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Potential Application of Agriculture by-Products in Heavy Metals Bioremediation

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ABSTRACT

Possible inexpensive adsorbent sources are urgently required to remove metals and pollutants from aquaculture effluents. Thus, this study evaluated the intensive bacterial production and metal removal (Mn, Co, Ni, Cu, Zn, Pb and Cd) from marine water, which will be used for the culture of aquatic animals using biofloc technology (BFT). In the presence of sugarcane bagasse (T1) and rice-bran (T2) as organic carbon sources, marine polluted water was treated for 6 weeks. As expected, the bacterial numbers increased throughout the experimental period, which helped in breaking down the carbon source and metal removal. Overall, the percentages of heavy metal removal from water samples with different biofloc, and the sequence of adsorption of heavy metals from polluted marine water were Pb > Cu > Ni > Cd > Mn > Co > Zn for T1. Meanwhile, a slight difference was detected in the sequences of T2 as: Pb > Ni > Cu > Cd > Mn > Zn > Co. Furthermore, the relationships among different carbon sources, heavy metals and microbes were also analyzed using the principal component analysis (PCA) and correlation matrix. However, the bacterial and metal profiles have variability by 97.20% at the end of the experiment. The results of this study indicate that the heavy metals concentrations are affected by the bacteria and carbon sources directly via changes in their concentration which provides the opportunity to mitigate the impact of polluted water on the environment so far and provide the opportunity to use water free of heavy metals in aquaculture practices.

INTRODUCTION,

Water pollutions have become a significant global problem since water is an essential natural resource for human life. Furthermore, water is an important resource for developing economics and society in terms of agriculture, industry and various facilities. Heavy metals such as Mn, Co, Ni, Cu, Zn, Pb and Cd are environmental priority pollutants, forming one of the most serious environmental problems **(Ashruta *et al.*, 2014)**.

Metals can be carried away from their sources by wind, depending on their form (gaseous form or as particulates). Several heavy metals have become a significant matter of concern due to their toxicity and tendency to accumulate in food chains. Fishes, molluscs, and other aquatic life located at the end of the food chain may accumulate metals and pass them to humans through food causing chronic or acute diseases **(Zhang, 2014)**.

Biosorption is a promising technique for the removal of heavy metals from aqueous environments, especially when adsorbents are derived from lignocellulosic materials. Sugarcane bagasse (SB) and rice-bran (RB) are agricultural waste substances, structurally, consisting of cellulose, hemicellulose and lignin **(Coelho *et al.*, 2007)**.

Agricultural by-products can be used as a bio-sorbent for heavy metals from the water to purify it to the greatest possible degree. Sugarcane bagasse (SB) is one of the major residues obtained from agriculture; every year millions of tons of SB have been produced by sugarcane agribusiness. I addition, rice brane (RB) is a by-product of the rice milling industry, and the amount of RB available is far in excess of any local uses, thus frequently causing disposal problems (**Rahmani *et al*., 2019**). Both SB and RB were chosen as sorbent materials for metal ions removal due to their granular structure, insolubility in water, chemical stability and local availability in addition to the price.

In aquaculture, the use of microbes to improve water quality has been known as probiotics. Probiotics are expected to have beneficial effects through the production of inhibitory compounds, competition for chemicals or available energy, competition for adhesion sites, enhancement of the immune response, and improvement of water quality. The use of various types of bacterial strains has been investigated, and scientists reported that such an activity could eliminate some metal elements in a bioreactor **(Wu *et al*., 2011).**

We hypothesized that biofloc itself can adsorb metals from water. Therefore, the present study aimed to verify the potential use of biofloc technology (BFT) produced by SB and RB as a cheap source of carbon to reduce the concentrations of some heavy metals or not in the polluted seawater, which can be used for different practices such as mariculture, especially in coastal areas.

MATERIALS AND METHODS

1. Experimental facilities and design

Two treatments were conducted using flow-through shrimp culture effluents with two BFT systems (SB and RB) as bio-sorbent for water treatment. However, Agriculture by-products (SB and RB) were used as organic carbon sources to improve the growth of total bacteria (TB). In BFT treatments, the carbon and nitrogen concentration (C: N ratio) were calculated to be 16:1 using the addition of SB and RB (for more information about the system see **Sharawy *et al.*, 2022**). All tanks were aerated and mixed continuously using an air blower of 5 hp to maintain the growth of bacteria and did not permit sedimentation in the tank bottom. BFT treatment systems were maintained for 6 weeks without any water exchange (zero water exchange), except to compensate for the evaporation losses and to maintain a salinity of 32 ppt.

2. Water sampling collection

Water samples were collected weekly for 6 weeks from all treatments in sterilized autoclave bottles, cooled in an ice bag and transferred to the microbiology laboratory, National Institute of Oceanography and Fisheries (NIOF), Suez Branch for further water (heavy metal determination) and microbial analysis.

3. Microbial analysis

Levels of viable Total bacteria (TB) were determined by counting the colonies that grew on Trypticase soy agar plates supplemented with (50: 50) marine water: distilled water **(Kelany *et al.,* 2019 and Asaduzzaman *et al.,* 2008)**. The technique of bacterial count was performed using the serial dilution technique according to **Schneider *et al.* (2006)**. TB of water samples were determined every 7 days of the initial and 6 weeks by collecting 30 ml water samples in a sterile polypropylene bottle and subsequently folded to 10-fold serial dilution. Then, 1 ml of appropriate dilutions was applied in triplicates over the plates of Trypticase soy agar containing 1.0 % w/v NaCl for TB counts **(Thomsen, 2005)**. Each colony in the incubated plates (at 37 ^{o}C for 24 h) was then counted in the range of 30 – 300 cfu (colony-forming unit) **(Ali *et al.,* 2021)**.

4. Heavy metals analysis

Water samples were filtered through FG/C filter paper (0.45 Micron). The filtrate was treated with Ammonium Pyrrolidine Dithiocarbamate (APDC) to complex the heavy metals after well shacked. However, methyl isobutyl ketone (MIBK) was used for extraction according to **APHA, 2005**. Furthermore, concentrations of metals (Cu, Zn, Pb, Cd, Ni, Co and Mn) were measured against external calibration standards using PerkinElmer A Analyst 100 Atomic Absorption Spectrometer. The removal efficiency is calculated as the following formula:

$$\% \text{ Removal} = (Ci\text{- } Ce/ Ci) \times 100$$

Where; Ci and Ce are the initial and final heavy metals concentration (µg L^{-1}).

Deionized distilled water was used to prepare all aqueous solutions. Samples were measured against acid blank. The accuracy and precision of the method were verified by analysis of certified sediments reference materials (SD-M-2/IM) provided by the International Atomic Energy Agency (IAEA), Vienna. Analytical results of the quality control samples indicated a satisfactory performance of heavy metals determination within the range of certified values with 90.4-97.5% recovery for metals studied.

5. Statistical analysis

Significant analysis, principal component analysis (PCA) and correlation matrix were determined using PRIMER V7 to find the association between the bacterial foundation and selected heavy metal in different trials as a variable at a set of 1000 permutations and a 5% significance level **(Vidal *et al.,* 2020)**. Heavy metal concentration was the first variable (F1) and the bacterial count was the second variable (F2) while the trials (C, T1 and T2) were the host variables.

RESULTS

1. Bacterial analysis

Fig. (1) shows the total bacterial (TB) count during the experimental time (weekly); TB in SB treatment (T1) was gradually increased by increasing the time of the experiment until week 4 (W4) compared with the control (C) by 3300±126, 9546±271, 15441±190, 27150±125 and 57873±130 cfu/ml for C, W1, W2, W3 and W4 respectively, while the abundance decreased after that to 47650±314 and 46574±0238 cfu/ml for W5 and W6 by the end of the experiment.

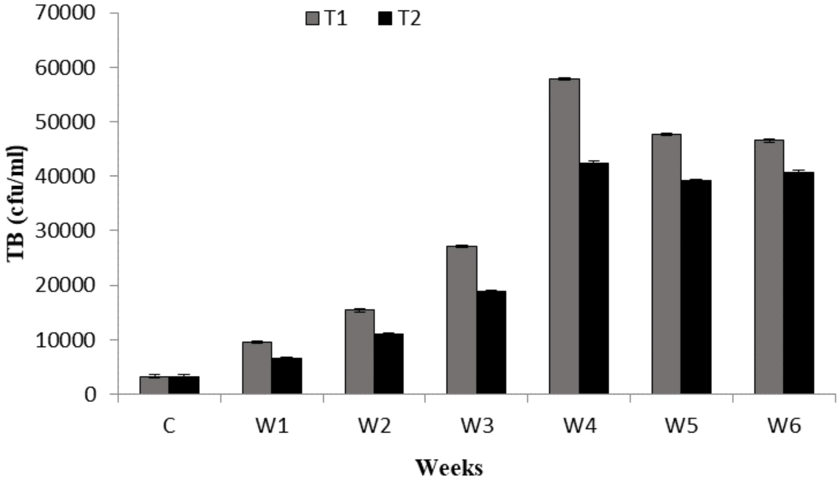

Fig. 1. Total bacterial (TB) count during the experiment. C: Control treatment (polluted seawater); T1: SB treatment and T2: RB treatment, as sources of carbon.

In the same manner, the bacterial abundance of RB treatment (T2) was less than other treatment bacteria, but T1 gradually increased to reach the highest number on the 30th day reaching 42483±147 cfu/ml. The abundance decreased to 39166±252 and 40780±274 cfu/ml for W5 and W6, respectively at the end of the experiment (Table 1).

Table 1. Effects of biofloc on bacterial growth in the water supplemented with different carbon sources

Weeks	Total bacteria (cfu/ml)	
	T1	**T2**
C	3300±126.23	3300±126.23
W1	9547±271.67^{e}	6592±256.7^{d}
W2	15442±190.94^{d}	11000±450^{c}
W3	27150±125^{c}	18867±202.75^{b}
W4	57873±130.41^{a}	42483±147.522^{a}
W5	47650±314.58^{b}	39167±252.75^{a}
W6	46574±0238.72^{b}	40781±274.55^{a}

***C:** Control (only polluted seawater), **T1:** seawater with sugarcane bagasse (SB) treatment and **T2:** seawater with rice-bran (RB) treatment; *Mean ± SD followed by different letters in the same row are significantly different (p < .05).*

2. Heavy metals analysis

Table (2) shows the mean concentrations of metals for polluted seawater without any treatment (C), SB treatment (T1) and RB treatment (T2). It was found that the mean value of Cu for C was (0.85μg L^{-1}) while these values were from the beginning 1st week (W1) (0.69 and 0.73μg L^{-1}) to the end of the study at the 6th week (W6) (0.16 and 0.28μg L^{-1}) but at W5 of the experiment these concentrations were (0.13 and 0.20μg L^{-1}) for T1, and T2, respectively. It means that the recovery percentage of Cu from water samples at W5 (84.42 % and 75.93 %) was higher than that of W6 (81.19 % and 66.75 %) for T1 and T2 respectively (Table 3). Zn concentration for C was (0.53μg L^{-1}) while, for T1 and T2, concentrations were (0.28 and 0.18μg L^{-1}), and (0.45 and 0.24μg L^{-1}) from the start (W1) to the end of the experiment (W6), respectively, while at W5 Zn Concentrations were (0.14 and 0.16μg L^{-1}) for T1 and T2 respectively (Table 2).

Table 2. Metals concentrations (μg L^{-1}) in polluted seawater (C), SB treatment (T1) and RB treatment (T2).

	Metals Weeks	Cu	Zn	Pb	Cd	Ni	Co	Mn
C	**W1**	0.850	0.529	0.403	0.231	0.107	0.028	0.077
T1		0.686	0.276	0.343	0.149	0.082	0.023	0.056
T2		0.735	0.448	0.343	0.195	0.091	0.021	0.063
T1	**W2**	0.563	0.334	0.240	0.119	0.059	0.019	0.033
T2		0.609	0.405	0.300	0.018	0.060	0.015	0.046
T1	**W3**	0.248	0.291	0.204	0.072	0.036	0.013	0.036
T2		0.404	0.381	0.260	0.165	0.050	0.013	0.054
T1	**W4**	0.148	0.217	0.071	0.048	0.029	0.010	0.023
T2		0.306	0.318	0.180	0.069	0.033	0.011	0.030
T1	**W5**	0.133	0.139	0.027	0.040	0.015	0.005	0.015
T2		0.205	0.156	0.049	0.052	0.022	0.009	0.024
T1	**W6**	0.159	0.181	0.060	0.042	0.021	0.006	0.017
T2		0.283	0.236	0.090	0.064	0.029	0.010	0.029

C: control (polluted seawater), **T1:** polluted seawater with SB treatment; **T2:** polluted seawater with RB treatment and W: weeks.

Table (3) shows that the percentage of treatment of Pb using carbon source in T1 was 93.43 % while that of T2 was 87.88 % at W5 and this was declared from the decreasing of Pb concentration from 0.40µg L^{-1} (C) to 0.03 and 0.05 µg L^{-1} for T1 and T2, respectively, while at the end of experiment (W6) concentrations of Pb were higher than that of it at W5 (0.06 and 0.09µg L^{-1}) with recovery Percentage (85.11 % and 77.67 %) for T1 and T2 respectively. Table (2) showed that the concentration of Cd for the control sample (C) was 0.23µg L^{-1} while for T1 values of Cd from W1 to W6 were 0.15 and 0.04µg L^{-1} with 36 % at week 1 to 82.06 % treatment percentage at the end of the study (W6), but in T2 the percent of treatment at the end of the study was 72.37% (Table 3). The recovery percentages of Ni, Co and Mn at the end of the experiment (W6) were found to be (80.67 %, 75.63 % and 78.43% and 72.55 %, 64.16 % and 61.70 %), while at W5 there were found to be (86.18 %, 81.72 % and 81.05 % and 79.18 %, 65.59 % and 68.24 %) for T1 and T2 respectively. Table (3) also shows the percentage of heavy metal removal from water samples with different Biofloc and the sequence of adsorption of heavy metals in the water of aquaculture ponds as follows: Pb > Cu > Ni > Cd > Mn > Co > Zn for T1 (SB treatment). While for T2 (RB treatment) the sequence was found as follow: Pb > Ni > Cu > Cd > Mn > Zn > Co. As a result, lead (Pb) is more adsorbed than other metals; whereas Co and Zn showed the lowest adsorption. These results indicated that Biofloc is more effective in adsorbing Pb than other metals. In general, it was found that the recovery percentage of metals at W5 was higher than that of the end of the experiment W6 and T1 has a percentage higher than that of T2 for all metals.

Table 3. Removal (%) of metal ions using bacteria in presence of SB (T1) and RB (T2).

	Removal (%)			
	T1		T2	
Metal Ion	**W 5**	**W6**	**W5**	**W6**
Cu	84.42	81.19	75.93	66.75
Zn	73.63	65.77	70.47	55.49
Pb	93.43	85.11	87.88	77.67
Cd	82.66	82.06	77.60	72.37
Ni	86.18	80.67	79.18	72.55
Co	81.72	75.63	65.59	64.16
Mn	81.05	78.43	68.24	61.70

3. Correlation matrix between bacterial and metal variables

Correlation matrixes of SB and RB bacterial and metal variables were shown in Table (4). For T1, the Pearson correlation coefficients shown in a half matrix are the results of statistical analyses for possible relationships between microbial counts and the studied heavy metals. There was a positive correlation between bacteria and Cu, Zn, Pb, Cd, Ni, Co and Mn with coefficients (r) of 0.88, 0.65, 0.93, 0.81, 0.82, 0.85 and 0.77, respectively. The positive correlation between Cu and Zn, Pb, Cd, Ni, Co and Mn has coefficients (r) of 0.73, 0.93, 0.95, 0.97, 0.96 and 0.86, respectively. Also, there is a positive correlation between Zn and Pb, Cd, Ni, Co and Mn by coefficients (r) 0.73, 0.86, 0.78, 0.78 and 0.79, respectively. Lead demonstrated a positive correlation with Cd, Ni, Co and Mn by coefficients (r) 0.91, 0.94, 0.94 and 0.93, respectively. The correlations of Cd with Ni, Co and Mn have positive correlation by coefficients (r) 0.97, 0.93 and 0.94, respectively. Nickel achieved a correlation between Co and Mn by (r) 0.98 and 0.94, respectively. Finally, cobalt correlates with Mn by (r) 0.89.

On the other hand, RB (T2) Pearson correlation matrix between bacteria and Cu, Zn, Pb, Cd, Ni, Co and Mn by coefficients (r) 0.88, 0.86, 0.87, 0.97, 0.85, 0.73 and 0.87, respectively. The positive correlation between Cu and Zn, Pb, Cd, Ni, Co and Mn has coefficients (r) of 0.89, 0.86, 0.87, 0.96, 0.92 and 0.85, respectively. Also, there is a positive correlation between Zn and Pb, Cd, Ni, Co and Mn by coefficients (r) 0.98, 0.93, 0.93, 0.87 and 0.92, respectively. Lead demonstrated a positive correlation with Cd, Ni, Co and Mn by coefficients (r) 0.94, 0.88, 0.78 and 0.86, respectively. The correlation of Cd with Ni, Co and Mn has a positively coefficient (r) of 0.88, 0.77 and 0.91, respectively. Nickel achieved a correlation between Co and Mn by (r) 0.96 and 0.91, respectively. Finally, cobalt correlates with Mn by (r) 0.87. All significance variables have a significant level at n= 6 and p-vlaue ≤ 0.05.

4. Relations between bacteria and heavy metals

Principle component analysis (PCA) was investigated between bacterial abundance and metal concentration using different carbon sources. Fig. (2) shows the variability and correlation between the bacterial analysis and heavy metal using sugarcane bagasse compared to control in different weeks. The correlation of metal to bacterial species achieved a variability of 97.20 %. All variables have different percentages of variability among each other by weeks with achieving 46.74, 10.90, 2.21, 0.59, 10.53, 15.69 and 13.33 % for C, W1, W2, W3, W4, W5 and W6. Total bacteria (TB) in T1 treatment have high variability percentage of 11.76 % by square cosines 0.89.

The highest variables effect was Cd and Ni by 13 and 13.01 % with square cosines 0.98 and 0.98. About the correlation, W4, W5 and W6 were more affected by total bacteria by 10.53, 15.69 and 13.33 %, while the bacterial abundances have the most correlation value to other variables by 46.74 %.

Fig. (3) shows the variability and correlation between the bacterial analysis and heavy metal using rice-bran compared to control in different weeks. Statistical analysis demonstrated that the sugarcane bagasse correlation variables were higher in variability correlation than rice-bran variables. The correlation of metal to bacterial species achieved a variability of 85.26 %. All variables have different percentages of variability among each other by weeks with achieving 38, 14.22, 2.02, 0.05, 9.49, 19.36 and 16.84 % for control, W1, W2, W3, W4, W5 and W6, respectively. Total bacteria in T2 treatment have a variability percentage of 12.21 % by square cosines 0.93. Other variables demonstrated variability by 12.57, 12.84, 12.45, 12.63, 12.81, 11.98 and 12.51 % with square cosines 0.95, 0.97, 0.94, 0.96, 0.97, 0.91 and 0.95 for Cu, Zn, Pb, Cd, Ni, Co and Mn, respectively. The highest variables effect was Cd and Ni by 12.63 and 12.81 %. About the correlation, week 1 was more affected by variables with 14.21 %, while the bacterial abundances have the most correlation value to other variables at 38 %.

Table 4. Correlation matrix among biofloc treatments, heavy metals and microbial abundance during the whole experimental time.

Correlation matrix for SB

Variables	**TB**	**Cu**	**Zn**	**Pb**	**Cd**	**Ni**	**Co**	**Mn**
TB	**1**	**0.0016**	**0.0286**	**0.0004**	**0.0059**	**0.0053**	**0.0033**	**0.0099**
Cu	0.8835	**1**	**0.0145**	**0.0004**	**0.0002**	**< 0.0001**	**0.0001**	**0.0026**
Zn	0.6497	0.7288	**1**	**0.0141**	**0.0025**	**0.0088**	**0.0087**	**0.0072**
Pb	0.9303	0.9324	0.7318	**1**	**0.0008**	**0.0003**	**0.0002**	**0.0004**
Cd	0.8087	0.9523	0.8630	0.9139	**1**	**< 0.0001**	**0.0003**	**0.0004**
Ni	0.8161	0.9705	0.7765	0.9414	0.9759	**1**	**< 0.0001**	**0.0003**
Co	0.8469	0.9608	0.7774	0.9472	0.9391	0.9765	**1**	**0.0013**
Mn	0.7657	0.8599	0.7925	0.9326	0.9362	0.9379	0.8944	**1**

Correlation matrix for RB

bacteria	**1**	**0.0016**	**0.0025**	**0.0023**	**0.0000**	**0.0032**	**0.0140**	**0.0022**
Cu	0.8836	**1**	**0.0013**	**0.0029**	**0.0021**	**< 0.0001**	**0.0006**	**0.0034**
Zn	0.8631	0.8942	**1**	**< 0.0001**	**0.0004**	**0.0005**	**0.0022**	**0.0006**
Pb	0.8680	0.8550	0.9803	**1**	**0.0003**	**0.0017**	**0.0083**	**0.0024**
Cd	0.9712	0.8712	0.9340	0.9392	**1**	**0.0020**	**0.0095**	**0.0008**
Ni	0.8481	0.9647	0.9276	0.8824	0.8753	**1**	**< 0.0001**	**0.0008**
Co	0.7325	0.9242	0.8686	0.7812	0.7692	0.9623	**1**	**0.0021**
Mn	0.8697	0.8453	0.9207	0.8648	0.9136	0.9124	0.8705	**1**

Bold numbers = the p-value (level alpha=0.05); **None Bold numbers** = Coefficients of determination (R^2).

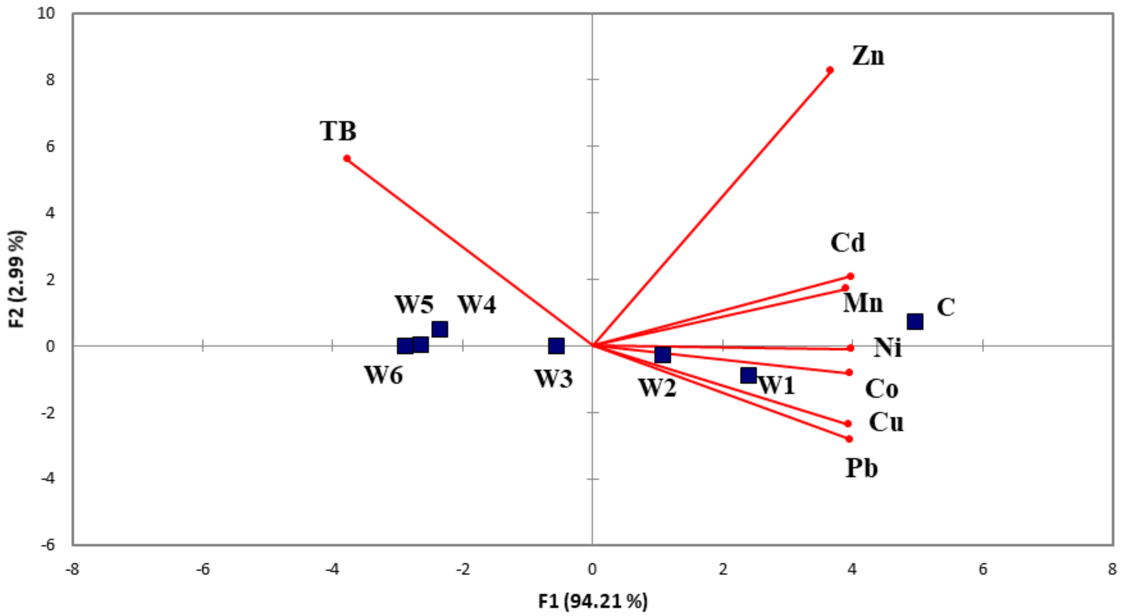

Figure 2: Principle component analysis of bacterial abundance and metal with sugarcane bagasse carbon sources during the time of the experiment (97.20 % variability).

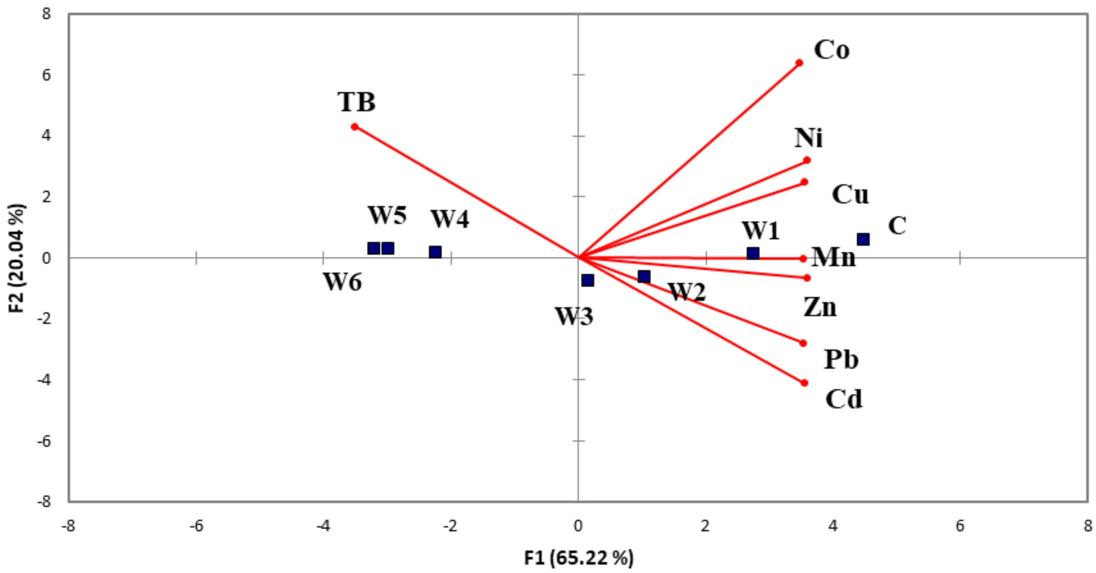

Figure 3: Principle component analysis of bacterial abundance and metal with rice-bran carbon sources during the time of the experiment (85.26 % variability)

DISCUSSION

It was noticed that, by using the carbon source, the bacterial numbers were increased than the control throughout the experiment period, which was used for breaking down the carbon source to increase the surface area on which heavy metals were adsorbed

(Sharawy *et al.*, 2020). Also, Bacteria remove metal ions by precipitation and these depend on the number of bacteria in the examined samples **(Asaduzzaman *et al.*, 2008)**. The higher TB count developed in sugarcane bagasse would have increased the metal removal ratio to other treatments **(Schneider *et al.*, 2006)**.

The use of carbon source allows the growth of large numbers of bacterial species so that the carbon source is broken into small parts that can bind with metals, this performs a phenomenon called multi-reactivation, bio-methylation, and the binding of metal ions to the surfaces of cells leads to a reduction in the concentration of heavy metals using sugarcane and rice-bran treatments **(Thomsen, 2005)**. Microbes are capable to utilize the diverse range of carbon sources originating from agricultural products **(Bothner *et al.*, 1998)**. However, the type of carbon sources seems to affect the Biofloc production rate as molasses containing sucrose, a disaccharide was more effective compared to rice flour having starch, a polysaccharide composition and functional properties of rice **(Jais *et al.*, 2017)**. This indicates that the nature of carbohydrates affects the quantum of Biofloc production with a higher level of production by application of simple sugar.

Heavy metals are dangerous because they are taken up and stored faster than they are broken down or extracted (metabolized) they tend to accumulate, and they alter the structure of bacterial cells by affecting their enzyme activity, and structure and attaching to the surface of the bacterial cell. The presence of Cu and Zn may have resulted from their release from the extensive use of anti-fouling paints by shipping activities found in the Suez Bay **(Zyoud *et al.*, 2019)**. It was found that; rice husk showed high efficiency for the sorption of the investigated.

Heavy metals tend to accumulate in food chains, this led to an increase in the concentration of metals in the C sample, while a decrease in the concentration of metals in the water was observed in the Biofloc basins, which confirms the effectiveness of these compounds in removing metals from the water, this can be explained by the presence of some types of bacteria that are resistant to different concentrations of heavy metals and can grow in the presence of heavy metals with feed on them, which led to a reduction in the percentage of heavy metals during the time of the experiment **(Abdi and Kazemi, 2015)**. This is in agreement with that of **(Qasem *et al.*, 2021)** who stated that bacteria are classified as gram-positive bacteria or gram-negative bacteria, the most significant difference between these two types is in their cell wall composition and thickness. Gram-positive bacteria have a greater potential for removal of heavy metal cations due to the significant electronegative charge density they hold due to the presence of teichoic and teichuronic acids (polyalcohol) which are linked by phosphodiester bonds, attached to peptidoglycan of the cell wall **(Xiang *et al.*, 2000 and Prangchumpol, 2018)**.

Heavy metal contamination is considered one of the most critical environmental problems. Thus, convenient steps need to be considered to decrease heavy metals and metalloid concentrations in water to appropriate levels. Cu, Zn, Pb, Cd, Ni, Co and Mn are considered the most popular heavy metals. Although these heavy metals can be detected in traces; however, they are still hazardous so they have been chosen for this study. It was noticed that the heavy metal readings in aquaculture water gradually decreased through the experimental period. The adsorption mechanism is defined by the physicochemical properties of adsorbent and heavy metals and operating conditions and the resistance of microbes to heavy metal concentrations led to an increase in the microbial communities which can grow in the high concentration of heavy metals **(Artusi *et al.*, 2002)**, this may explain why the metal removal percentage by Biofloc treatments recorded a maximum percentage for Pb (93 %) and the percentage of metal adsorption came in this order Pb > Cu > Ni > Cd > Mn > Co > Zn for T1 and Pb > Ni > Cu > Cd > Mn > Zn > Co, this may be due to that, the difference of the ability of each metal to bind to the molecules of the Biofloc treatment compounds than other ones and the presence of some bacterial isolates which can mitigate the concentration of heavy metal as sulphate-reducing bacteria, oxidizing bacteria **(Astuti *et al.*, 2021)**. In general, it was found that sugarcane bagasse has a treatment (T1) percentage higher than that of rice-bran (T2) for all metals from the start to the end of the experiment, this was most probably due to the strong interaction between metal ions and active functional groups inside sugarcane bagasse than that of rice-bran. The principal component analysis is a descriptive, exploratory technique that expresses the greatest amount of variance in the different variables. This is determined in this research by carbon sources, bacterial abundance and metals. The model is converting the variables into new axes that are orthogonal and the results presented in those axes are not related to each other **(Ture *et al.*, 2021)**. Also, the Pearson correlation coefficient is a suitable measure of association when n couples of continuous data collected on the same experimental unit, follow a bivariate normal distribution. In this study, the only relationship that can be postulated is the table **(Xiang *et al.*, 2000)**. There are positive correlations between SB and Ni & Co. The use of sugar cane in aquaculture with the addition of bacteria-induced microorganisms for producing several enzymes to break down lignin in sugarcane shoots especially peroxidase enzyme-containing Ni and Co which leads to lower the concentration of heavy metals in water throughout the experiment **(Ajewole *et al.*, 2021)**. The microbial association with the heavy metal especially cobalt, nickel and manganese metals during the experiment is the result of the presence of different bacterial genes that are resistant to heavy metals. These genes percentage are increases in the presence of a large proportion of heavy metal resistance genes in the water that associated with copper (*copA*), cadmium-zinc-cobalt (*czc*) and nickel-cobalt-cadmium (*ncc*) genes which were documented by **Ture *et al.*, 2021**, who explained that the occupation of *copA*, *czc* and *ncc* genes in the muscle of fish and water. So, cobalt, nickel and manganese led to an increase in the proliferation of bacteria and their growth in aquaculture experiments. Copper concentrations are positively correlated by lead, and cobalt with nickel and this may be due to that, The interaction between the essential metals lead (Pb) and copper (Cu) is thought to be due to the induction by led of A cytosolic metallothionein protein. This protein can then bind Zn and Cu as well as Pb **(Xiang *et al.*, 2000)**. Bacteria are adapted in presence of heavy metals in aquaculture farms. This is demonstrated by **Zhou *et al.*, 2002** who scientifically proved that all bacterial isolates were metal tolerant by significance correlation ($p < 0.05$, 0.01) with *E. coli* abundance in aquaculture in developing countries **(Ajewole *et al.*, 2021)**. Contamination of fish farms with heavy metals is one of the problems that lead to damage to the product and its quantities. In this experiment, Bioremediation design can reduce the proportion of metals using different by-products of carbon sources at biofloc technology. The number of bacteria positively affects the heavy metals concentrations without differences in nutrients used for aquaculture purposes.

Generally, the removal of metals with sugarcane was higher than that of rice-bran and this may be due to that in sugarcane the metal ion-binding mechanism of adsorption on sugarcane is attributed to its abundance of hydroxyl groups from cellulose, in which aqueous medium favours ion exchange or complexation with metal, while in rice-bran the metal ion binding capacity was dependent on the metal ion (Table 5).

Table 5: Chemical composition of sugarcane bagasse (SB) and rice-bran (RB) modified after **Rocha *et al.* (2011)** and **Ali *et al.* (2017)**.

Ingredient	SB	RB
Cellulose (%)	42.50	33.60
Hemicellulose (%)	24.88	21.8
Ash (%)	7.60	8.00
Protein (%)	1.50	14.00
Lipid (%)	1.50	15.00
Carbohydrates (%)	24.40	22.00
Fibre (%)	65.00	8.00

CONCLUSION

In conclusion, the results support the use of biofloc (organic carbon sources after bacterial utilization) as natural sorbents for heavy metals from polluted water before using it for aquaculture in a period not exceeding thirty days, and more investigations are needed toward the treatment conditions such as temperature, pH and time of adsorption using removal isotherm equations (Langmiur and ffreundlich).

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