presence of these metals, raising concerns about potential environmental and health risks. Cadmium, in particular, is a highly toxic metal that can accumulate in organisms and cause severe harm to both aquatic life and humans if ingested. On the other hand, Manganese and Lead exhibit lower mean concentrations of 0.1003 mg/L and 2.826 mg/L, respectively. Although their levels are comparatively lower, it is crucial to continue monitoring to ensure they remain within acceptable limits. Chromium and Nickel fall into an intermediate range, indicating a moderate presence (Fig. 4**).** All these metals may have adverse effects on the environment and on human health, necessitating careful management. Copper also presents a moderately elevated mean concentration, highlighting the importance of maintaining a balance to prevent potential toxicity.

Although Zinc is an essential nutrient for many organisms, elevated levels of Zinc and Cadmium can have adverse effects on aquatic life and human health if the water source is used for consumption or irrigation. On the other hand, the negative mean concentration of Manganese at -2.3435 mg/L, suggests that the concentrations of manganese in the water samples are lower than the background levels (Fig. 5**).** Low Mn concentration could be due to natural variations or dilution effects in the environment, which may be influenced by geological factors or human activities. Cadmium is detected in the water samples, with a mean concentration of 0.831 mg/L, which is a highly toxic metal. Even at relatively low levels, its presence could potentially pose risks to aquatic life and human health. Chromium is also present in the samples, with a mean concentration of 1.992 mg/L. Depending on the specific form of chromium, elevated levels can have adverse effects on both the environment and human health. Nickel exhibits a mean concentration of 0.137 mg/L which is not as elevated as some other metals, but continued monitoring is important as nickel can have adverse effects on both human health and the environment. Lead is present in the water samples, with a mean concentration of 0.446 mg/L, which is relatively lower in concentration. However, lead is a toxic metal, and its presence above background levels warrants attention. Copper is moderately elevated, with a mean concentration of 2.965 mg/L. While copper is an essential nutrient for many organisms, excessive levels can be toxic, underscoring the importance of maintaining an appropriate balance.

Table 2.0 presents an essential evaluation of the Pollution Contamination Index (PCI) for different metals in the water samples. The data generated from **equ. 6** highlights varying levels of contamination, with Zinc, Manganese, and Chromium exhibiting low CPI values, indicating that their concentrations are within acceptable limits and pose a lower risk of contamination. Nickel and Copper display moderate CPI values, suggesting slightly elevated concentrations that require attention and potential management strategies to prevent any adverse environmental impacts. Lead stands out with a high CPI value, indicating a significant concentration of lead in the water samples, raising concerns about potential environmental and health risks associated with lead contamination. Urgent action may be required to address this issue and mitigate potential harm. However, the most alarming finding is the extremely high CPI value for Cadmium, indicating a severe concentration of this metal. This level of contamination poses a significant risk to the environment and demands immediate attention and comprehensive remediation efforts. The results from this Table shows critical importance of continuous monitoring and effective management of metal concentrations in water samples.

The mean concentration of Vibrio, at approximately 2.25×10^2 Cfu/mL, suggests its moderate presence. Vibrio species can pose a health risk to humans, particularly those with weakened immune systems, potentially leading to gastrointestinal infections. In animals and aquatic organisms, exposure to Vibrio can lead to infections, affecting their overall health and potentially disrupting the ecosystem (Olalemi et al., 2020). Low mean concentration of E. coli at 1×10^{-1} Cfu/mL indicates a lower risk. While certain strains of E. coli can cause illnesses in humans through fecal-oral transmission, at this concentration, the risk to both humans and animals is relatively low. In comparison, the control samples for E. Coli indicate a similar low presence, suggesting that the environment from which the samples were collected may not pose an elevated risk (in agreement with Soleimani et al., 2021). The most concerning finding is the high mean concentration of Salmonella at approximately 3.825×10^2 Cfu/mL. Salmonella is a highly pathogenic bacterium known to cause severe foodborne illnesses in humans (Samuel et al., 2019). Ingesting contaminated food or water can lead to symptoms ranging from gastroenteritis to more severe cases of salmonellosis. For animals and aquatic organisms, Salmonella infection can result in signif icant economic losses to the agricultural industry and potential disruptions in the ecosystem. Similarly, the presence of Shigella at an average concentration of 6×10^1 Cfu/mL is alarming. Shigella is highly contagious and can cause severe gastrointestinal infections in humans (Nisa et al., 2020), with symptoms including diarrhea, fever, and abdominal cramps. Exposure to Shigella can also impact animals and aquatic organisms, potentially affecting their health and survival. Comparing these results to the control samples, it was found that there was a significantly higher bacterial concentrations, particularly for Salmonella and

Shigella which implies a higher potential risk to both humans and animals in the sampled environment. It is crucial to address and rectify the sources of contamination to prevent further spread of these pathogens. Implementing strict sanitation and food safety measures is imperative, especially in environments where microbial contamination can pose a substantial risk.

Conclusion

Heavy metals such as Cadmium, Chromium, Nickel, Lead, and Copper were identified as contaminants, emphasizing potential ecological risks and the importance of targeted intervention strategies. Zinc, Manganese, and Cadmium show moderate contamination levels, while Chromium, Nickel, Lead, and Copper display varying degrees of contamination with associated ecological risks. Nickel, Copper, and Manganese emerge as elements of particular concern, imposing focused attention and intervention at specific locations. The integration of diverse indices, including the Contamination Factor, Pollution Load Index (PLI), Heavy Metal Potential Ecological Risk Coefficient, Potential Ecological Index, Geo-Accumulation Index, and Pollution Contamination Index, gives a detailed understanding of pollution levels and associated risks. In the microbial assessment, it becomes evident that the samples collected exhibit significantly higher bacterial concentrations, particularly for Salmonella and Shigella. This indicates a higher potential risk to both humans and animals in the sampled environment. Moreso, regular monitoring and testing for microbial contaminants are essential for promptly identifying and mitigating potential health risks associated with these bacteria for humans, animals, and aquatic organisms alike.

Recommendations

The following recommendations were made in the course of the study. Further study should be carried out:

1. To conduct a detailed investigation into how anthropogenic activities, including agriculture and urban development, influence environmental factors such as tides, water flow, and geological features.
2. To assess the bio-accumulation of heavy metals in aquatic biota, in fish and invertebrates as well as plants.

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