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APPLICATION OF BIOFILMS IN REMOVAL OF HEAVY METALS FROM WASTE WATER UNDER STATIC CONDITION

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The aim of the research was to apply biofilms as a model in ecotoxicology to remove selected heavy metals (Cd, Cu, Cr, Zn and Pb) from the wastewater under a static conditions. Biofilms were grown in three graded concentrations of the metal leachates (0.625, 0.417 and 0.250%), harvested after 1, 2 and 3 weeks and analyzed for heavy metals. Mean accumulations peaked on Day 21, and of Cd ranged from 0.000 to 0.040 (mean = 0.00837 ± 0.002), Cu from 0.000 to 0.212 (meam = 0.03929 ± 0.012), Cr from 0.000 to 0.500 (mean = 0.05821 ± 0.021), Zn from 0.000 to 1.456 (mean = 0.31833 ± 0.109) and Pb from 0.000 to 0.099 (mean = 0.02129 ± 0.006) mg/g in resultant biofilm formations. Accumulation of the metals increased significantly with time [F(205.59) > Fcrit(3.95)] at the 95% confidence interval. Those of Pb was significantly higher in the 0.625% leachate mixture than control (Sig F = 0.034) at $P < 0.05$, even as those of Cd and Cu were slightly higher in the concentrations than control. Biofilm model removed small amounts of metals from waste water stream in static condition.

Key words: heavy metals, biofilms, bioaccumulation, waste water, static condition.

Heavy metals such as Cu, Fe, Mn, Mo, Zn and Ni can play a role as micronutrients, even though others such as Hg, Pb, Cd, Cu, Ni and Co can be toxic to humans [1]. Due to the discharge of large amounts of metal-contaminated waste waters in recent times, heavy metal pollution of aquatic systems has become one of the most serious environmental problems of concern. Industries bearing such heavy metals as Cd, Cr, Cu, Ni, Pb and Zn are the most hazardous among the chemical-intensive industries because these metals have high toxicity and solubility in the aquatic environment [2]. Heavy metals can be absorbed by living organisms once they enter the food chain, and large concentrations of them may accumulate in aquatic biotopes, as well as human body due to their recalcitrance in the environment [3]. When their concentrations are beyond tolerable levels, they can cause serious health disorders like gastro-intestinal effects, chronic renal disease [4] and central nervous system disorders [5]. Morphological and behavioural abnormalities in fish such as alteration in their sensory reception, reduced responses to normal olfactory function (such as feeding, mating, selection or homing), reduction in swimming performance, gills purge, and ventilation could also result. Other higher organisms may suffer learning impairment, loss of equilibrium that can lapse into paralysis, loss of reproductive efficiency, and irregular metamorphosis as symptoms of toxic exposure to metals [6].

Decontamination of heavy metals from waste water has been a challenge for a while in that, most of the heavy metal salts are soluble in water and form aqueous solution and so, cannot easily be separated using ordinary physical means. However, several different conventional treatment processes are commonly employed to remove heavy metals from industrial waste water before their discharge into the environment [7]. These methods include chemical precipitation, ion exchange, electrochemical treatment,

membrane technologies, and adsorption on activated carbon among others. Each of these methods has significant disadvantages. For instance, chemical precipitation and electrochemical treatments are ineffective, especially when metal ion concentration in aqueous solution is lower than 50mg/l [8]. Moreover, such treatments produce large amounts of sludge that are not environmentally friendly and need to be treated with great difficulties. Ion exchange membrane technologies and activated carbon adsorption processes are extremely expensive [9].

Therefore, there is a need for new, novel, efficient, eco-friendly and cost effective approaches in the treatment, minimization or even elimination of heavy metals in the environment. In this sense, biological alternatives such as the utilization of biofilms have shown promising results even when the metals are present in very low concentrations. Accordingly, the application of these microorganisms in the removal of heavy metals from waste water has been effective and widely recommended [10].

Biofilms are consortia of microbial cell that are attached on solid surfaces or wet environment [11]. In most natural environments, microbes are commonly found in close association with surfaces and interfaces in the form of multicellular aggregates glued together with the slime they secrete [12]. They occur nearly in every moist environment where sufficient nutrient flow is available and surface attachment can be achieved. Biofilms can be formed by a single bacteria cell species, although they can also consist of many species of bacteria, fungi, algae and protozoa [13]. Approximately 97% of the biofilm matrix is either water, which is bound to the capsules of microbial cells or solvent, the physical properties of which (such as viscosity) are determined by the solutes dissolved in it [14]. The formation of Extracellular Polymeric Substances (EPS) enhances the ability of cell to adhere to surfaces with the presence of flagella, pili, fimbriae, or glycocalyx [15]. The diffusion processes that occur within the biofilm matrix are dependent on the water binding capacity and mobility of the biofilm.

Heavy metals uptake by these microbial biomass is a new eco-compatible and economically feasible application that has been develop to remove heavy metals from waste water [16], and studies have shown that interaction of microbial substance with heavy metals reduced heavy metal ion concentrations in solution [17, 3]. This bioremediation option is based on the high metal binding capacity of biological agents, which remove heavy metals from waste water or contaminated sites with high efficiency. Research has revealed that they act as metal biosorbent as they have metal-sequestering properties [18]. Biofilms can decompose or transform hazardous substances into less toxic metabolites or degrade them to nontoxic end products. They can also survive in contaminated habitats because they are metabolically able to exploit contaminants as potential energy sources [19, 20]. In biological treatment or removal of heavy metals, microorganisms with biological activity such as algae, bacteria, fungi and yeast can be used in their naturally occurring forms.

The efficient removal of heavy metals from waste water is dependent on several factors, including sludge concentration, the solubility of metal ions, pH, the metallic concentration and waste water pollution load [21]. However this study was focused on effective removal of heavy metals from static waste water using biofilms; a bioremediation technology that is very important especially in developing countries such as Nigeria where waste water discharge regulations are flouted and treatment does not have top priority due to high cost of treatment facilities.

Meylan et al. [17] and Ogbuagu et al. [3] have conducted experiments on metal accumulation in algal biofilm in lotic streams and observed that biofilms are efficient model for the removal of metals in solution. However, reports on the application of this biological technique in static environments which mimic industrial effluent reservoirs are lacking. It is in this regard that the current study was conducted to investigate possible biosorption of metal contaminants in static set-up.

Materials and Methods

Preparation of metals leachates

Ten grams of soil sample collected from a waste dumpsite that had been in use for over 15 years, situated along Owerri-Aba Road in Owerri was mixed with 1000 ml of surface water sourced from Otamiri River and thoroughly stirred to attain homogeneity. The resulting solution was decanted into 1 litre plastic container as stock solution.

Establishment of leachate concentrations.

Serial concentrations of 75, 50 and 30 ml of the stock solution were made up to 12,000 ml with water sourced from the Otamiri River in 3 different 30 litres aquaria. The aquaria were labeled as Bexp A, Bexp B and Bexp C,

representing the 75ml (0.625%), 50 ml (0.417%) and 30 ml (0.250%) stock leachate-water mixtures respectively. There was also a 4th aquarium designated as Bexp control which served as a control and contained 100% diluent water only. The mixtures were stirred properly.

Shortly after preparations, samples were collected from each of the aquarium in 30 ml sterile plastic bottles, fixed with two drops of concentrated HNO_3, and sent to the laboratory as soon as possible for analysis of heavy metals.

Growth of Biofilms. The biofilms were formed from waste water leachates and were made up of same consortia of bacteria, fungi, etc that have already been established by earlier researchers, as stated in the Introduction of this article.

Biofilms were allowed to grow and investigated under relatively natural conditions in microcosms. The microcosms consisted of sterile plastic containers housing serially arranged sterile glass slides according to the method of Meylan et al. [17] and Ogbuagu et al. [3]. Three replicate microcosms were installed in each aquarium.

Harvest of Biofilms

Serial harvests were made after 1, 2 and 3 weeks from the date of installation. At each time, temperatures and pH of water in the aquaria were taken *in situ*. Biofilms were scraped off the surfaces of glass slides and introduced into sterile sample bottles that had been pre-rinsed with distilled water. The samples were then fixed with 2 drops of conc. HNO_3 for laboratory analysis.

Laboratory Analysis

The metals (Pb, Cd, Cu, Cr and Zn) in the stock solution and biofilm samples were determined using Atomic Absorption Spectrophotometer (AAS) (Varian 600 Spectra AA) after digestion and in keeping with the method of Karvelas et al. [22]. Centrifugation of the biofilm samples was completed during a 30 minutes period at 400 rpm and at 4 °C. A nitrate cellulose filter (0.45μm diameter) was used to filter the content prior to completion of a digestion procedure. Heated mixture of conc. HNO_3 was used for digestion, and the digestion mixture was prepared with 6 ml of 65% HNO_3 and 2 ml of 30% H_2O_2. After centrifugation, distilled water was added to make sample up to 20 ml. The mixture was used for quantification of the metals. Analytical blanks were run in the same way as the samples and concentrations were determined using standards prepared in the acid matrix. The concentration represented the dissolved metals in solution while those collected on the filter paper were digested with aqua regia before AAS analysis. The heavy metals concentrations in solution and filter paper were considered to be the total heavy metal concentrations in biofilm samples, and expressed in mg/g.

Statistical Analysis

The SPSS© V.22.0 and MS Excel© statistical softwares were used to analyze data. The student's *t*-test of significant variation was used to compare heavy metal biosorptions in biofilm formations, while the one way ANOVA and Duncan Multiple Range tests were used to establish homogeneity in mean variance and mean separations of biosorptions respectively at $P < 0.05$. Variation plots were used to represent accumulations of the metals in graded biofilm formations.

Results and Discussion

Water temperature and pH

Water temperature ranged between 27.4 and 35.5 °C in the four aquaria. In Bexp A, Bexp B and Bexp C experimental setups, it ranged from 27.5–35.5 (31.0±4.03), 27.4–35.4 (31.1±4.03) and 27.8–35.3 (31.3±3.78) °C respectively. However, in the Bexp Control setup, it ranged from 27.9–35.4 (31.4±3.77) °C. pH ranged from 4.30–6.45 (5.42±0.06) in the aquaria; with mean values of 6.40±0.01 (Bexp A), 5.21±0.02 (Bexp B), 5.10±0.01 (Bexp C), and 4.33±0.01 (Bexp Control).

Biosorption of heavy metals in biofilms

In the replicates of the 0.625% (Bexp A) biofilm formations, mean accumulations of Cd were 0.0183±0.011 and 0.01±0.008 mg/g, of Cu were 0.098±0.058 and 0.08933±0.051 mg/g, and of Cr were 0.044±0.024 and 0.18533±0.111 mg/g. Mean accumulations of Zn and Pb in the same biofilms were 0.420±0.390 & 0.521±0.386 and 0.0587±0.025 & 0.0400±0.025 mg/g respectively. In Bexp B (0.417%) biofilm formations, mean accumulations of Cd, Cu, Cr, Zn and Pb were 0.0097±0.005 & 0.0103±0.004, 0.0297±0.028 & 0.0277±0.026, 0.0563±0.045 & 0.0477±0.035, 0.463±0.044 & 0.4503±0.042 and Pb 0.020±0.013 & 0.021±0.015 mg/g in the replicates.

In the 0.250% (Bexp C) biofilm formations, mean accumulations of Cd were 0.006±0.004 and 0.012±0.008, Cu 0.050±0.038 and 0.0183±0.007, Cr 0.064±0.026 and 0.067±0.025, Zn 0.340±0.033 and 0.350±0.033, and Pb 0.015±0.008 and 0.015

±0.008 mg/g in the replicates. However, in the control aquarium, mean accumulations of Cd were 0.00067±0.0003 and 0.000±0.000, Cu 0.00067±0.0003 and 0.00033 ±0.0002, Pb 0.00067±0.0003 and 0.00033±0.0002, Cr 0.00033±0.0002 and 0.00033±0.0002, Zn 0.00067±0.0003 and 0.00033±0.0002 mg/g.

Comparison of metal accumulations in biofilms

The one way ANOVA test revealed that the accumulation of Pb was significantly different in the graded biofilm formations (Sig. F = 0.034; $P < 0.05$). A post-hoc Duncan Multiple Range Test revealed that accumulations of Cd and Cu differed significantly between the Bexp A and control biofilm formations at the 95% confidence limit (Table). The accumulation of Pb in the Bexp A biofilms also differed significantly from those of the Bexp C and Bexp Control setups.

Values with same superscript along same rows are not significantly different at P<0.05, Bexp A=0.625% leachate mixture, Bexp B=0.417% leachate mixture, Bexp C=0.250% leachate mixture and Bexp Control= 0.000% concentration.

Effect of time on accumulations of heavy metals in biofilms

Bexp A leachate mixture

On Day 7, mean accumulations of Cd, Cu, Cr, Zn and Pb were 0.0045±0.0005, 0.0200±0.001, 0.0005±0.0003, 0.022±0.001 and 0.0081±0.001 mg/g respectively in the biofilm formations (Fig. 1). On Day 14, mean accumulations of the respective metals were 0.008±0.002, 0.056±0.009, 0.0695±0.015, 0.0625±0.026 and 0.048±0.034 mg/g in the biofilm formations (Fig. 2).

On Day 21 mean accumulations of the metals were 0.030±0.001, 0.206±0.006, 0.274±0.226, 1.328±0.128 and 0.092±0.007 mg/g respectively in the biofilm formations (Fig. 3).

Bexp B leachate mixture

On Day 7, mean accumulations of the metals (Cd, Cu, Cr, Zn and Pb) were 0.005±0.001, 0.0005±0.0002, 0.000±0.000, 0.016±0.004 and 0.0005±0.0003 mg/g respectively (Fig. 1). On Day 14, mean accumulations of the metals were 0.006±0.001, 0.0025±0.0005, 0.0255±0.0025, 0.030±0.001 and 0.013±0.001 mg/g respectively in the biofilm formations (Fig. 2). On Day 21, mean accumulations of the respective metals were 0.019±0.001, 0.083±0.003, 0.1305±0.0155, 1.324±0.024 and 0.048±0.003 mg/g (Fig. 3).

Bexp C leachate mixture

Accumulations also varied in the 0.250% leachate mixture. On Day 7, mean accumulations of Cd, Cu, Cr, Zn and Pb were 0.002±0.001, 0.055±0.002, 0.0325±0.004, 0.0085±0.005 and 0.0005±0.0002 mg/g respectively (Fig. 1). Mean accumulations of the metals were 0.003±0.001, 0.0195±0.003, 0.0495±0.0005, 0.0235±0.004 and 0.017±0.003 mg/g respectively on Day 14 in the biofilm formations (Fig. 2). However, on Day 21, mean accumulations of the metals were 0.022±0.007, 0.0775±0.048, 0.1155±0.001, 1.004±0.004 and 0.028±0.003 mg/g (Fig. 3).

Bexp Control leachate mixture

On Day 7, mean accumulations of Cd, Cu, Cr, Zn and Pb were 0.0005±0.0003, 0.001±0.000, 0.000±0.000, 0.0005±0.004 and 0.0001±0.000 mg/g respectively (Fig. 1). On Day 14, mean accumulations of the respective

Table. **Mean separation of accumulation of heavy metals in biofilms formed in graded leachate mixtures using Duncan Multiple Range Test ($P < 0.05$)**

	Graded concentrations			
Heavy metals	Bexp A	Bexp B	Bexp C	Bexp Control
Cd	0.014167^{a}	0.010000^{ab}	0.009000^{ab}	0.000400^{b}
Cu	0.093833^{a}	0.028667^{ab}	0.034167^{ab}	0.000600^{b}
Cr	0.114667^{b}	0.052000^{b}	0.065833^{b}	0.000400^{b}
Zn	0.470833^{b}	0.456667^{b}	0.345333^{b}	0.000600^{b}
Pb	0.049333^{a}	0.020333^{ab}	0.015000^{b}	0.000600^{b}

Values with same superscript along same rows are not significantly different at $P < 0.05$, Bexp A=0.625% leachate mixture, Bexp B=0.417% leachate mixture, Bexp C=0.250% leachate mixture and Bexp Control= 0.000% concentration.

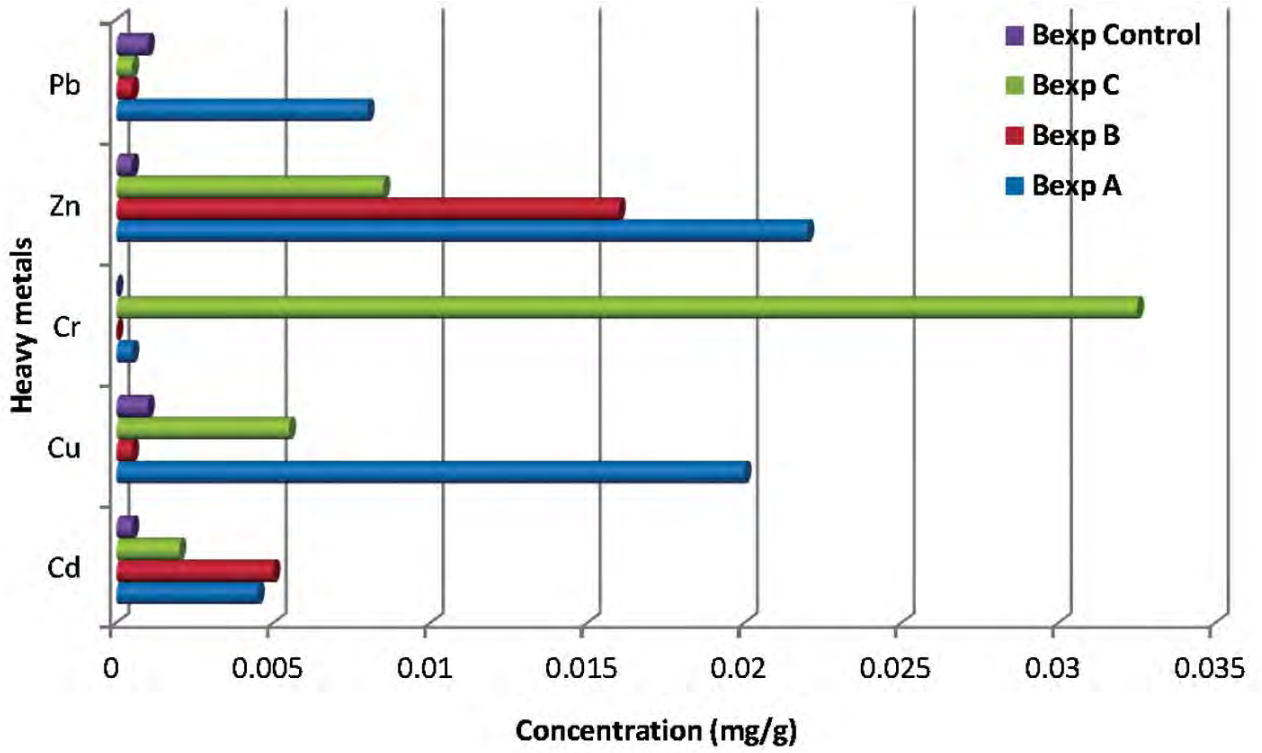

Fig. 1. **Mean accumulation of metals in biofilms formed in graded leachate mixture after 7 days**

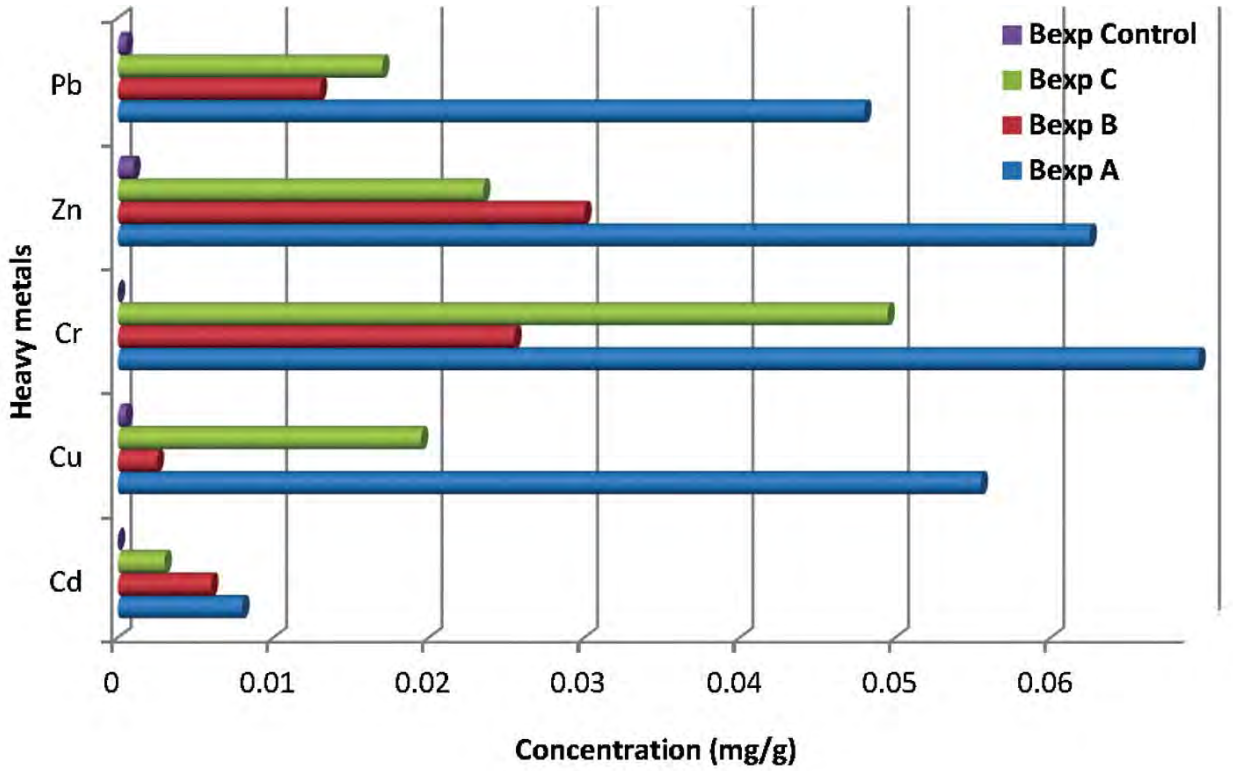

Fig. 2. **Mean accumulation of heavy metals in biofilms formed in graded leachate mixture after 14 days**

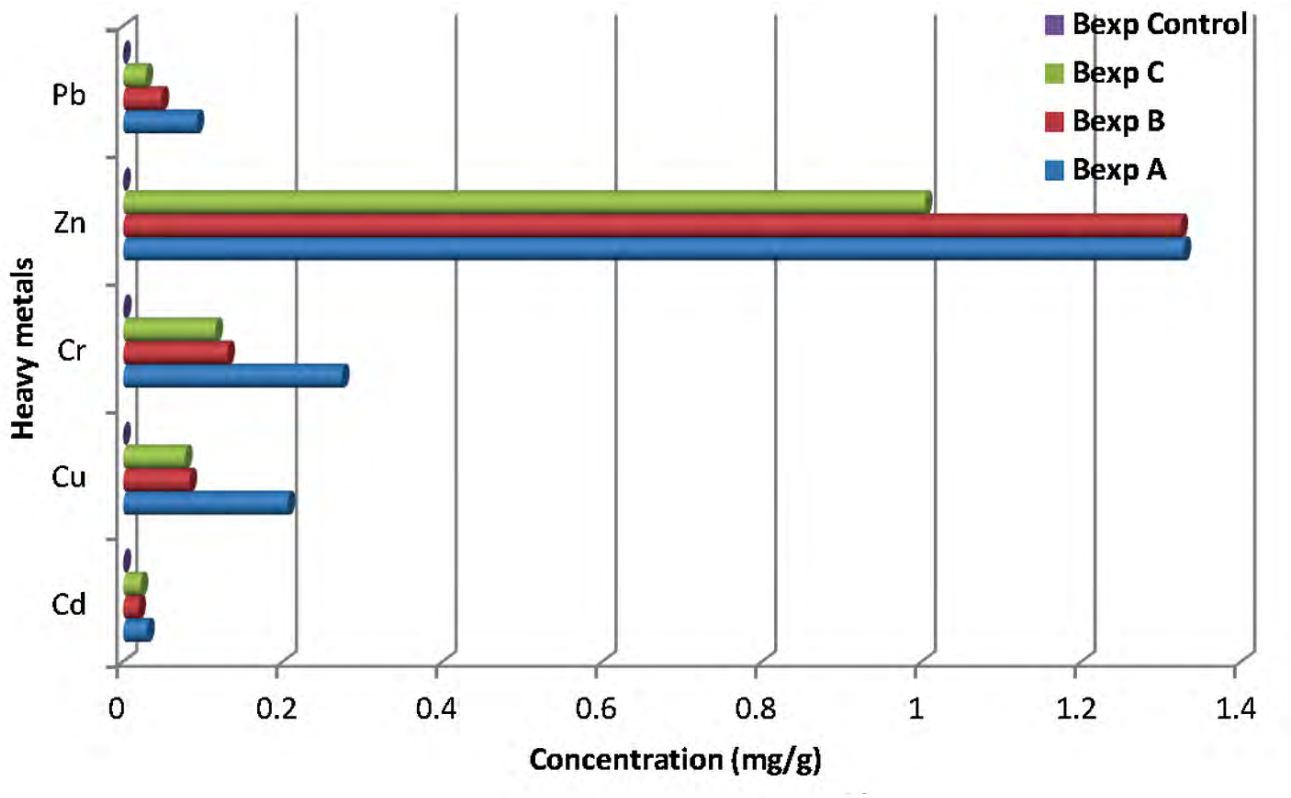

Fig. 3. **Mean accumulation of heavy metals in biofilms formed in graded leachate mixture after 21 days**

metals were 0.000±0.000, 0.005±0.001, 0.000±0.000, and 0.001±0.000 and 0.0005±0.0003 mg/g in the biofilm formations (Fig. 2). On Day 21, mean accumulations of the metals were 0.0005±0.0002, 0.000±0.000, 0.000±0.000, 0.000±0.000 and 0.000±0.000 mg/g (Fig. 3).

The ANOVA test of homogeneity revealed that accumulations of the metals differed significantly over the 21 days experimental period [F(205.59)>Fcrit (3.95); $P < 0.05$].

The mere observation of accumulation of some trace metals in this work confirmed that biofilm models can offer some solution in the removal of heavy metals from waste water streams even in static conditions. However, the rate and amount of accumulations were less than those observed in lotic aquatic environments by Doering and Uehlinger [23] in the Tagliamento River in Europe, Ogbuagu et al. [3] in Otamiri River in Nigeria and Meylan et al. [17] in the Furtback, Canton of Zurich. This technique therefore holds promises for effective, inexpensive and eco-friendly metal bioremediation technology for the removal of recalcitrant contaminants such as the persistent organic pollutants (including heavy metals) from complex industrial effluents, and hence can offer pollution free environment if optimized. Less biofilm formations were observed in this work and it could be attributed to absence of renewal and replenishment of biomass which are usually associated with lotic, but lacking in static conditions.

The observed significantly higher accumulations of Cd, Cu and Pb in the Bexp A than Control biofilms reflect bioavailability of the trace element in the treatment mixture. However, Cd, Cu and Pb were more readily removed from the leachate mixtures than Zn and Cr. This is similar to the observation of Azizi et al. [24], that Cu, among other metals was more readily removed from waste water stream. This research [24] presents the results of an evaluation of the removal of selective heavy metals (Cd, Cu, Ni and Zn) from waste water through a Modified Packed Bed Biofilms Reactor (PBBR).

The graded leachate mixtures were also associated with different pH levels, but their resultant biosorption trend did not support the observation of UNEP GEMS

[25] that heavy metals are usually more bioavailable in acidic media, and so would get more biosorbed. Rather, it appeared to be in consonance with the observation of Huang et al. [26] that pH had no significant effects on heavy metals (Cr, Ni, Cu, Zn, Cd, Pb) release in Huangpu River sediments, East China. The reason for this non-release could be attributed to metal speciation. In that work, Huang et al. [26] observed that even when available, the exchangeable fraction of the metals was only about 0.06 to 2.63% of all the metals in the sediment, while the residual fraction, which is the most stable one, accounted for about 51.50 to 86.45% of all the metals in the sediment. These reasons may have contributed to the low release flux of the metals studied.

Time played a vital role in the uptake of the metals in that development of biofilms took some time and at early stages, there were little or no accumulations of the metals. This was because biofilm communities require time to establish themselves hence, microbial dose or concentration also determined rate of metal uptake in the aquaria. However, data on the accumulation of Cr and Cu especially on Days 7 and 14 days does not seem to indicate the existence of a dose-dependent effect. This could reflect the early presence of little or no biofilm formations on those days to cause accumulation.

Heavy metal removal efficiency in this study was dependent on the presence of microorganisms as well as concentration of metal ions in solution. Deibel and Schoeni [13] had documented that biofilms can consist of many species of bacteria, fungi, algae and protozoa, though their composition in the current study was not determined. Accordingly, Chipasa [21] observed that biosorption became apparent as higher metal concentrations stimulated increased microbial activity, and so, increased removals from leachate mixture. This again is related to bioavailability of the trace elements. However, this was partially in consonance with the observations of Piccirillo and Pereira [27] and Karvelas et al. [22] wherein heavy metal removal efficiency was also dictated by the influence of microorganism but became apparent at lower metal concentrations. They rather observed that higher concentrations of metals reduced their removal from waste water stream, and so explained that this could be due to reduced activity of microorganisms in the system due to higher concentration of metals inducing stress on the microorganisms by reducing their action towards the metals. This in turn would negatively affect the functioning of biological treatment process.

The following observations were made in the study:

1. Biofilm model removed some heavy metals, especially Pb, Cd and Cu in static condition.
2. Less biofilm formations and bioaccumulation of metals were observed in static than reference lotic conditions.
3. Removal of metals was dose dependent in the biofilms; and
4. Removal of metals was also time dependent.

This study revealed that the application of biofilms can offer some solutions in the removal of heavy metals from waste waters in static condition.

Recommendations

Based on these findings, it is recommended that biosorptive method of metal removal from effluent streams in static condition should be encouraged and optimized as a more attractive and economic alternative in environmental solutions. Further kinetic studies on the relationship between metals accumulation in biofilms and time should be carried out.

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ЗАСТОСУВАННЯ БІОПЛІВОК ДЛЯ ВИДАЛЕННЯ ВАЖКИХ МЕТАЛІВ ЗІ СТІЧНИХ ВОД ЗА СТАТИЧНИХ УМОВ

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Метою дослідження було використання біоплівок як екотоксикологічної моделі для видалення відібраних важких металів (Cd, Cu, Cr, Zn і Pb) зі стічних вод за статичних умов. Біоплівки вирощували в трьох відградуйованих концентраціях фільтратів металів (0,625, 0,417 і 0,250%), збирали через 1, 2 і 3 тижні й аналізували на вміст важких металів. Середні значення піків в утворених біоплівках досягли максимуму на 21-й день: Cd — від 0,000 до 0,040 (середнє значення = 0,00837 ± 0,002), Cu — від 0,000 до 0,122 (середнє значення = 0,03929 ± 0,012), Cr — від 0,000 до 0,500 (середнє значення = 0,05821 ± 0,021), Zn — від 0,000 до 1,456 (середнє значення = 0,31833 ± 0,109) і Pb — від 0,000 до 0,099 (середнє значення = 0,02129 ± 0,006) мг/м. Накопичення металів істотно збільшилось із часом [F (205,59) > Fcrit (3,95)] за 95%-го довірчого інтервалу. Значення для Pb були значно вищі в 0,625%-й вилуговуваній суміші фільтрату, ніж контроль (Sig F = 0,034) за $P < 0,05$, тимчасом як для Cd і Cu — трохи вищі, ніж контрольні. Застосування біоплівки призвело до невеликого видалення важких металів зі стічних вод за статичних умов.

Ключові слова: важкі метали, біоплівки, біоакумуляція, стічні води, статичні умови.

ПРИМЕНЕНИЕ БИОПЛЕНОК ДЛЯ УДАЛЕНИЯ ТЯЖЕЛЫХ МЕТАЛЛОВ ИЗ СТОЧНЫХ ВОД В СТАТИЧЕСКИХ УСЛОВИЯХ

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Целью исследования было использование биопленок в качестве экотоксикологичекой модели для удаления отобранных тяжелых металлов (Cd, Cu, Cr, Zn и Pb) из сточных вод в статических условиях. Биопленки выращивали в трех градуированных концентрациях фильтратов металлов (0,625, 0,417 и 0,250%), собирали через 1, 2 и 3 недели и анализировали на содержание тяжелых металлов. Средние значения пиков в образовавшихся биопленках достигли максимума на 21-й день: Cd — от 0,000 до 0,040 (среднее значение = 0,00837 ± 0,002), Cu —от 0,000 до 0,122 (среднее значение = 0,03929 ± 0,012), Cr — от 0,000 до 0,500 (среднее значение 0,05821 ± 0,021), Zn — от 0,000 до 1,456 (среднее значение = 0,31833 ± 0,109) и Pb — от 0,000 до 0,099 (среднее значение = 0,02129 ± 0,006) мг/г. Накопление металлов существенно увеличилось со временем [F (205,59) > Fcrit (3,95)] при 95%-м доверительном интервале. Значения для Pb были значительно выше в 0,625%-й выщелачивающей смеси фильтрата, чем контроль (Sig F = 0,034) при $P < 0,05$, в то время как для Cd и Cu несколько превышали контрольные. Применение биопленки привело к небольшому удалению тяжелых металлов из сточных вод в статических условиях.

Ключевые слова: тяжелые металлы, биопленки, биоаккумуляция, сточные воды, статические условия.