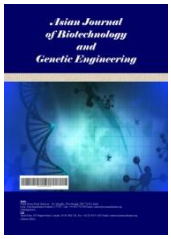

Asian Journal of Biotechnology and Genetic Engineering

5(2): 45-52, 2022; Article no.AJBGE.86860

Physico-Chemical Analysis and Aerobic Bioremediation of Paint Effluent Using *Pseudomonas aeruginosa and Bacillus subtilis*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/86860

Original Research Article

Received 07 March 2022
Accepted 10 May 2022
Published 13 May 2022

ABSTRACT

Bioremediation uses biological techniques to reduce the harmful effects of pollutants in the environment, including wastewater. It is a handy tool for various applications in environmental protection. In developing strategies for bioremediation of paint effluent, untreated paint effluent from Dulux Plc was inoculated with two aerobic microorganisms, *Pseudomonas aeruginosa* and *Bacillus subtilis.* Microbiological and physicochemical analysis was carried out for five weeks upon inoculation of effluent with *Pseudomonas aeruginosa* P, *Bacillus subtilis* B, in separate setups, effluent sample inoculated with both organisms M, to observe their synergy and effluent sample without inoculation, served as a control sample to compare the abilities of the native effluent microflora with that of the introduced microorganisms. The cultures of microorganisms were observed in nutrient broth until the exponential phase before their inoculation into the effluent sample. The bacterial cultures of *Pseudomonas aeruginosa, Bacillus subtilis* and *consortium* contained 4.2 x 10^8cfu/ml, 3.8 x 10^9cfu/ml and 5.2 x 10^9cfu/ml, respectively, when inoculated into the experimental setups P, B and M, in the experimental samples, BOD and all the inorganic nutrient sources tested decreased rapidly with a proportional increase in the population densities. This trend was firm for PO_4^{3-} and NO_3, which eventually became

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limiting in the experimental and control samples. Inoculating microorganisms with the addition of appropriate inorganic nutrients may be a suitable method for rapid bioremediation of paint effluent.

Keywords: Bacterial; bioremediation; paint effluent; waste water management; physicochemical analysis.

1. INTRODUCTION

There is no primary stream of wastewater associated with the production of paint and allied products because all the constituents entering mixers or reactors come out as products [1]. Cleaning operations of mixers, reactors, blenders, packing machines and floors are the primary wastewater sources. Wastewater contains concentrations of biological oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, toxic compounds and colour because of the varying degree of chemicals used [1]. The discharge of such coloured wastewater into the environment is aesthetically displeasing, but impedes light penetration, damages the quality of the receiving streams, and maybe toxic to treatment processes, food chain organisms and aquatic life [2]. Before they discharge into the environment, effluent treatment is necessary.

Bioremediation uses living microorganisms or microbial processes to detoxify and degrade environmental pollutants to revive the environment [3]. This biotechnological process helps to facilitate the biodegradation of contaminants or toxic materials, causing environmental pollution and health hazards [3]. Biodegradation of pollutants in the effluent is becoming an increasingly important wastewater treatment method [4], thus restoring the original surroundings [5]. Pollutants are degraded naturally using active microbial strains. The merits of these options include that they appear to be the most environmentally friendly method for removing pollutants since other methods, such as chemicals, introduce more toxic compounds to the environment [5]. Biological treatment is advantageous for removing pollutants, mainly when the wastewater contains considerable amounts of organic solvent found in paint effluent. However, paint effluent is co-contaminated with organic and metal pollutants. Metallic powders used as additives have a biostatic and biocidal effect on microorganisms [6]. Hence, this study aimed at the aerobic bioremediation of paint effluent.

2. MATERIALS AND METHODS

2.1 Sources of Effluent

For this study, untreated paint effluent was collected from Dulux Cap Plc 2, Adeniyi Jones Avenue, Ikeja Lagos, Nigeria. The sample was collected in a clean 5 Liters bottle and repeatedly rinsed with the wastewater. Afterwards, the sample was transported in an ice chest and transported to the laboratory. It was stored in the refrigerator at 4oC prior to analysis on arrival.

2.2 Source of Microorganisms

The microorganisms used – *Pseudomonas aeruginosa* and *Bacillus subtilis* were collected on nutrient agar slants manufactured by Titan Biotech Limited in India prepared in labelled different McCartney bottles. These microorganisms were obtained from FIIRO (Federal Institute of Industrial Research, Oshodi). Thereafter, they were transported to the laboratory on the slant and stored a 4^0C. Confirmation of organisms was done by culturing the organisms overnight at 37^0C using cetrimide agar and chloramphenicol (5mg/ml) supplemented Luria-Bertani (L.B.) agar, which are selective for *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively. All media used were prepared according to the manufacturers' instructions.

2.3 Sub-culturing of Microorganisms

The stored microorganisms on slants were revived on freshly prepared separate nutrient agar plates labelled P and B for *Pseudomonas aeruginosa* and *Bacillus subtilis,* respectively, using the streak plate techniques with a sterile wire loop, and incubated overnight at 37^0C. To obtain pure isolates of the microorganisms, isolates were sub-cultured onto fresh labelled nutrient agar plates and incubated overnight at 37^0C. The sub-culturing was done according to the labels on the plates. Eight nutrient agar plates were prepared; four were labelled B1, B2, B3, B4 and the other four were P1, P2, P3, and

P4. Isolates from P were sub-cultured in replicates on the Ps, while isolates from B were sub-cultured on the Bs.

2.4 Inoculation of Nutrient Broth with Microorganisms

Our conical flasks (calibrated 500mls) containing 250mls of prepared nutrient broth were labelled separately as P_1, P_2 – *Pseudomonas* and B_1, B_2 for *Bacillus.* The eight nutrient agar plates were used; four plates were inoculated with *Bacillus subtilis,* while the other four were with *Pseudomonas aeruginosa.* 2mls of sterile distilled water were poured into each plate under aseptic conditions. Afterwards, a flamed inoculating loop was used to scrape the microbial growth displayed on plates and was poured into the conical flasks as directed by the labels. The flasks were incubated overnight at 37^0C after inoculation. After incubation, microbial growth was measured by taking the turbidity reading twice at a daily interval of 6 hours, using the UV-Vis spectrophotometer (Model: 1-290). Reading was taken aseptic condition using Bunsen burner and cleaning the bench with cotton wool moistened with 70% alcohol.

2.5 Inoculation of Effluent with Broth

Four clean plastic containers washed with detergent (so Klin, Nigeria) were labelled P-*Pseudomonas*, B-*Bacillus*, C-Control containing effluent alone, and M-*Bacillus* + *Pseudomonas*. Two (2) liters of fresh untreated effluent were then measured using a measuring cylinder and poured into each bowl. The inoculated nutrient broths in conical flasks were poured into the bowls as directed by the labels. The setup (samples) was opened and placed in a confined area in the laboratory, where it was monitored for five weeks [7].

2.6 Enumeration of Microbial population

2.6.1 Total bacteria (viable) count

The total bacterial counts of the samples were obtained by aseptically introducing aliquots (0.1ml) of appropriate dilution on a sterile nutrient agar plate by spread plate technique after serial dilution. The plates were incubated at 37^0C for 18-24 hours. The total viable count of the samples was estimated based on the colony counts and multiplied by the reciprocal of the dilution factor and reported as a colony-forming unit. This was taken from each of the labelled containers from which the mean population was taken using the formula;

$$\text{CFU/mL}=\frac{(no.\ of colonies * dilution factor)}{volume of culture plate} \quad [8]$$

2.7 Determination of Physico-Chemical Parameters

2.7.1 Hydrogen ion concentration

The pH of the wastewater samples was determined using a portable pH meter with combined glass and calomel electrodes. The pH meter was standardized with buffer solution pH 4.0, 9.2 and 7.0, after which the samples were tested in turn.

2.7.2 Electrical conductivity

The electrical conductivity was measured using a portable electrical conductivity meter (H.M. Digital COM-100). The electrical conductivity meter was standard with 342 ppm sodium chloride solution, after which the different samples solution were analyzed for electrical conductivity.

2.7.3 Temperature

The temperature of effluent samples was determined using the mercury bulk thermometer. The probe was dipped into the effluent samples left for 3 minutes. Readings were determined from the rise in the level of mercury. The samples were stirred using a stirring rod before analyzing the temperature.

2.7.4 Appearance and odour

The appearance and odour of the samples were determined by an organoleptic method using physical observation and by smelling the samples.

2.7.5 Titratable acidity

The titratable acidity of the sample solution was determined by the methyl orange method. To $50cm^3$ of the sample, 2 drops of methyl orange indicator were added in a conical flask placed over a white surface. The solution was then titrated with standard 0.02N sodium hydroxide (NaOH) until the colour became faint orange.

2.7.6 Sulphate (SO_4^{2-}), Nitrate (NO_3-) and Phosphate (PO_4^{3-})

The SO_4^{2-}, NO_3- and PO_4^{3-} were determined using the HACH DR 2000 direct reading spectrophotometer, which is internally calibrated. For each of the parameters, the HACH reagents in pillows were added to 25ml of water samples, and after the reaction time, the concentration was read directly in the spectrophotometer after zeroing with the blank. Each parameter, SO_4^{2-}, NO_3- and PO_4^{3-} has their respective wavelength of best absorption, SO_4^{2-}(450nm), NO_3- (500nm) and PO_4^{3-} (890nm).

2.7.7 Dissolved oxygen (D.O.)

The D.O. of the water sample was determined using a portable dissolved oxygen meter.

2.7.8 Total bicarbonate (HCO^{3-})

The total bicarbonate was determined by titrating $50cm^3$ of the wastes water sample with $0.1NH_2SO_4$ in a conical flask using 5 drops of phenolphthalein indicator until a colourless solution was obtained (V_1). At the end of the titration, 2 drops of methyl red were added to the same flask. The titration continued until the solution changed from yellow to rose red (V_2).

Calculation|:

1ml of 0.1N H_2SO_4 = 0.0061g HCO^{3-}
V_1 = Vol. of 0.1N H_2SO_4 used in phenolphthalein titration
V2 = Vol. of 0.1N H_2SO_4 used in red methyl titration.

2.7.9 Residual oil content (ROC)

The residual oil content was determined by extraction of samples with N-hexane followed by quantification by gravimetric method. About $100cm^3$ water sample was extracted with 50cm of n-hexane twice, and the extract collected was passed through a packed column of anhydrous sodium sulphate to remove water (dehydration) and collected in a dry beaker. The resulting solution was then evaporated to dryness over a hot water bath. The increase in the mass of the beaker gave the mass of oil extracted from the $100cm^3$ of the wastewater sample.

Calculation:

ROC (mg/l) = (M2-M1) x 104

M2 = Mass of oil + beaker
M1 = Mass of beak

2.8 Biochemical Oxygen Demand (BOD_S^{20})

The BOD_s^{20} was carried out by determining the dissolved oxygen content of the sample at zero-days, day of sampling and the fifth day after incubation at 20^0C in a dark bottle. The difference between dissolved oxygen at zero and that of the firth, multiplied by the dilution factor, gives the BOD_s^{20}.

3. RESULTS

3.1 Changes in Physicochemical and Microbiological Parameters

The table below shows the changes in the mean population of the microorganisms as well as the physicochemical parameters, which include sulphate, phosphate, nitrate, total bicarbonate, residual oil content, dissolved oxygen, biochemical oxygen demand, and pH of the three samples P, B, and M compared with the control (C).

3.2 Dissolved Oxygen (D.O.)

The figure below shows the measures of dissolved oxygen in the samples for the five weeks. Dissolved oxygen measurement in wastewater is essential and serves as an indicator for ecosystem conditions and the survival of aquatic life (State of Narragansett Bay and its Watershed 2017 Technical Report).

3.3 Biochemical Oxygen Demand (BOD)

The amount of oxygen used by microbes in water to decompose organic matter is called BOD, and it is essential in determining the extent of wastewater pollution and the effectiveness of the treatment method used. The figure below shows the BOD of the samples compared with the control for five weeks.

4. DISCUSSION

Insufficiency of water for domestic uses and the harm it may cause in the future, just as estimated by a recent U.N. report that indicates that by 2025, two-thirds of the world's population could face water stress. Out of this little water accessible for use, industries consume a

reasonable amount, mostly in cleaning, which returns into the environment as effluents/wastewater after treatment [9-11]. This present study analyzes *Pseudomonas aeruginosa* and *Bacillus subtilis* in the microbial treatment of wastewater, which can restore the original natural surroundings, save cost, and prevent further pollution [12]. This study shows that the effluents of paint can be treated using *P. aeruginosa* and *B. subtilis.* In this present study, the analysis results of the microbial inorganic sources, namely HCO_3-, PO_4^{-3}, NO_3^{-1} and SO_4^{-2}, were low in effluent samples at week 5 (Table 1), which is low in agreement with the studies of Tolulopeet al. [13]. A significant amount of remediation was recorded in M with PO_4 almost completely deleted, showing that the organisms' synergistic degradation of the effluents is more efficient and effective. Nitrate and phosphate are given special attention in water pollution because they play a significant role in eutrophication, leading to algae growth enhancement if not handled, which causes the death of aquatic animals [14].

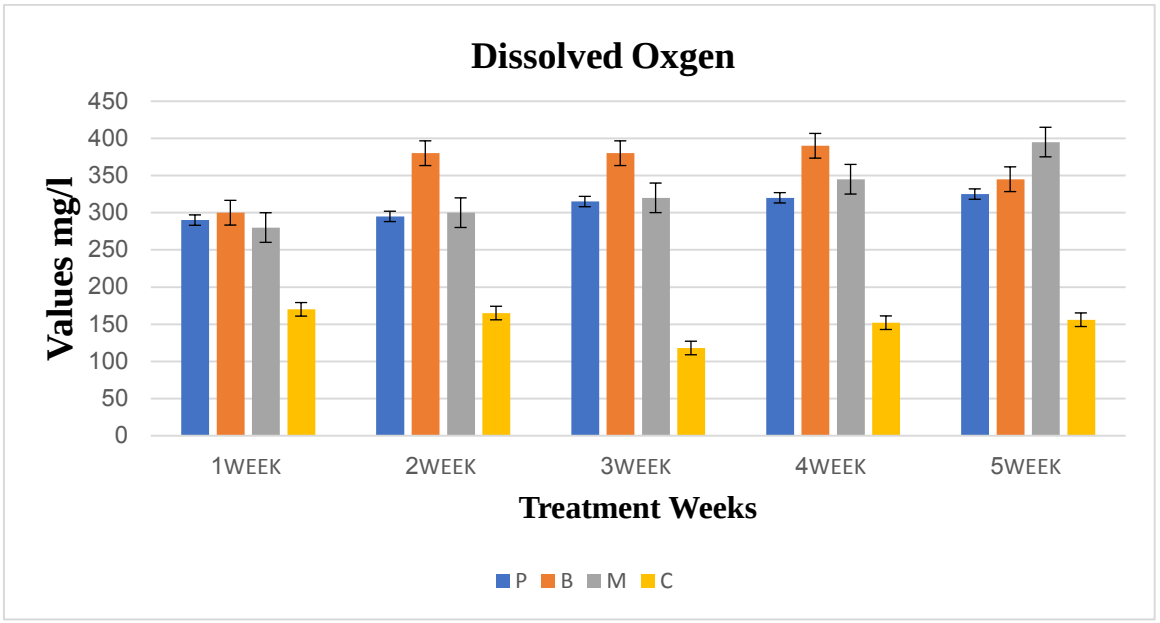

Fig. 1. Measures of dissolved oxygen in the samples for the five weeks

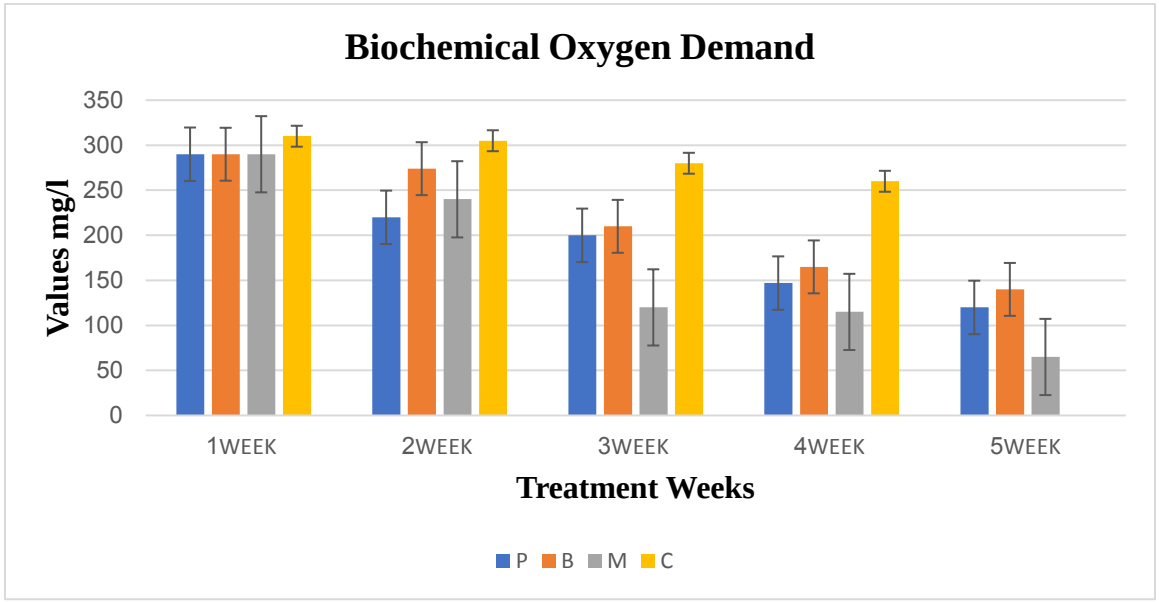

Fig. 2. Measures of BOD in the samples for the five weeks

Table 1. Mean changes in physicochemical and microbiological parameters

Weeks	Sample	SO_4^{-2} mg/l	NO_3^{-1} mg/l	PO_4^{-3} mg/l	HCO_3 mg/l	ROC mg/l	Ph	Conductivity μ Scm-1	Titratable acidity	Mean population
1	P	4.0	48	43	0.06	0.02	7.4	2200	210	3.2×10^9
	B	6.0	46	43	1.0	1.0	7.4	2190	272	4.1×10^8
	M	6.0	46	48	1.90	1.90	6.9	2000	200	5.0×10^5
	C	6.9	48	46	1.20	1.20	7.2	2200	272	3.1×10^2
2	P	7.2	37	40	0.10	0.05	7.1	2250	240	3.8×10^9
	B	5.1	32	41	0.2	0.03	7.2	2230	279	4.7×10^9
	M	5.8	43	32	1.02	0.01	7.1	1887	188	4.7×10^{11}
	C	6.2	46	37	1.10	0.02	7.2	2187	270	4.2×10^7
3	P	6.0	30	32	0.34	0.03	6.8	1950	270	4.5×10^9
	B	6.2	32	36	0.2	0.025	7.1	2100	289	5.2×10^9
	M	5.6	30	26	0.56	0.01	7.1	1880	164	6.8×10^{11}
	C	5.0	25	32	1.02	0.01	6.6	2192	274	3.4×10^{10}
4	P	3.2	28	30	0.54	0.04	6.8	1940	290	2.6×10^{11}
	B	5.1	30	30	ND	0.01	6.6	2050	269	3.2×10^{11}
	M	4.2	26	16	0.21	0.01	6.9	1740	140	6.9×10^{11}
	C	4.2	25	30	0.56	0.01	6.9	2050	281	4.0×10^8
5	P	3.0	22	27	0.33	0.02	6.9	1930	275	1.8×10^{11}
	B	4.9	26	27	ND	0.01	7.2	1900	288	1.8×10^{10}
	M	4.1	20	6	0.21	0.03	6.9	1650	125	6.9×10^{11}
	C	1.6	20	27	0.56	0.002	7.1	1990	200	3.5×10^4

Fig. 1 and 2 deplete the changes in D.O. and BOD over time in the setups. In M dissolved oxygen, data obtained showed increasing trends with a corresponding significant decrease in BOD; asimilar inverse relationship between D.O. and BOD has been reported by Ram et al. [7] and Aboulhassan et al. [8]. In P and B, the BOD reduction was not so rapid compared with M. In C, the trends of the results were different, particularly in the BOD, as there was no appreciable reduction compared with the experimental samples. A high BOD level in water means a problem, and higher aquatic animals suffer from stress, suffocate and die due to low dissolved oxygen [13].

The mean changes in the population of bacteria displayed a similar growth pattern in P, B, and C in week 3, suggesting the presence of contaminants in C from the laboratory environment as microbial load in the control sample was infinitesimal in week 1. The changes in ROC and pH in the setup over time are summarized in Table 1. Finally, there was an observable decrease in the volume of effluent and week 5, and the effluent was utterly dried up, probably due to evaporation as raw materials used in paint formulations contain effervescent chemicals.

5. CONCLUSION

In conclusion, wastewater management is done poorly in Nigeria, and it causes a significant hazard to the environment, especially to water bodies where most industries channel their wastewater, causing eutrophication, algal boom, reduction in dissolved oxygen, and an inverse rise in biochemical oxygen demand. This results in the death of aquatic life, causing a reduction in aquatic food and environmental pollution. Instead of using chemicals to treat paint effluents, this study suggests using *P. aeruginosa* and *B. subtilis,* an environmentally friendly approach that is efficient through their ability to increase the D.O. while decreasing the BOD of the paint effluent studied.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dey BK, Hashim MA, Hasan S, Sen-Gupta B. Microfiltrationof water-based paint effluents, Adv. Environ. Res. 2004;8:455–466.
2. Mahdavi TA, Donnelly T, Andersonm GK. Colourremovalfrom simulated dye wastewater using a two-phase anaerobic packed bedreactor, Water Res. 2001;35: 425–432.
3. Ojoawo SO, Udayakumar G, Naik P. Phytoremediation of phosphorus and nitrogen with canna x generalis reeds in domestic wastewater through NMAMIT constructed wetland. Aquatic Procedia. 2015;4:349–356.
4. Worku A, Tefera N, Kloos H, et al. Biosremediation of brewery wastewater using hydroponics planted with vetiver grass in Addis Ababa, Ethiopia. Bioresour. Bioprocess. 2018;5:39-43.
5. Sasikumar CS, Papinazath T. Environmental management: Bioremediation of Environment" Martin, J., Bunch, V. eds. Proceedings of the third international conference on environment and health, Chennai, India. 2003;465-469.
6. Melcalf L, Eddy HR. Wastewater engineering, treatment and reuse (4th edition), Tale McGraw-Hill, New York; 2003.
7. Ram SL, Pravin US, Deepali SP. Study on Physico-chemical Parameters of Waste Water Effluents from Taloja Industrial Area of Mumbai, India. Int. J. Ecosyst. 2011;1: 1–9.
8. Aboulhassan MA, Souabi S, Yaacoubi A, Baudu M. Treatment of Paint Manufacturing Wastewater the Combination of Chemical and Biological Processes. Inter. J. Sci. Environ. Technol. 2014;3:1747–1758.
9. Nwachukwu SCU, Akpata TVI. Principles of Quantitative Microbiology; 2003.
10. Central California Area Office- American River Water Education Center. Water

facts- worldwide water supply. Bureau of reclamation; 2020.
11. Usharani K, Umarani K, Ayyasamy PM, Shanthi K, Lakshmanaperumalsamy P. Physico-chemical and bacteriological characteristics of Noyyal River and groundwater quality of Perur, India. J. Appl. Sci. Environ. Managccc. 2010;14:29–35.
12. Endeshaw A, Birhanu G, Zerihun T, Misganaw W. The role of microorganisms in bioremediation- A review. Open Journal of Environmental Biology. 2017;7.20-25.
13. Tolulope E, Aniyikaiye T, Oluseyi J, Odiyo O, Joshua NE. Physico-Chemical Analysis of Wastewater Discharge from Selected Paint Industries in Lagos, Nigeria. Intl. J.Envir. Res. Public Health. 2019;16:123-127.
14. Rachna B, Disha J. Water Quality Assessment of Lake Water: A Review. Sustain. WaterResour. Manag. 2016;2: 161–173.

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