

Efficiency of the WWTP in a Small Town of South Africa to Eradicate *E. COLI*

Makuwa S.¹, Green E.², Fosso-Kankeu E.³ and Tlou G⁴.

Abstract— *E. coli* is a common Gram-negative facultative anaerobic bacterium. Its principal habitat has been identified in the intestinal tracts of mammals and birds as well as non-host environment (water/sediments). It is widely used as an indicator bacterium and its detection in water indicates possible pollution with waterborne pathogens. The organism's quantification after post treatment (chlorination) serves to evaluate the performance of a WWTP for microbial reduction. South African General Authorisation and Special standards stipulate that treated sewage effluents must have a limit of 1000 and 0 (zero) *E. coli* (per 100mL) respectively. This study aims at determining the efficiency of the WWTP in a small town of South Africa in eradicating the presence of *E. coli* through its contact tank disinfection (chlorination) process. Samples were collected for detection of *E. coli* using Colilert 18 method, from wastewater before chlorination and final effluent after chlorination. Physicochemical analyses were also conducted from the final effluent discharged to the river (Mooi River). The plant applies an average of 10 KG per hour of chlorine to eradicate *E. coli*. Since the inception of the study, up to date the plant is at an average of 48 *E. coli* count detected (8% compliance to zero *E. coli*) at the final effluent. Suspended solids which enhance the presence of these organisms were within the plant WUL at all time. An average of 3.7 mg/L of turbidity was detected. In comparison with the plant WUL, other physicochemical determinants such as pH, conductivity, nitrate, ammonia, ortho-phosphate and COD were at 100%, 100%, 7%, 97%, 100% and 100% respectively. Nitrate is also one determinant affecting the plant optimal performance. Although the average detected organisms showed to be lower, however the plant efficiency of eradicating the organisms is not efficiently meeting the regulated required limits of the plant. Such investigation is crucial in determining the compliance of the plant to its Water Use License (WUL).

Keywords— *E. coli*, Wastewater Treatment Plant (WWTP), Chlorination, Water Use License (WUL), Physicochemical.

I. INTRODUCTION

Wastewater is a complex mixture of both inorganic and organic materials. It is divided into domestic waster (also known as sewage), industrial wastewater, and municipal

wastewater which is a mixture of the two [1]. There are two crucial reasons for treatment of wastewater: to prevent pollution, by protecting the environment; and protecting public health by safeguarding water supplies and preventing the spread of waterborne diseases [2]. According to General Authorisation in terms of section 39 of the national Water Act of 1998, Table 1 indicates the compliance limit required before a wastewater can be discharged into the environment. However, certain plants can be regulated differently on their Water Use License depending on the capacity and design of the plant. Effluent discharges from treatment process can be an important source of pathogenic bacteria in surface waters [3, 4], however like any other WWTP in South Africa, the basic function of a WWTP is to speed up the processes of purifying wastewater. *E. coli* is widely used as an indicator bacterium [5] and its detection in water indicates possible pollution with waterborne pathogens if treatment process is insufficient [6, 7, 8, 9]. The quality water is important for the well-being of the environment, society and economy [10]. Being exposed (consuming or in contact) to water contaminated with *E. coli* is deleterious to human health as well as aquatic life [11]. The organism's quantification after chlorination which is widely used to disinfect wastewater prior to discharge, serves to evaluate the performance of a WWTP for microbial reduction [12]. The growing demand for water for industrial, agricultural, environmental, municipal and domestic requirements has extended the requirements for an improvement in water treatment processes [13]. Due to certain factors such as resistance of microbial community [9, 11, 14], malfunction or poor management of wastewater systems [15], effluent containing pathogenic bacteria are discharged into receiving water bodies [16, 17]. Section 24 (a) of the South African constitution [18] state that "Every human has the right to an environment that is not harmful to human health or well-being". The very same constitution [18] on section 24 (b) further state that "Everyone has the right to have the environment protected". This study aims at determining the efficiency of the WWTP at a small town in North West Province of South Africa in eradicating the presence of *E. coli* as per the plant WUL requirements. The physicochemical compliance to WUL was also assessed in this study.

Manuscript received September 27, 2019. This work was supported in part by the University of Johannesburg.

S. M. Makuwa Author is with the Department of Biotechnology, University of Johannesburg, Doornfontein, South Africa.

E. Green is with Department of Biotechnology, University of Johannesburg, Doornfontein, South Africa.

E. Fosso-Kankeu is with the Water Pollution Monitoring and Remediation Initiatives Research Group in the School of Chemical and Minerals, Engineering at the North-West University, Potchefstroom, South Africa; Email: elvisfosso.ef@gmail.com; Elvis.FossoKankeu@nwu.ac.za.

G. Tlou is with School of Chemical and physical sciences at the North-West University, Mafikeng, South Africa.

TABLE I: WASTEWATER LIMIT VALUES APPLICABLE TO DISCHARGE OF WASTEWATER INTO A WATER RESOURCE

SUBSTANCE/PARAMETER	GENERAL LIMIT	SPECIAL LIMIT
Faecal Coliforms (per 100 mL)	1000	0
Chemical Oxygen Demand (mg/L)	75	30
pH	5.5-9.5	5.5-7.5
Ammonia (ionised and un-ionised) as Nitrogen (mg/L)	6	2
Nitrate/Nitrite as Nitrogen (mg/L)	15	1.5
Chlorine as Free Chlorine (mg/L)	0.25	0
Suspended Solids (mg/L)	25	10
Electrical conductivity (mS/m)	70 mS/m above intake to a maximum of 150 mS/m	50 mS/m above background receiving water, to a maximum of 100 mS/m
Ortho-Phosphate as phosphorous (mg/L)	10	1 (median) and 2.5 (maximum)
Fluoride mg/L	1	1

II. METHODOLOGY

A. Study Area

A Municipality's WWTP, at a small town in North West Province of South Africa was selected as a study area for collection of samples. The area is roughly 120 km (75 mi) west-southwest of Johannesburg and 45 km (28 mi) east-northeast of Klerksdorp. The town itself and surrounding suburbs have a population of 123,669. It is an industrial, service and agricultural growth point of North West province.

B. Sample Collection

All procedures were performed according to the protocols described in the Standard Methods for the Examination of Water and Wastewater [19].

Water samples were obtained from the WWTP at one of municipality's in North West Province, South Africa. Samples were collected aseptically using sterile sampling bottles 1L and 250 mL, for physicochemical and microbiological tests respectively. Samples were collected at the following points:

- At the influent before contact tank (before disinfection);
- At the discharge point after contact tank (after disinfection).

III. DETECTION OF *E. COLI*

Collected samples were analysed for presence of *E. coli* using Colilert® method [20]. The Quanti-trays were incubated at 37 °C for 18-24 hours. The results of the quantifications were reported as *E. coli* count/100 mL.

IV. PHYSICOCHEMICAL TESTING

Temperature, pH and chlorine parameters were measured *on site* using XS instrument Bench Meter for temperature and pH, while HACH colorimeter was used to measure chlorine (HACH instrument, Pocket Colorimeter™, Loveland, USA). Chlorine was measured onsite. Other Physicochemical tests were done for all the samples at the Municipality's Laboratory using Gallery Discrete Analyser (Thermo Scientific).

V. RESULTS

A. Detection of *E. coli*

Colilert method is based on Defined Substrate Technology®. The product utilizes nutrient indicators that produce color and fluorescence when metabolized by *E. coli*. It can detect *E. coli* at 1 CFU/100 mL within 18-22 hours. In all the 119 samples analysed for *E. coli*, only 9 samples were negative for *E. coli*. As expected the number of *E. coli* present in the samples declined from samples before disinfection to final effluent disinfected samples. The compliance of the plant to its WUL limits of zero *E. coli* was 8% (see Table 2). From the number of *E. coli* which entered the contact tank before disinfection, the plant managed to eradicate 99 % in all the month studied (see Table 3).

The study also measured the number of *E. coli* entering the contact tank before disinfection in relation to the number of this organisms surviving disinfection. The analyses were done for a period of four month (January – April 2019). In all the months studied, the plant was not able to obtain 100% reduction of *E. coli*, instead a reduction of around 99% was achieved.

TABLE II: COMPLIANCE OF *E. COLI* TO THE WWTP'S WUL

Date	<i>E. coli</i>			
Jan 2019-Apr 2019	Total Samples Tested	No. of zero <i>E. coli</i> samples	Avg	% Compl to WUL Limit
	119	9	48	8%

Avg = Average

Compl = Compliance

WUL = Water Use License

TABLE III: AMOUNT OF *E. COLI* ERADICATED THROUGH CHLORINATION TREATMENT PROCESS

Date	<i>E. coli</i> count/100m L before chlorination	<i>E. coli</i> counts/100m L after chlorination	Eradiated <i>E. coli</i> %
Jan-19	330095	47	99.99
Feb-19	726795	10	99.99
Mar-19	378106	96	99.97
Apr-19	20934	37	99.82

B. Physicochemical Quality of the Final Effluent

Water samples were tested for a wide range of physicochemical parameters. The findings were compared against the plant WUL limits. Table 4 indicates the limits from the plant WUL.

TABLE IV: RECOMMENDED LIMITS FOR DISCHARGE INTO THE ENVIRONMENT

SUBSTANCE/PARAMETER	WUL LIMITS
Faecal Coliforms (per 100 mL)	0
Chemical Oxygen Demand (COD) (mg/L)	≤75 after applying chloride correction
pH	5.5-9.5
Ammonia (as N) (mg/L)	10
Nitrate (as N) (mg/L)	3
Suspended Solids (mg/L)	≤25
Electrical conductivity (mS/m)	≤75 mS/m above intake water
Ortho-Phosphate as phosphorous (mg/L)	<3
Fluoride mg/L	1

As shown in Table 5, eight parameters were analysed and compared with the plant WUL limits stipulated in Table 4. pH (6.74 – 7.9), suspended solids (0.6 – 17 mg/L), electrical conductivity (72.27 – 117.7 mS/m) and ortho phosphate (0-1.93 mg/L) were always within the required limits and achieved compliance of 100%, while ammonia (0 – 10.59 mg/L) and Chemical Oxygen Demand (COD) (3 – 90 mg/L) compliances were 97% and 98% respectively. Since the free residual chlorine (0.16 – 2.65 mg/L) is not regulated on the municipal WUL, it was then measured in line with General Authorization (G.A) limits of 0.25 mg/L. Of the 120 samples analysed for free residual chlorine, 12% of them complied with G.A limits. Nitrate was the parameter with lowest compliance, detected between 0.1-11.4 mg/L, with a compliance of 9% to the plant WUL limits.

Turbidity and temperature were the other studied physicochemical parameters not regulated in South African wastewater final effluent. Temperature measured was between 19 and 26°C. Detected turbidity was between 1 and 4 NTU, with an average of 3.7 NTU.

TABLE V: PHYSICO-CHEMICAL PARAMETERS VALUES OF WATER

Parameters	Avg	No. of failures	No. of samples analysed	% compl
Cl ₂ mg/L	0.83	106	120	12%
pH	7.465	0		100%
S.S mg/L	7.64	0		100%
E.C mS/m	88.37	0		100%
Nitrate mg/L	5.756	109		9%
NH ₃ (mg/L)	2.688	3		97%
Ortho P (mg/L)	0.428	0		100%
COD mg/L	26.2	2		98%

VI. DISCUSSION

One of the ways to evaluate water quality is to measure water quality variables and assess values for benchmarks, such as guidelines. The wastewater treatment plant at the study area is regulated by South African Department of Water and Sanitation through its WUL which stipulates limits mentioned in Table 4. The License was obtained in 2016 and amended in 2017 due to set limits that were not in line with the plant design. Previously the system was operating under G.A limits. The plant maintained its Green Drop status for several years since its inception in 2008 except in 2009 [22, 23, 24, 25]. Its compliance limits for *E. coli* therefore moved from 1000 *E. coli* count/100mL (G.A) to 0 *E. coli* count/100 mL (WUL).

E. coli

Water quality is a newsworthy issue in the health of public due to concerns arising from the none-quality discharge of inadequately treated sewage into water bodies, which can be of health concern to human and the environment [26, 27]. Microbiological water quality standards are majorly based on faecal indicators; though they signify a minor part of the total bacterial in aquatic environment and *E. coli* is a frequently used indicator organism to monitor the microbial quality of water [28]. Even though the plant managed to eradicate *E. coli* completely in 9 times out of the 119 tests, however, from the counts exposed to disinfection, the plant is able to eradicate more than 99%. It has been known for many years that the number of bacteria present in the effluent of an activated-sludge plant is reduced 90 to 99% as compared with the influent [29, 30]. Analysing the effectiveness of *E. coli* reduction, Bonde, 1990 observed that the number of *E. coli* decreased by 95% [31], whereas Szumilas study reported that, modern sewage treatment plants are able to reduce more than 99.99% of coliform bacteria [32]. Study by Budzinska and colleagues also showed elimination of *E. coli* in treated wastewater within the range from 99.40 to 99.92% [33]. Similar findings of above 99% *E. coli* reduction was also observed in this study. When the presence of this organism is not completely eradicated, it therefore indicates possible pollution with pathogenic waterborne organisms [7, 8, 9]. According to a study by Samie, only 2 out of the 14 studied plants complied with the South African General and Special standards which stipulate that treated sewage effluents must have a standard of nil faecal coliforms (Act 96 of 18 May 1984 No. 9225, Regulation 991) [34]. Zero *E. coli* detection was also observed in this study in few occasions.

The average count discharged into the environment is less than 100 (48 *E. coli* counts/100 mL) which therefore meets the compliance to the limits of 1 000 cfu/ 100 mL set for agricultural purposes (DWAf, 1996). Of the 14 plants studied by Samie, 9 of them complied with the agricultural purpose limit [34].

Physicochemical Parameters

Apart from chlorine, turbidity and temperature, out of the 8 physicochemical parameters studied, Ammonia, COD and Nitrate were the only parameters that did not comply to set WUL limits. Osulale and Okoh study outcomes showed that

six (6) out of the twelve (12) parameters tested complied with the South African (SA) effluent GA discharge standard limits as outlined in DWAF [21].

The nitrate level ranged between 0.1 mg/L to 11.4 mg/L compared to 8.03 mg/L to 18.70 mg/L detected in Osuolale and Okoh study. Elevated levels of nitrates can result in eutrophication, giving rise to increase algae growth and eventually leading to reduced dissolved oxygen levels in the water [37]. Osode [38] and Odjadjare et al. [39] also reported compliance of some of the WWTPs in the Eastern Cape Province with respect to nitrate, while in this study, only 11 out of the 120 samples analysed complied with the plant WUL limit.

The disposal of untreated high strength ammonium wastewater in water bodies is a significant environmental problem because of the harmful effect of the free ammonia (FA) on the aquatic life and environment [40, 41]. Study by Agoro and colleagues indicated effluents discharges of ammonia between 0.06 and 112 mg/L [42] compared to 0 – 10.59 mg/L in the current study. Chemical Oxygen Demand (COD) is a measure of the oxygen equivalent of organic materials in wastewater and a widely used indicator of wastewater quality [43, 44]. Compliance for COD in the current study (98%) was higher compared to Osuolale and Okoh study in the Eastern Cape Province of South Africa with compliance of 77% to its regulatory standard [35]. As compared to the China's discharge standard limit for wastewater of 60 and 120 mg/L for Grade 1 and 2 respectively [45], while in Germany, the COD discharge limits range from 75 mg/L to 150 mg/L for different scales WWTPs [46]. The current plant WUL limits is stated in table 4. All COD values were higher than the maximum accepted values (125 mg O₂/L) of the Romanian Law in Popa and colleagues study [47].

Due to the lack of regulatory standards limit at the study plant's WUL, chlorine was compared against South African GA limits. According to South African Regulation Standards (G.A limits) as indicated in Table 2, a WWTP is allowed to discharge residual chlorine of less 0.24 mg/L. The studied plant discharge limit is not within the required limit, therefore literature state that many of these chemical disinfectants if overdosed or used inappropriately can react with organic and inorganic precursors and bring the formation of disinfection by-products (DBPs) with adverse health effects to aquatic life [48], therefore affecting the limited water resources in the country [. About 67% of the WWTP-A samples had low free chlorine concentration below the recommended set limit while WWPT-B samples had acceptable levels of free chlorine concentration in a study by Osuolale and Okoh [21].

Turbidity of the final effluent can serve as a measure of treatment efficiency [69-71]. Due to the lack of regulatory standard, the turbidity levels were compared against South Arabian limit for irrigation as well as aesthetic limit from South African Standard for Drinking Water of 5 NTU as there are no set limits for these parameter in SA. In this study, the turbidity detected was less compared to the above limits.

VII. CONCLUSION

The peculiar challenges facing the water and sanitation sector in South Africa are still paramount. In South Africa water is most critical resource and its quality is a prerequisite for all forms of life. Treated wastewater is used to alleviate challenge of water shortages.

Effluent discharges from treatment processes can be an important source of pathogenic bacteria in surface waters however like any other WWTP in South Africa, the basic function of the studied plant WWTP is to speed up the processes of purifying wastewater. Although the treatment plants succeeded in removing majority of *E. coli* from the influents which however were not mostly in line with its WUL limit, effluent discharges are only occasionally devoid of the organisms, thus constituting a potential threat of incidences of infectious diseases.

The high non-compliance with regard to nitrate level suggests that inorganic matters are present in high amounts in the effluent discharge. The continuous discharge of this poor quality effluent will contribute to the eutrophication of the surface water body and subsequently lead to the death of aquatic organisms.

REFERENCES

- [1] Odlare M. (2014). Introductory Chapter for Water Resources. Earth Systems and Environmental Sciences. <https://doi.org/10.1016/B978-0-12-409548-9.09035-7>.
- [2] Singureanu C., Woinaroschy A. (2017). Simulation of Bardenpho Wastewater Treatment process for Nitrogen removal using Superpro Designer Simulator. *UPB Scientific Bulletin, Series B* 79 (4).
- [3] Okoh A.I., Odjadjare E.E., Igbinosa E.O., Osode A.N. (2007). Wastewater treatment plants as a source of microbial pathogens in receiving watersheds. *African Journal of Biotechnology* 6 (25): 2932-2944. <https://doi.org/10.5897/AJB2007.000-2462>
- [4] Auerbach E.A., Seyfried E.E., McMahon K.D. (2007). Tetracycline resistance genes in activated sludge wastewater treatment plants. *Water Research* 41: 1143-1151. <https://doi.org/10.1016/j.watres.2006.11.045>
- [5] Rompré A., Servais P., Baudart J., de-Robine M. R., Laurent P. (2002). Detection and enumeration of coliforms in drinking water: current methods and emerging approaches, *Journal of Microbiological Methods.*, 49 (1): 31-54. [https://doi.org/10.1016/S0167-7012\(01\)00351-7](https://doi.org/10.1016/S0167-7012(01)00351-7)
- [6] George I., Crop P., Servais P. (2002): Fecal coliform removal in wastewater treatment plants studied by plate counts and enzymatic methods. *Water Research* 36: 2607-2617. [https://doi.org/10.1016/S0043-1354\(01\)00475-4](https://doi.org/10.1016/S0043-1354(01)00475-4)
- [7] Scott T.M., Rose J.B., Jenkins T.M., Farrah S.R., Lukasik J. (2002). Microbial Source Tracking: Current Methodology and Future Directions. *Applied and Environmental Microbiology*, 68 (12): 5796-5803. <https://doi.org/10.1128/AEM.68.12.5796-5803.2002>
- [8] Yassi A., Kjellström T., De Kok T., Guidotti T. (2001). Basic environmental health, Oxford University Press. New York. p.210. <https://doi.org/10.1093/acprof:oso/9780195135589.001.0001>
- [9] Zhi S., Banting G., Li Q., Edge T.A., Topp E., Sokurenko M., Scott C., Braithwaite S., Ruecker N.J., Yasui Y., McAllister T., Chui L., Neumann N.F. (2016). Evidence of Naturalized Stress-Tolerant Strains of *Escherichia coli* in Municipal Wastewater Treatment Plants. *Applied and Environmental Microbiology* 82 (18): 5505-5518.
- [10] UNEP, 2010. Study on mercury sources and emissions, and analysis of cost and effectiveness of control measures "UNEP Paragraph 29 study".

- United Nations Environmental Programme, Chemicals Branch, DTIE, Geneva, Switzerland, November 2010.
- [11] Owoseni M.C., Olaniran A.O., Okoh A.I. (2017). Chlorine Tolerance and Inactivation of *Escherichia coli* recovered from Wastewater Treatment Plants in the Eastern Cape, South Africa. *Applied Science* 7 (8): 810. <https://doi.org/10.3390/app7080810>
- [12] Davies C.M., Long J.A., Donald M., Ashbolt N.J. (1995). Survival of fecal microorganisms in aquatic sediments of Sydney, Australia. *Applied and Environmental Microbiology* 61: 1888–1896.
- [13] Levantesi C., La Mantia R., Masciopinto C., Beockelmann U., Ayuso-Gabella M.N., Salgot M., Tandoi V., Van Houtte E., Wintgens T., Grohmann E. (2010). Quantification of pathogenic microorganisms and microbial indicators in three wastewater reclamation and managed aquifer recharge facilities in Europe. *Science of the Total Environment* 40(21): 4923–4930. <https://doi.org/10.1016/j.scitotenv.2010.07.042>
- [14] Dungeni M., Van der Merwe R., Momba M. (2010). Abundance of pathogenic bacteria and viral indicators in chlorinated effluents produced by four wastewater treatment plants in the Gauteng Province, South Africa. *Water Research* 36: 607–614. <https://doi.org/10.4314/wsa.v36i5.61994>
- [15] Ahmed W., Neller R., Katouli M. (2005). Evidence of septic system failure determined by a bacterial biochemical fingerprinting method. *Journal of Applied Microbiology* 98: 910–920. <https://doi.org/10.1111/j.1365-2672.2004.02522.x>
- [16] Geary P.M., Gardner E.A. (1998). Sustainable On- site Treatment Systems, Individual and Small Community Sewage Systems. St Joseph, Michigan: *American Society of Agricultural Engineers*.
- [17] Wakelin S., Colloff M.J., Kookana R.S. (2008). Effect of Wastewater Treatment Plant Effluent on Microbial Function and Community Structure in the Sediment of a Freshwater Stream with Variable Seasonal Flow. *Applied and Environmental Microbiology* 74 (9): 2659–2668.
- [18] Constitution of the Republic of South Africa, 1996.
- [19] Standard Method for the Examination of Water and Wastewater. 2008.
- [20] Sartory D.P., Allaert Vandevenne C. (2009). Improved methods for detecting *E. coli* and coliforms in drinking water: AFNOR validation of Colilert®-18/Quanti-Tray®. *Journal of Microbiological Methods* 68(3): 522-9
- [21] DWAF, Department of Water Affairs and Forestry 1996, South African water quality guidelines (2nd edition), Vol. 2, Domestic Use. Pretoria, RSA (1996). *Compilation of Water Quality Guidelines and Standard*.
- [22] DWA, Department of Water Affairs 2009, *Municipal Wastewater Treatment: Base information for targeted risk-based regulation: (Limpopo Province)*, 2009.
- [23] DWA, Department of Water Affairs 2011, *Green drop report 2011: Wastewater service regulation*, Republic of South Africa.
- [24] DWA, Department of Water Affairs 2012, *Green drop progress report 2012: Wastewater service regulation*, Republic of South Africa.
- [25] DWA, Department of Water Affairs 2014, *Green drop progress report 2012: Wastewater service regulation*, Republic of South Africa.
- [26] Suthar S., Sharma J., Chabukdhara, M., Nema A.K. (2010). Water quality assessment of river Hindon at Ghaziabad, India: Impact of industrial and urban wastewater. *Environmental Monitoring* 165: 103–112. <https://doi.org/10.1007/s10661-009-0930-9>
- [27] Calijuri M.L., do Couto E.D.A., Santiago A.D.F., Camargo R.D.A., e Silva M.D.F.M. (2011). Evaluation of the Influence of Natural and Anthropogenic Processes on Water Quality in Karstic Region. *Water Air Soil Pollution* 223: 2157–2168. <https://doi.org/10.1007/s11270-011-1012-5>
- [28] Turolla A., Cattaneo M., Marazzi F., Mezzanotte V., Antonelli M. (2018). Antibiotic resistant bacteria in urban sewage: Role of full-scale wastewater treatment plants on environmental spreading. *Chemosphere* 191:761–769. <https://doi.org/10.1016/j.chemosphere.2017.10.099>
- [29] Kabler P. 1959. Removal of pathogenic microorganisms by sewage treatment processes. *Sewage Ind. Wastes* 31:1373-1382.
- [30] Kampelmacher E. H., van Noorle Jansen L.M. (1970). Salmonella - its presence in and removal from a wastewater system. *Journal of Water Pollution Control Federation* 42:2069-2073.
- [31] Bonde G.J. (1990). Pollution of a marine environment, *Water Poll. Contr. Fed. Washington* 2(45).
- [32] Szumilas T., Michalska M., Bartoszewicz M. (2001). Characteristics of bacterial contamination of municipal wastewater from a large urban agglomeration and evaluation of the reduction of pollution in wastewater treatment. *Roczn. PZH* 52(2): 155 (in Polish).
- [33] Budzińska K., Szejniuk B., Jurek A., Traczykowski A., Michalska M., Berleć K. (2014). Effectiveness of removing microbial pollutants from wastewater by the activated sludge method. *Environment Protection Engineering* 40 (4): 53-67.
- [34] Samie A., Obi C.L., Igumbor J.O., Momba M.N.B. (2009) Focus on 14 sewage treatment plants in the Mpumalanga Province, South Africa in order to gauge the efficiency of wastewater treatment. *African Journal of Biotechnology* 8: 3276–3285.
- [35] Osuolale O., Okoh A. (2015). Assessment of the Physicochemical Qualities and Prevalence of *Escherichia coli* and *Vibrios* in the Final Effluents of Two Wastewater Treatment Plants in South Africa: Ecological and Public Health Implications. *International Journal of Environmental Research and Public Health* 12: 13399-13412 <https://doi.org/10.3390/ijerph121013399>
- [36] Department of Water Affairs and Forestry. Revision of General Authorisations in Terms of Section 39 of the National Water Act, 1998 (ACT NO. 36 OF 1998) (THE ACT). South African Gov. Gaz. 2013, 3–32.
- [37] Adeyemo O.K., Adedokun, O.A., Yusuf, R.K., Adeleye, E.A. (2008). Seasonal changes in physico-chemical parameters and nutrient load of river sediments in Ibadan city. *Nigeria. Glob. Nest J.* 10: 326–336. <https://doi.org/10.30955/gnj.000458>
- [38] Osode A. N. (2014). The impact of wastewater quality on receiving water bodies and public health in Buffalo City and Nkonkobe Municipalities, University of Fort Hare, 2007. Available online: <http://hdl.handle.net/10353/69> (accessed on 3 January 2014)
- [39] Odjajare E.E., Igbinsola E.O., Mordi R., Igere B., Igeleke C.L., Okoh A.I. (2012) Prevalence of multiple antibiotics resistant (MAR) *Pseudomonas* species in the final effluents of three municipal wastewater treatment facilities in South Africa. *Int. J. Environ. Res. Public Health* 9: 2092–107. <https://doi.org/10.3390/ijerph9062092>
- [40] Carrera J., Baeza J. A., Vicent T., Lafuente, J. (2003). Biological nitrogen removal of high strength ammonia industrial wastewater with two-sludge system. *Water Res.* 37: 4211–4221. [https://doi.org/10.1016/S0043-1354\(03\)00338-5](https://doi.org/10.1016/S0043-1354(03)00338-5)
- [41] Lay-Son M., Drakides C. (2008). New approach to optimize operational conditions for the biological treatment of a highstrength thiocyanate and ammonium waste: pH as key factor. *Water Res.* 42: 774–780. <https://doi.org/10.1016/j.watres.2007.08.009>
- [42] Agoro M.A., Okoh O.O., Adefisoye M.A., Okoh A.I. (2018). Physicochemical Properties of Wastewater in Three Typical South African Sewage Works. *Polish Journal of Environmental Studies* 27(2): 491-499. <https://doi.org/10.15244/pjoes/74156>
- [43] Kang Y.W., Cho M.J., Hwang K.Y. (1999). Correction of hydrogen peroxide interference on standard chemical oxygen demand test. *Water Res.* 33: 1247-1251. [https://doi.org/10.1016/S0043-1354\(98\)00315-7](https://doi.org/10.1016/S0043-1354(98)00315-7)
- [44] Metcalf L., Eddy H.P. (2004). *Wastewater Engineering: Treatment and Reuse*. 4th Edition, McGraw-Hill, New York, USA, pp: 93-94.
- [45] Zhou Y., Duan N., Xuefang Wu X., Fang H. (2018). COD discharge limits for Urban Wastewater Treatment Plants in China based on statistical methods. *Water* 10 (777): 2-10. <https://doi.org/10.3390/w10060777>
- [46] Federal Ministry for the Environment, Nature Conservation and Nuclear Safety. Promulgation of the New Version of the Ordinance on Requirements for the Discharge of Waste Water into Waters (Waste

- Water Ordinance—ABWV); Federal Ministry for the Environment, Nature Conservation and Nuclear Safety: Berlin, Germany, 2004.
- [47] Popa P., Timofiti M., Voiculescu M., Dragan S., Catalin Trif C., Georgescu L.P. (2012). “Study of Physico-Chemical Characteristics of Wastewater in an Urban Agglomeration in Romania” – *The scientific world journal* pp. 1-10.
<https://doi.org/10.1100/2012/549028>
- [48] Collivignarelli M.C., Abbà A., Benigna I., Sorlini S., Torretta V. (2017). Overview of the Main Disinfection Processes for Wastewater and Drinking Water Treatment Plants. *Sustainability* 10 (86): 1- 21.
<https://doi.org/10.3390/su10010086>
- [49] Elvis Fosso-Kankeu. 2019. Nano and Bio-based Technologies for wastewater treatment: Prediction and Control Tools for the dispersion of Pollutants in the Environment. Wiley Scrivener. ISBN: 978-1-119-57709-6. Pp 301-336.
- [50] Elvis Fosso-Kankeu. 2019. New Horizons in Wastewaters Management: Emerging Monitoring and Remediation Strategies. Nova Science Publishers. ISBN: 978-1-53615-659-1.
- [51] J.G. Redelinghuys, E. Fosso-Kankeu, G. Gericke, F. Waanders. 2019. Coal Power Plant Wastewater Treatment by Thermal and Membrane Technologies. In Nano and Bio-based Technologies for wastewater treatment: Prediction and Control Tools for the dispersion of Pollutants in the Environment. Editor: Elvis Fosso-Kankeu. Wiley Scrivener. ISBN: 978-1-119-57709-6. Pp 149-168.
<https://doi.org/10.1002/9781119577119.ch5>
- [52] N. Mukwevho, E. Fosso-Kankeu, F. Waanders. 2019. PAHs Released from Coal Tars and Potential Removal Using Nanocatalysts. In Nano and Bio-based Technologies for wastewater treatment: Prediction and Control Tools for the dispersion of Pollutants in the Environment. Editor: Elvis Fosso-Kankeu. Wiley Scrivener. ISBN: 978-1-119-57709-6. Pp 169-203.
<https://doi.org/10.1002/9781119577119.ch6>
- [53] E. Fosso-Kankeu, P. Jagals, H. Du Preez, Exposure of rural households to toxic cyanobacteria in container-stored water. *Water SA*, Vol. 34, no. 5, pp. 631-636, 2008.
<https://doi.org/10.4314/wsa.v34i5.180660>
- [54] E. Fosso-Kankeu, A. Mulaba-Bafubiandi, B.B. Mamba, T.G. Barnard, Mitigation of Ca, Fe, and Mg loads in surface waters around mining areas using indigenous microorganism strains. *Journal of Physics and Chemistry of the Earth*, Vol. 34, pp. 825-829, 2009.
<https://doi.org/10.1016/j.pce.2009.07.005>
- [55] E. Fosso-Kankeu, H. Du Preez, P. Jagals, The health implication of relationships between bacterial endotoxin, cyanobacteria, coliforms and water stored in domestic containers of rural households in South Africa. *Journal of Water and Health*, Vol. 8, no. 4, pp. 601-610, 2010.
<https://doi.org/10.2166/wh.2010.094>
- [56] E. Fosso-Kankeu, A. Mulaba-Bafubiandi, B.B. Mamba, L. Marjanovic, T.G. Barnard, A comprehensive study of physical and physiological parameters that affect biosorption of metal pollutants from aqueous solutions. *Journal of Physics and Chemistry of the Earth*, Vol. 35, pp. 672-678, 2010.
<https://doi.org/10.1016/j.pce.2010.07.008>
- [57] E. Fosso-Kankeu, A.F. Mulaba-Bafubiandi, B.B. Mamba and T.G. Barnard, Prediction of metal-adsorption behaviour in the remediation of water contamination using indigenous microorganisms. *Journal of Environmental Management*. Vol. 92, no. 10, pp. 2786-2793, 2011.
<https://doi.org/10.1016/j.jenvman.2011.06.025>
- [58] H. Mittal, E. Fosso-Kankeu, Shivani B. Mishra, Ajay K. Mishra, Biosorption potential of Gum ghatti-g-poly (acrylic acid) and susceptibility to biodegradation by *B. subtilis*. *International Journal of Biological Macromolecules*. Vol. 62, pp. 370-378, 2013.
<https://doi.org/10.1016/j.ijbiomac.2013.09.023>
- [59] E. Fosso-Kankeu, F. Waanders, E. Maloy, Copolymerization of ethyl acrylate onto guar gum for the adsorption of Mg(II) and Ca(II) ions. *Desalination and Water Treatment*. doi: 10.1080/19443994.2016.1165147: pp. 1-10, 2016.
- [60] E. Fosso-Kankeu, F. Waanders, C.L. Fourie, Adsorption of Congo Red by surfactant-impregnated bentonite clay. *Desalination and Water Treatment*. doi: 10.1080/19443994.2016.1177599: pp. 1-9, 2016.
- [61] E. Fosso-Kankeu, A.F. Mulaba-Bafubiandi, L.A. Piater, M.G. Tlou, Cloning of the *cnr* operon into a strain of Bacillaceae bacterium for the development of a suitable biosorbent. *World Journal of Microbiology and Biotechnology*. DOI 10.1007/s11274-016-2069-5. 2016.
- [62] A. Manyatshe, E. Fosso-Kankeu, D. van der Berg, N. Lemmer, F. Waanders, H. Tutu, Metal retention potential of sediment and water quality in the Mooi River, South Africa. *Desalination and Water Treatment*. doi: 10.5004/dwt.2017.20222. 2017.
- [63] E. Fosso-Kankeu, A. Manyatshe, F. Waanders, Mobility potential of metals in acid mine drainage occurring in the Highveld area of Mpumalanga Province in South Africa: Implication of sediments and efflorescent crusts. *International Biodeterioration and Biodegradation*. Vol. 119, pp. 661-670, 2017.
<https://doi.org/10.1016/j.ibiod.2016.09.018>
- [64] E. Fosso-Kankeu, H. Mittal, F. Waanders, S.S. Ray, Thermodynamic properties and adsorption behaviour of hydrogel nanocomposites for cadmium removal from mine effluents. *Journal of Industrial and Engineering Chemistry*. Vol. 48, pp. 151-161, 2017.
<https://doi.org/10.1016/j.jiec.2016.12.033>
- [65] A. Manyatshe, E. Fosso-Kankeu, D. van der Berg, N. Lemmer, F. Waanders, H. Tutu, Dispersion of inorganic contaminants in surface water in the vicinity of Potchefstroom. *Physics and Chemistry of the Earth*. Vol. 100, pp. 86-93, 2017.
<https://doi.org/10.1016/j.pce.2017.04.008>
- [66] C. de Klerk, E. Fosso-Kankeu, F.B. Waanders, Evaluation of the antibacterial activity of metal impregnated multi-walled carbon nanotubes: impact of domestic wastewater as supporting medium. *Desalination and Water Treatment*. Vol. 99, pp. 272-281, 2017.
<https://doi.org/10.5004/dwt.2017.21735>
- [67] A. Manyatshe, E. Fosso-Kankeu, D. van der Berg, N. Lemmer, F. Waanders, H. Tutu, Metal speciation in the rivers around Potchefstroom based on seasonality. *Water Environment Research*. Vol. 90, no. 1, pp. 84-95, 2018.
<https://doi.org/10.2175/106143017X15054988926587>
- [68] L.P. Simelane, E. Fosso-Kankeu, P. Njobeh, S. Pandey, Response of bacterial biosorbents to chemical treatment as influenced by cell membrane structure and impact on the adsorption behaviour of dyes. *Current Science*. Vol. 114, no. 4, pp. 826-834, 2018.
<https://doi.org/10.18520/cs/v114/i04/826-834>
- [69] Barth E., Ettinger M. (1965). Summary report on the effects of heavy metals on the biological treatment processes. *J. Water Pollut. Control Fed.* 37: 86–96.
- [70] Hussain S.A. (2010). Isolation and Identification of Pathogenic Bacteria from Drinking Water of Khairpur, Sukkur and Rohri; Shah Abdul Latif University: Khairpur, Pakistan.
- [71] “Norms for establishing pollutant load limits of industrial and municipal wastewater to discharge into the natural receivers,” Romanian GD 352 from 21 April 2005, which modify and complete the GD 188/2002—Appendix 3, NTPA-001/2002.