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Linear alkyl benzene sulfonate concentration varies daily, weekly and seasonally in Pittsburg's waste water: it is possible that the college student population influences the variation, Crawford County, Kansas.

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LINEAR ALKYLBENZENE SULFONATE CONCENTRATION VARIES DAILY, WEEKLY AND SEASONALY IN PITTSBURG'S WASTEWATER: IT IS POSSIBLE THAT THE COLLEGE STUDENT POPULATION INFLUENCES THE VARIATION. CRAWFORD COUNTY, KANSAS

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An Abstract of the Thesis by
Balsam Alessa

Linear Alkylbenzene Sulfonate (LAS) is a biodegradable surfactant commonly used industrially and domestically. Concentrations had never been examined in Pittsburg's Wastewater Treatment Plant because the National Pollution Discharge Elimination System permit does not specify any standard for LAS in Pittsburg's wastewater. This study monitored influent and effluent LAS concentrations three times per day, every day for 12 weeks (Feb 24 – May 18, 2014). Concentrations were determined by the Methylene Blue Active Substances method.

There was very little variation in the effluent concentrations which were one tenth of those in the influent. LAS concentrations in influent were highly variable by the time of the day, day of the week, and week of the study period. Three weeks that were lower in influent LAS concentrations coincided with university activities. College students may have a large impact on influent LAS concentrations because they make up 25% of Pittsburg's population. One week was spring break, and the other two weeks were during final exams.

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CHAPTER I

1. INTRODUCTION

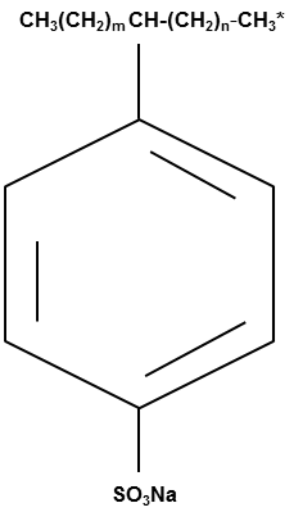
1.1 Uses

Linear Alkyl Benzene Sulfonate (LAS) is a biodegradable surfactant, which is commonly utilized industrially and domestically. LAS has been broadly utilized for more than 30 years. The global annual production of LAS is 2 million tons (Dionex.com., 2009). Eighty percent of which is used for household detergents, laundry detergent, and dishwashing products. The remaining 20% is used for industrial and institutional cleaners, textile production, agricultural processes, pesticides and as emulsifiers (Sablayrolles et al, 2009).

1.2 Chemical structure and properties

The chemical structure of LAS is $CH_3(CH_2)_mCH_-(CH_2)_n$ –CH_3SO_3Na (m+n= 7-10). The LAS is composed of a sulfonated aromatic ring attached to a linear alkyl chain containing between 10 to 13 carbon units. The length of alkyl chain determines the cleaning capability, biodegradability and toxicity (Figure 1) (Dionex.com., 2009). A sodium salt of LAS is utilized in detergents and cleaning products for domestic and industrial applications. It is a non-volatile anionic amphiphilic surfactant (Huang & Wang, 1994).

Figure 1 Structure of LAS



* (m + n= 7-10)

1.3 Biodegradability

Branched alkylbenzene sulfonate (BAS) is slow to biodegrade in waste treatment plants because of the branched alkyl chain. It has been replaced with Linear alkylbenzene sulfonate (LAS) over the past 25 years because LAS is more biodegradable and less toxic due to the linear alkyl chain. (Cler.com., 2014). LAS incorporating a benzene ring, which resists biodegradation. Bacterial degradation starts at the alkyl chain therefore all degradation products include benzene rings (Ying, 2006), and the final degradation product is benzene.

1.3.1 In laboratory

Biodegradation was tested in the laboratory using two bacterial species isolated from a wastewater treatment plant (Khleifat, 2006). Isolates of *Pantoea agglomerans* and *Serratia odorifera* tested individually were able to degrade LAS but a combination of the two species was much more effective, so the two species were cultured together in two media both containing 199.7 mg/L of LAS: minimal medium and nutrient broth. In the minimal medium containing LAS as the only carbon source, the bacterial combination was able to grow but only degraded 36% of the LAS. In the nutrient broth 70% was degraded. In another lab test degradation was up to 79% in 165 days (Olkowska et al, 2014).

1.3.2 In nature

The anaerobic biodegradation was studied in Upflow Anaerobic Sludge Blanket Reactors (UASB) (Sanz et al, 2003). Reactor 1 contained LAS and other carbon sources for three months while Reactor 2 was fed with a LAS solution without other carbon sources for four months. LAS was measured by High-performance liquid chromatography (HPLC) in influent and effluent streams of the liquid phase and in the solid phase (granular sludge used as biomass). The primary biodegradation of LAS was high (64 –85%). Biodegradation was higher when other carbon sources were absent. Thus, the surfactant can be partly used as carbon and energy source by anaerobic bacteria.

By comparing the biodegradation of LAS in a laboratory and in nature, biodegradation in nature was higher when LAS was only the source of carbon but was higher in a laboratory when there were many sources of carbon.

1.4 Toxicity

1.4.1 Microbes

Due to LAS surfactant properties, it adsorbs into sediment (Sanderson et al. 2006). Using a microbial community, which was isolated from polluted sediment, Flores et al. (2010) found that microbial growth was reduced by LAS and showed that LAS denatured proteins in the cell membrane, altering the permeability of the membrane to nutrients and other chemical substances. They determined the half maximal inhibitory concentration (IC_{50}) of LAS was 8.22 mg/L.

1.4.2 Algae

Varsha et al, (2011) demonstrated that LAS is equally toxic to fish and invertebrates, but toxicity to algae varied widely. Holt et al, (1992) demonstrated that the median lethal concentration (LC_{50}) at 72-96 hours ranged from (0.89 – 299 mg/L) for the fresh water algae and (0.024 – 9.9) mg/L for the marine groups.

1.4.3 Fish

The toxic effects of LAS were examined in the respiratory functions of tigerperch (*Terapon jarbua*) by three approaches (LC50, respiratory curve, and pathomorphological changes in gills after exposure to sublethal concentrations of LAS) (Huang & Wang,1994). Respiratory rate decreased from 0.018 ppm/min to 0.012 ppm/min, when LAS concentration changed from 3.5 mg/L to 5.0 mg/L. The secondary lamellae in gill epithelium was destroyed when LAS concentration reached 2.5 mg/L. The LC_{50} value was 3.28 mg/L. LAS could be lethal because it decreases the respiratory function. Varsha et al., (2011) demonstrated that 100% of ticto barb (*Puntius ticto)* died in a concentration of 28 mg/L at 24 hours. The LC_{50} was 25.5 mg/L. Thus, the overall LAS toxicity data concerning the aquatic organisms fluctuate between 1 and 10 mg per liter in brief durability experiments.

1.4.4 Mammals

LAS shows slight acute toxicity in Mammals. The oral the median lethal dose (LD_{50}) values for rats range from 1,080 to 1,980 mg/kg body weight (bw) (UNEP, 2005). While the oral LD_{50} values for mice are 2,160 mg/kg bw for males and 2,250 mg/kg bw for females. The dermal LD_{50} value for rat was greater than 2,000 mg/kg bw. Mortality occurring at respirable particle concentrations of 310 mg/m^3. All the studies about skin irritation on rabbits for LAS at a concentration of ~ 50% were consistent and showed similar irritation effects. In various repeated dose experiments with rats, mice, and monkeys who had been exposed to LAS via oral and dermal routes the lowest observed adverse effect level (LOAELs) ranged between 115 and 750 mg/kg bw/day. While no-observed adverse effect level (NOAELs) ranged between 40 and 250 mg/kg bw/day. The effects, which have been frequently observed, incorporated restrained body weight increase, diarrhea, raises in comparative liver weight, discrepancies in enzymatic and serum-biochemical criteria, and moderate degeneration and shedding of the tubular epithelium in the kidneys.

The activities of some enzymes, amino nitrogen, glutathione, lipid peroxidation, and histamine in skin, liver, and kidney for Guinea pigs showed increases after 30 days of topical treatment by 2.5 mg/kg and 5.0 mg/kg of LAS and quinalphos (a pesticide) alone and in combination (Marthur et al, 2000). The animal which had been receiving high doses showed erythema, edema, and hair loss. The treatment damaged skin, liver and kidney and was dose dependent. Skin was hyperkeratinized and contained increased levels of mononucleocytes. The liver cells were hypertrophic, and kidney tubules were necrotic and glomerular capsules were atrophied.

Forty-eight guinea pigs were subdivided equally into four groups and topically exposed to paraphenylenediamine (Paraphenylenediamine (p-PD) is the main aromatic amine used in the formulation of hair dyes) (PPD) (4 mg/kg), LAS (12 mg/kg) and PPD (4 mg/kg) plus LAS (12 mg/kg) for 30 days (Mathur et al, 2005). The enzymes activity, lipid per-oxidation, and histamine increased at the time when glutathione levels decreased in the skin. The histopathological investigation demonstrated serious hyperkeratosis, compression of collagen fibers and vacuolization of epidermal cells.

LAS is considered to be non-toxic for microbes. On the other hand, it is toxic to fish and invertebrates, but toxicity to algae varied widely. LAS shows slight acute toxicity in Mammals but it is toxic to Guinea pigs.

1.4.5 Toxicity of degradation products

Degradation products cause chronic and sub-lethal toxicities to aquatic animals, and some toxicity to the soil fauna (Ying, 2006). The terrestrial environment has appears to be the sink for the surfactants and degradation products. High concentrations of surfactants and their degradation products may negatively influence organisms in the environment (Ying, 2006). Benzene, which is the final degradation product, is a well-known human carcinogen and may cause leukaemia, aplastic anaemia and multiplex myeloma (Ying, 2006).

1.4.6 potentially toxic product resulting from the chlorination of benzene

Carlson & Kosian, (1987) determined the chronic toxicities of several chlorinated benzene compounds to fathead minnows (Pimephales promelas) from 32–33 day embryo through early juvenile development exposures.

1.5 Historical use

LAS has been utilized for more than 25 years industrially and domestically and usage is increasing (Lewis, 1991; Dionex.com, 2009). It has replaced the highly branched alkylbenzene sulfonate (BAS) which was created in 1964.

Despite the fact that utilizations of LAS will probably stabilize or even decrease somewhat in more developed countries, it will grow by at least 2.0–4.0% in the less-devolved regions such as: the Middle East, Africa, India, China, and Southeast Asia due to the fast increase in requirement for LAS in the Asia Pacific region (His.com, 2012). It is predicted that by 2016 the region will attribute for more than 50% of overall demand. Global increases in the demand for LAS are believed to have grown at an approximate annual average of 2% during 2011–2016. If current research discovers a better, less toxic surfactant, the LAS production will probably decrease.

1.6 Discharge

1.6.1 In environment

LAS is released directly into the environment as a component of fertilizers and pesticides (Sablayrolles et al, 2009). It also is directly discharged in untreated sewage, which is a common practice in many parts of the world (Whelan et al, 2008)

1.6.2 In wastewater

LAS enters wastewater treatment plants and aerobic treatment eliminate almost all of it. What remains is released into the surface water as effluent (Oliveira et al, 2010). Solids from the wastewater treatment will contain some LAS which disposed of, or maybe utilized as fertilizers (Sablayrolles et al, 2009).

1.7 The importance of this study

LAS, by comparison to other surfactants, is more biodegradable and is less harmful. However, it is still capable of interfering with several metabolic processes in aquatic life. The National Pollution Discharge Elimination System permit (NPDES) regulate and set standard for wastewater treatment. Pittsburg's permit does not require treatment for LAS.

Previous studies have shown that LAS removed by microbes. These studies have simply collected influent and effluent samples to determine the effectiveness of the wastewater treatment. This study examines the daily, weekly , and seasonal (12 weeks) variation of LAS concentration.

1.8 Purpose

The purpose of this study 1) to determine LAS concentration in wastewater coming into and being released from the WWTP to determine the effectiveness of the treatment plant. 2) to examine how LAS concentration varies through the day (10:00, 14:30, and 19:00), the week, and the study period (February 24, 2014 - May18, 2014).

1.9 Expectations

LAS concentration should be higher in the influent than in the effluent because previous studies have shown it is almost completely removed. Because people tend to do laundry and shower at predictable intervals, concentration should be different through times of the day, days of the week, and study period (12 weeks).

CHAPTER II

2. METHODS AND MATERIALS

2.1 Collecting samples

Influent and effluent samples were collected within five minutes at the bar screen and cascade respectively (Figure 2). Samples were collected three times each day (10:00, 14:30, 19:00)(Figure 3). Two replicates of each sample were collected using a bucket attached to a rope. 500 mL from the first replicate was immediately transferred to a container (HDPE plastic bottles), and the bucket was emptied. The second replicate was immediately collected and 500 mL transferred to a second container. Most samples were analyzed within five minutes after having been collected. If they were not analyzed immediately, they were stored at four degrees Fahrenheit in the dark and analyzed within 24 hours. Samples were collected three times every day between February 24 and May18, 2014 for 84 days (12 weeks).

2.2 Lab analyses

LAS concentration was analyzed by Methylene Blue Active Substance (MBAS)(Hon-Nami & Hanya, 2013; USEP, 1983; APHA,2000; ASTM, n.d) using a SAM Kit from CHEMetrics, Inc. (Catalog No. I-2017 for detergents and anionic surfactants and MBAS. Range: 0.25-2.50 ppm) (Chemetrics.com., 2010).

Each sample container was processed separately. The dropper bottle was rinsed with the sample to be tested, then filled with 15 mL of the sample (Figure 4). One ampoule of MBAS reagent was added to the sample in the dropper bottle which was then capped and shook vigorously for 30 seconds. Then the dropper bottle sat undisturbed for one minute as the layers separated. After the one minute the cap was removed, the dropper bottle was slowly inverted and the subnatant chloroform layer was squeezed into a test tube. The dark blue liquid remaining in the dropper bottle was disposed and the test tube stood undisturbed for four minutes. After four minutes, the instrument was calibrated (zeroed) with distilled water and the sample test tube was analyzed. The instrument was recalibrated before every sample.

Effluent samples were processed as above. Because influent LAS concentration exceeded the range of the MBAS instrument, the influent samples were diluted 1:2 (5 mL influent + 10 mL distilled water), then processed as above. Because of the dilution, the influent LAS concentrations reported in the graphs were three times the concentration tested by the instrument.

2.3 Statistical analysis

Nested ANOVA and Tukey's HSD were performed using SAS/STAT® software (SAS, 2013).

Figure 2. Pittsburg's Wastewater Treatment Plant illustrating location for Bar Screen and Cascade

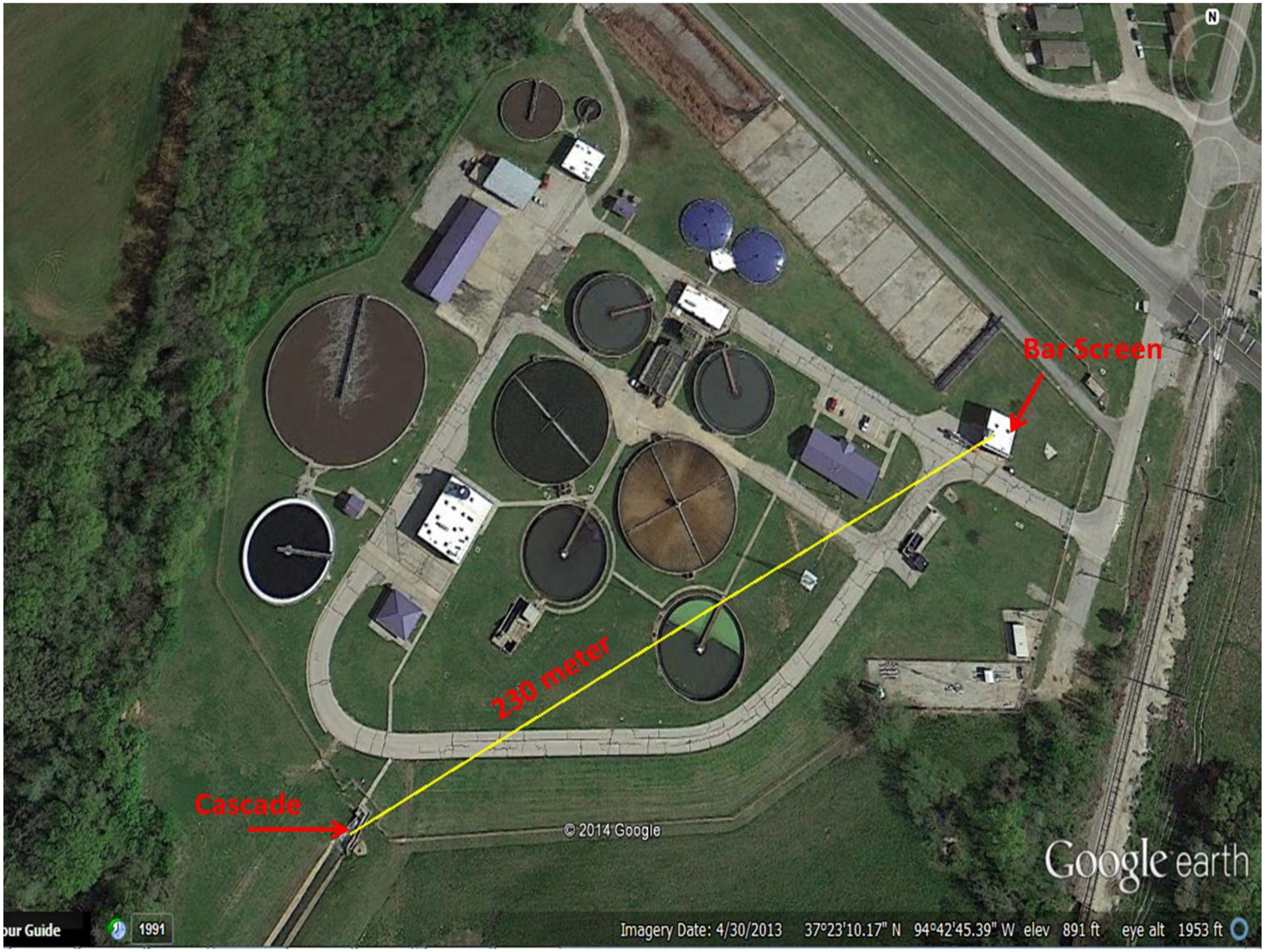
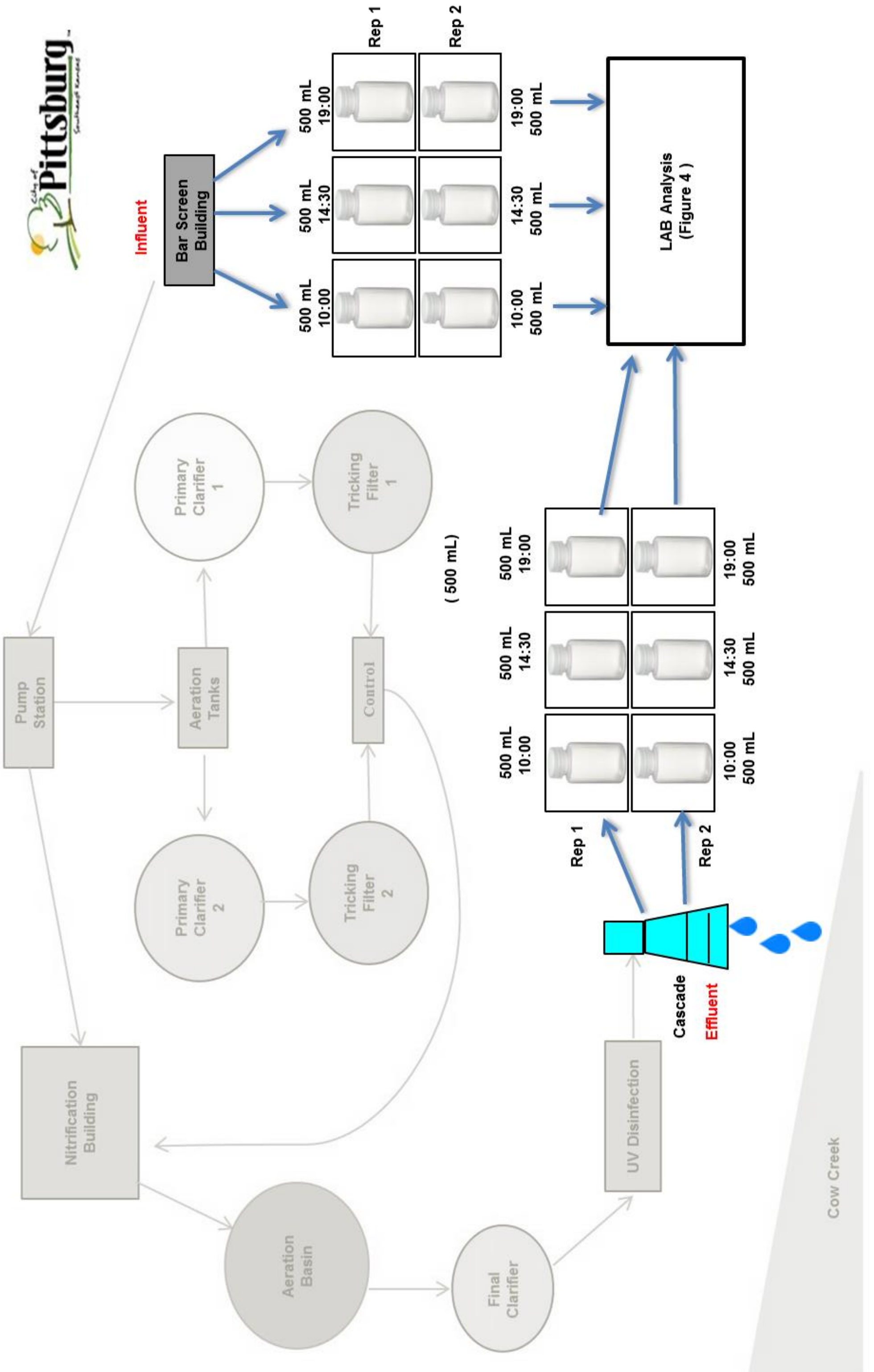
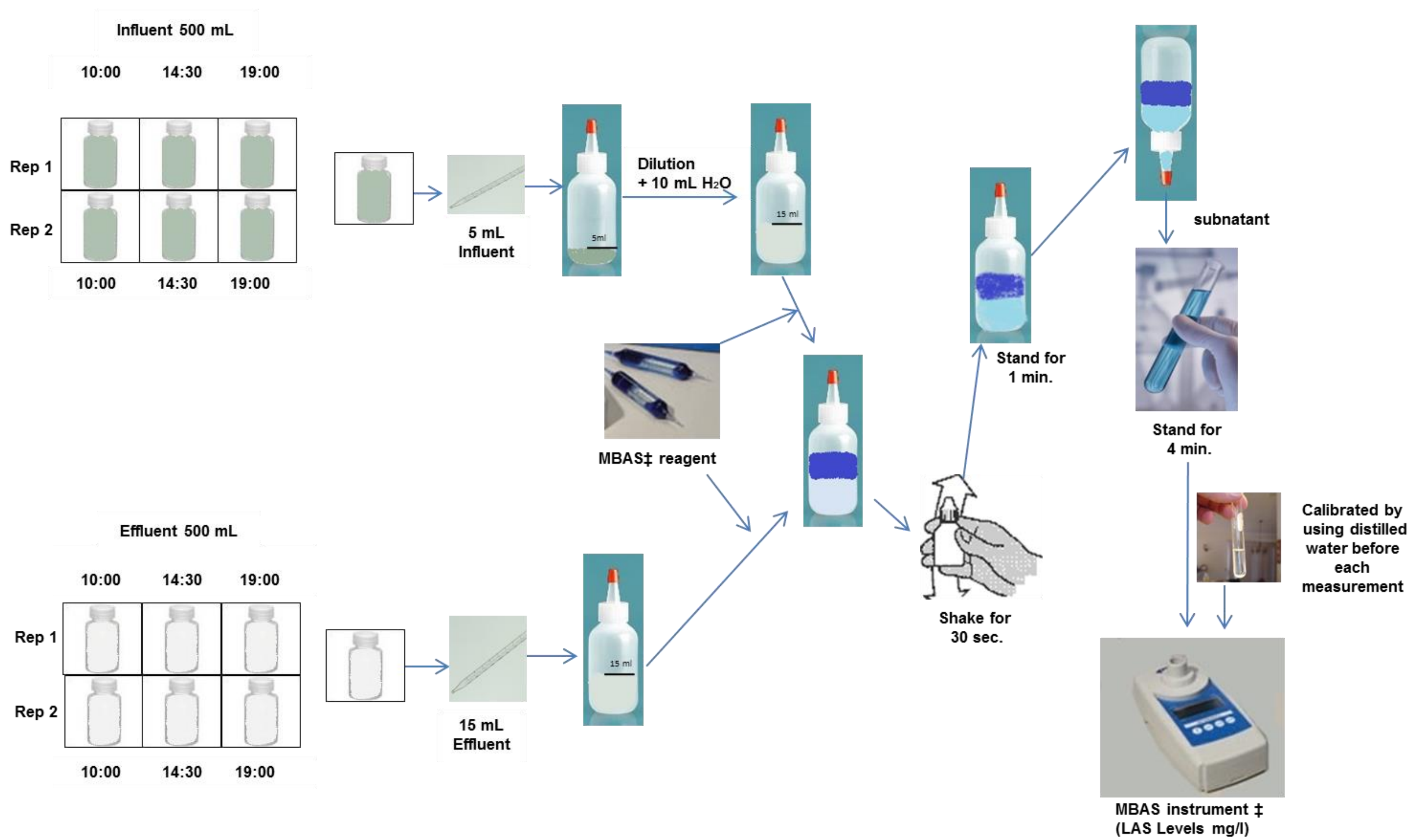


Figure 3 Collecting wastewater samples* at Pittsburg's Wastewater Treatment Plant



*The samples were collected at three times a day from influent and effluent.

Figure 4 Lab Analysis* of LAS† concentration



* Methylene Blue Active Substance

†LAS – Linear Alkylbenzene Sulfonate

‡ Detergents (anionic surfactants, MBAS) SAM Kit- Catalog No. I-2017-Range: 0.25-2.50 ppm-Methylene Blue Active Substance- CHEMetrics, Inc. - Midland, VA

CHAPTER III

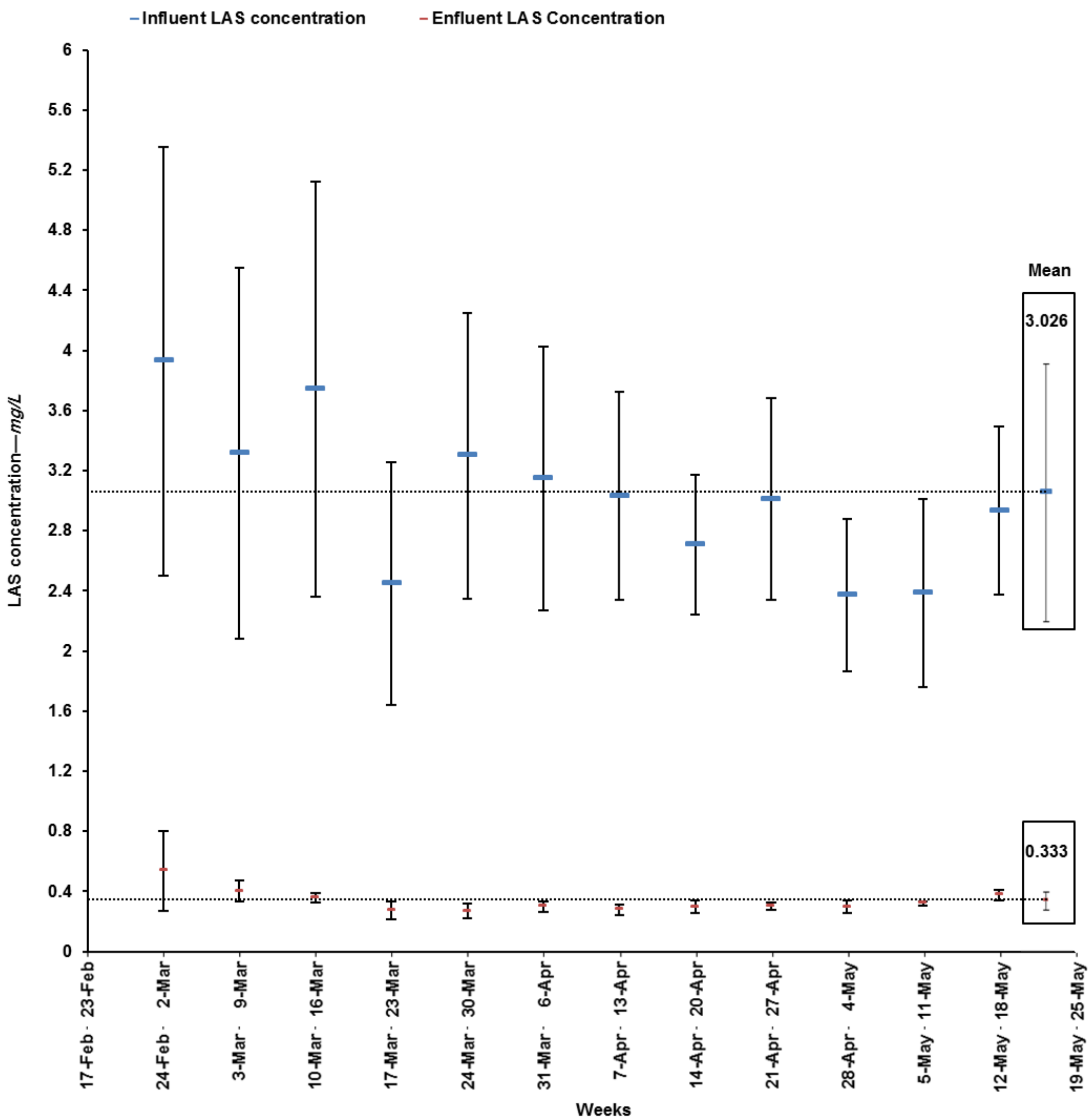
3. RESULTS AND DISCUSSION

3.1 Variation in influent and effluent LAS concentration through the study period

The weekly means (pooling each weekday and its three sample times) of influent LAS concentration (Figure 5) were very high and varied throughout the study period (2.4 – 3.9 mg/L). The standard deviation was also high and varied throughout the study period (0.5 – 1.4 mg/L). There was a significant difference in influent LAS in weeks (ANOVA f=3.99, df= 11, and P= 0.0001, Tukey's HSD).On the other hand, the weekly means of the effluent much lower (one tenth) and more consistent (0.27 – 0.53 mg/L) than the influent. The effluent standard deviation was also lower and more consistent (0.1 – 0.26 mg/L). There was a significant difference in effluent LAS by weeks (ANOVA f= 12.46, df=11, and P= 0.0001, Tukey's HSD).The effluent standard deviation was high in the first week, and then became smaller, probably because increasing experience in using the instrument.

Note that there are three weeks that the influent LAS concentration were lowest (17 March – 22 April – 5 May). These three weeks were significantly different from

Figure 5 Variation* in influent and effluent LAS† concentration through the study period



* Variation was calculated by pooling days and times for each week
† LAS is Linear Alkylbenzene Sulfonate

almost all the other weeks but were not significantly different from each other. They coincided with certain university activities. Because 25% of Pittsburg's population was students, it is possible they may have large impact on the LAS released into wastewater. The week of March 17 was spring break when many of students were out of town. Interestingly, the week before the concentration was much higher. This could be because students do a lot of laundry prior to leaving town. The week of April 17 was the week before final exams, and the following week (May 5) was the week for final exams. During these two weeks, students probably are not doing much laundry. Doing a full year study may reveal other changes in LAS that coincide with other university activities.

3.1.1 The effectiveness of wastewater treatment

In order to determine the reduction of LAS concentration, the effluent must be sampled two days after the influent because it takes that long to complete the water treatment process. Therefore the percent reduction in LAS could not actually be calculated. However, the effluent concentration was consistently very low(almost zero) throughout the study period. Using the mean influent and effluent concentrations to estimate the percent reduction, the effectiveness was 90%, which is similar to others reported in the literature (95-99%), (Doinex.com, 2009) .

3.1.2 Potential toxicity of wastewater before and after treatment

The influent LAS concentration was below the toxic level for the microbes (about half of the IC_{50}),and *Puntius ticto* (fish) (one seventh of the LC_{50}). It was three times greater than the LC_{50} for fresh water algae and 100 times greater than LC_{50} marine algae. It was slightly toxic for *Terapon jarbua* (fish) (greater than the LC_{50} by 0.62 mg/L).

After treatment, the effluent LAS concentration should be non-toxic for the microbes (15 times less than IC_{50}), the fresh water algae (about one fourth of the LC_{50}), *Terapon jarbua* (about one sixth of the LC_{50}), and *Puntius ticto(*about one fiftieth of the LC_{50}). It is apparently still toxic to marine algae (about 10 times the LC_{50}).

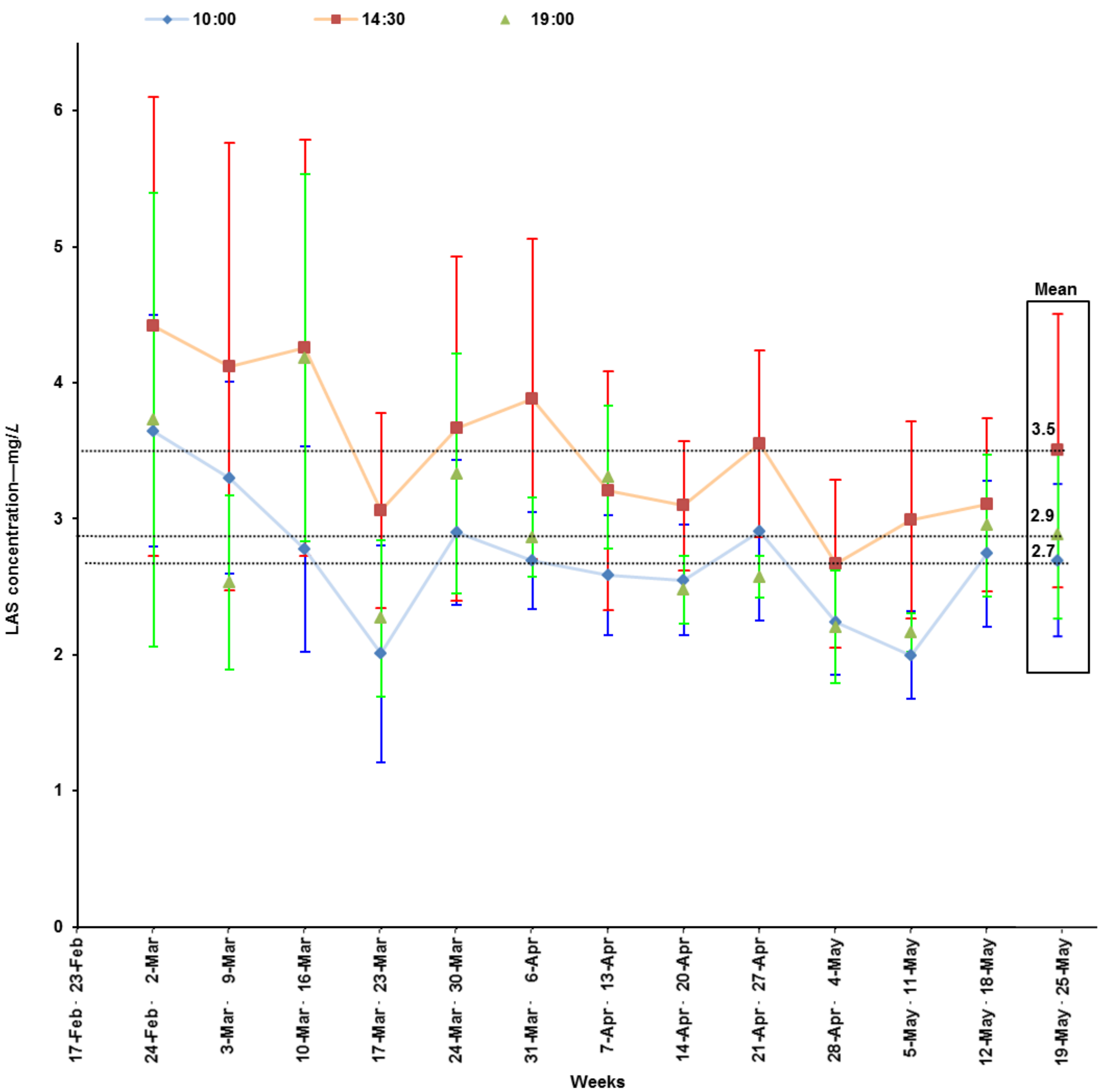
3.2 Variation in influent LAS concentration through the day

Examining the time of day for each week throughout the study period, Figure 6 shows the mean concentration for the entire week at each of the three sampling times (pooling seven days at 10:00, pooling seven days at 14:30, and pooling seven days at 19:00). For the first week, the LAS concentration was highest at 14:30 and lowest at 10:00. Examining sampling times throughout the study period, 14:30 was the highest concentration (ranging from 2.7 – 4.4 mg/L). The mean LAS concentration at 14:30 for the entire study period ($\bar{x}$ =3.5 ± 1 mg/L) was higher than the means of the other two sampling times. The lowest concentration throughout the study period was at 10:00 ($\bar{x}$ =2.7 ± 0.56 mg/L). However, examining each week, 10:00 was lowest only eight out of twelve weeks (ranging from 1.9 – 3.7 mg/L). It is not clear why 14:30 was highest every week when other values varied. There was a significant difference in influent LAS concentration at three times of day (ANOVA f=342.85, df=84, and $P<0.0001$, Tukey's HSD)

3.3 Variation in influent LAS concentration through the week

Examining each day within each week throughout the study period, Figure 7 shows the mean of the LAS concentration for each day of the week (pooling three sampling times for each day). The LAS concentration varied irregularly every day. For the first week, the LAS concentration was lowest on Sunday and highest on Tuesday.

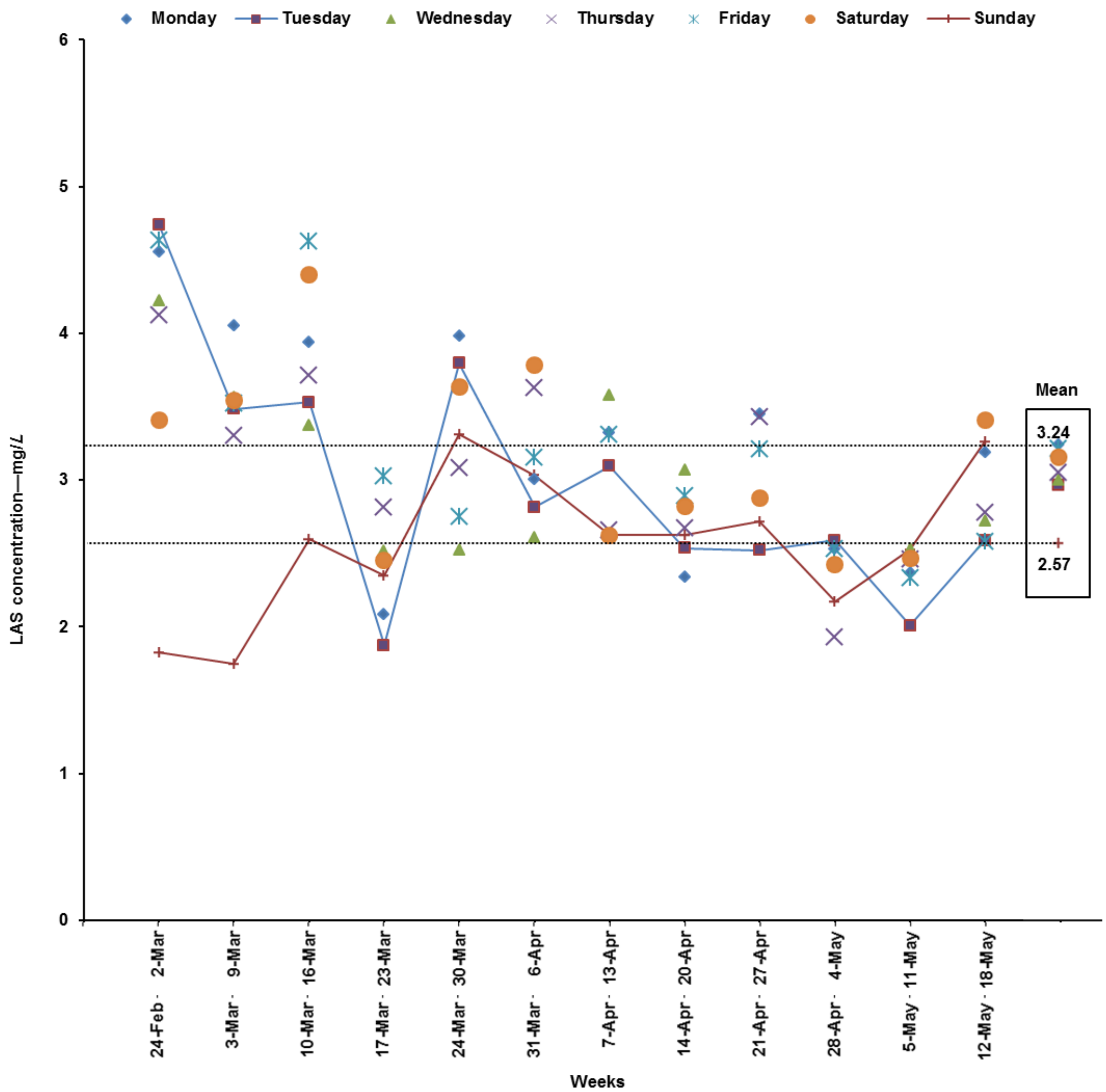
Figure 6 **Variation* in influent LAS† concentration though the day**



* Variation is weekly mean for time of day(10:00, 14:30, 19:00) calculated by pooling all days of the week)
† LAS is Linear Alkylbenzene Sulfonate

However, Sunday was lowest only three out of twelve weeks (ranging from 1.7 – 3.3 mg/L) and Tuesday was highest only two out of twelve weeks (ranging from 1.9 – 4.7 mg/L). Every other day of the week had the lowest LAS concentration at least once. Every day except Thursday and Sunday had the highest LAS concentration at least once. Therefore, there does not appear to be a consistent pattern for the highest and lowest concentration. However, the highest mean for the entire study period was on Monday ($\bar{x}$ =3.2 ± 1.1 mg/L), and lowest on Sunday ($\bar{x}$ =2.57 ± 0.70 mg/L). There was no significant difference in influent LAS by day (ANOVA f=0.82, df=72 , and P=0.8060, Tukey's HSD).

Figure 7 Variation* in influent LAS† concentration through the week



* Variation is $\bar{x}$ of LAS concentration for pooling all three samples (morning, afternoon, and evening) in each day for each week
† LAS is Linear Alkylbenzene Sulfonate

CHAPTER IV

4. CONCLUSION

4.1 Week

The weekly means of influent LAS concentration were very high and variable throughout the study period. By comparison, the weekly means of the effluent were much lower (one tenth) and more consistent than the influent. Some of the variation on influent LAS concentration appears to coincide with university activities. For instance, When students were on vacation, it was lowest. It is possible that students have large effect on the influent LAS because they comprise 25% of Pittsburg's population.

Pittsburg's Wastewater Treatment Plant reduced LAS concentration by 90%, which is similar to other published reductions (95-99%), (Doinex.com, 2009) .

4.2 Time

Examining the time of day for each week throughout the study period, the influent LAS concentration was consistently highest at 14:30, but the lowest concentration varied irregularly throughout the day. However, the mean for the entire study period was lowest at 10:00 and highest at 14:30. It is not clear why 14:30 was consistently highest every week.

4.3 Day

There does not appear to be a consistent daily pattern for the highest and lowest influent LAS concentration. However, the highest mean for the entire study period was on Monday and the lowest was on Sunday.

4.4 Toxicity

The influent LAS concentration levels were potentially toxic for fresh water algae, marine algae, and *Terapon jarbua* (fish) but it is non-toxic for the microbes and *Puntius ticto* (fish). After treatment, LAS concentration was reduced to non-toxic levels for the microbes, the fresh water algae, *Terapon jarbua, and Puntius ticto* but were potentially toxic to marine algae.

4.5 Future studies

- Study the whole year to see whether LAS concentration alters in synchrony with university activities (Fall Break, Christmas Holiday, Spring Break, and Summer Holiday).
- Determine the toxicity of effluent LAS to aquatic life.
- Examine degradation products in treatment plant.
- Examine the effect of chlorination on benzene.
- Study the time that LAS takes to degrade in treatment plant by examining the concentration in stages of the process at different times of the year because water temperature will vary.

CHAPTER V

5. REFERENCES

APHA Standard Methods, 2000 (22nd ed.) Method 5540 C.

ASTM D 2330-02, Methylene Blue Active Substances.

Chemetrics.com., 2010. Detergents (anionic surfactants, MBAS) SAM Kit. https://www.chemetrics.com/Detergents%20(anionic%20surfactants,%20MBAS)/Instrumental+Kits/I-2017 (accessed JAN 13, 2014).

CLER.com (The Council for LAB/LAS Environmental Research), 2014.Environmental And Other Benefits Of Replacing ABS With LAS. http://www.cler.com/facts/environ.html (accessed MAY 29, 2014).

Dionex.com., 2009. Determination of Linear Alkylbenzene Sulphonate in Treatment Plant Wastewater Streams Using On-Line Solid-Phase Extraction Followed by HPLC with Fluorescence Detection. www.dionex.com/en-us/webdocs. Retrieved from www.dionex.com: http://www.dionex.com/en-us/webdocs/81963-AN219-HPLC-LAS-Surfactant-Wastewater-05Oct2009-LPN2152.pdf (accessed Jan 13, 2014).

Flores, G.P.; Badillo, C.M.; Cortazar, M.H.; Hipolito, C.N.; Perez, R.S.; & Sanchez, I.G., 2010. Toxic Effects of Linear Alkylbenzene Sulfonate, Anthracene and Their Mixture on Growth of a Microbial Consortium Isolated From Polluted Sediment. *Revista internacional de contaminación ambiental*, 26:1:39-46.

Holt, M.S.; Mitchell, G.C.; & Watkinson, R.J., 1992. The Environmental Chemistry, Fate and Effects of Non Ionic Surfactants In Anthropogenic Compounds*, The Handbook of Environmental Chemistry- ,* Springer -Verlag ed, Berlin, 3:F:89144.

Hon-Nami, H; & Hanya, T., 2013. Linear Alkylbenzene Sulfonates in River, Estuary and Bay Water. *Water Research a journal of the International Water Association*, 14:9: 1251–1256

Huang, B.; & Wang, D., 1994. Effects of Linear Alkylbenzene Sulfonate (LAS) on the Respiratory Functions of Tigerperch (*Terapon Jarbua*). *Zoological Studies*, 33:3: 205-210.

Ihs.com., 2012. Linear Alkylate Sulfonates. http://www.ihs.com/products/chemical/planning/ceh/linear-alkylate.aspx (accessed Feb 10, 2014).

Khleifat, M.K., 2006. Biodegradation of Linear Alkylbenzene Sulfonate by a Two-Member Facultative Anaerobic Bacterial Consortium. *Enzyme and Microbial Technology*, 39:1030-1035.

Lara-Martin, P.A; Gómez-Parra, A.; Sanz, J.; & González-Mazo, E., 2010. Anaerobic Degradation Pathway of Linear Alkylbenzene Sulfonates (LAS) in Sulfate-Reducing Marine Sediments. *Environmental Science Technology*, 44:5:1670-1676.

Lewis, M.A., 1991. Chronic and Sublethal Toxicities of Surfactants to Aquatic Animals: A Review and Risk assessment. *Water Resourses*, 25:1:101-113.

McAvoy, D.C.; Dyer, S.D; Fendinger, N.J.; Eckhoff, W.S; Lawrence, D.L; & Begley, W.M., 2009. Removal of Alcohol Ethoxylates, Alkyl Ethoxylate Sulfates, and Linear Alkylbenzene Sulfonates in Wastewater Treatment. *Environmental Toxicology and Chemistry*, 17:1705-1711.

Mathur, A.K.; Gupta, B.N.; Singh, A.; Srivastava, M.K.; & Raizada, R.B., 2000. Dermal Toxicity of Linear Alkylbenzene Sulfonate and Quinalphos in Guinea Pigs. *Cutaneous and Ocular Toxicology*, 19:1:43-53.

Mathur, A.K.; Raizada, R.B.; Srivastava, M.K.; & Singh, A., 2005. Effect of Dermal Exposure to Paraphenylenediamine and Linear Alkylbenzene Sulphonate in Guinea pigs. *U.S. National Library Of Medicine National Institutes Of Health*, 18:4:238-40.

Oliveira, L.L.; Costa, R.B.; Duarte, C.S.; Silva, E.L.; & Varesche, M.B.A., 2010. Anaerobic Degradation of Linear Alkylbenzene Sulfonate in Fluidized Bed Reactor. *Brazillian Journal of Chemical Engineering*, 27:4:539-543.

Olkowska, E.; Ruman, M.; & Polkowska, Z., 2014. Occurrence of Surface Active Agents in the Environment. *Journal of Analytical Methods in Chemistry*, 2014:769708-15.

Rothschild, W.G., 2009. On Complex Formation in Equimolar Chloroform-Benzene Solution: A Revisit by Molecular Dynamics Simulation and Orientational Correlation States Superposition. *Journal of Molecular Liquids*, 150:1-3:68-72.

Sablayrolles, C.; Montréjaud-Vignoles, M.; Silvestre, J.; & Treilhou, M., 2009. Trace Determination of Linear Alkylbenzene Sulfonates: Application in Artificially

Polluted Soil – Carrots Systems. *International Journal of Analytical Chemistry*, 23:65-71.

Sanderson, H.1.; Dyer, S.D.; Price, B.B.; Nielsen, A.M.; van Compernolle, R.; Selby, M.; Stanton, K.; Evans, A.; Ciarlo, M.; & Sedlak, R., 2006. Occurrence and Weight-of-Evidence Risk Assessment of Alkyl Sulfates, Alkyl Ethoxysulfates, and Linear Alkylbenzene Sulfonates (LAS) in River Water and Sediments. *U.S National Library Of Medicine National Institutes Of Health*, 15;368(2-3):695-712.

Sanz, J. L.; Culubret, E.; Ferrer, J.d.; Moreno, A.; & Berna, J. L., 2003. Anaerobic Biodegradation of Linear Alkylbenzene Sulfonate (LAS) in Upflow Anaerobic Sludge Blanket (UASB) reactors. *Biodegradation*, 14:1:27-64.

SAS (Statistical Analysis System), 2013. SAS Institute Inc., Cary, NC, USA.

UNEP (United Nation Environmental Program), 2005. Linear Alkylbenzene Sulfonate. http://www.chem.unep.ch/irptc/sids/oecdsids/las.pdf (accessed Jan 29, 2014)

USEPA Methods for Chemical Analysis of Water and Wastes, 1983. Method 425.1.

Varsha, J.; Mishra, K.D.; Madhuri, S.; & Govind, P., 2011. Linear Alkyl Benzene Sulphonate, a Detergent Induced Toxicity on the Gill of *Puntius Ticto* Fish. *International Research Journal of Pharmacy*, 2:1:76-78.

Whelan, M.J.; Egmond, R.V.; Guymer, I.; J O Lacoursière, J.O.; Vought, L.B.; Finnegan, C.; Fox, K.K.; Sparham, C.; O'Connor, C.; Vaughan, M.; & Pearson, J.M., 2008. The Behaviour of Linear Alkyl Benzene Sulphonate Under Direct Discharge Conditions in Vientiane, Lao PDR. Water Research, 14:20:4730-40.

Ying, G.G., 2006. Fate, Behavior, and Effects of Surfactants and Their Degradation Products in the Environment. *Environment International*, 32(30), 417-431.

APPENDICES

Appendix 1. The Variation of influent LAS concentration through the week

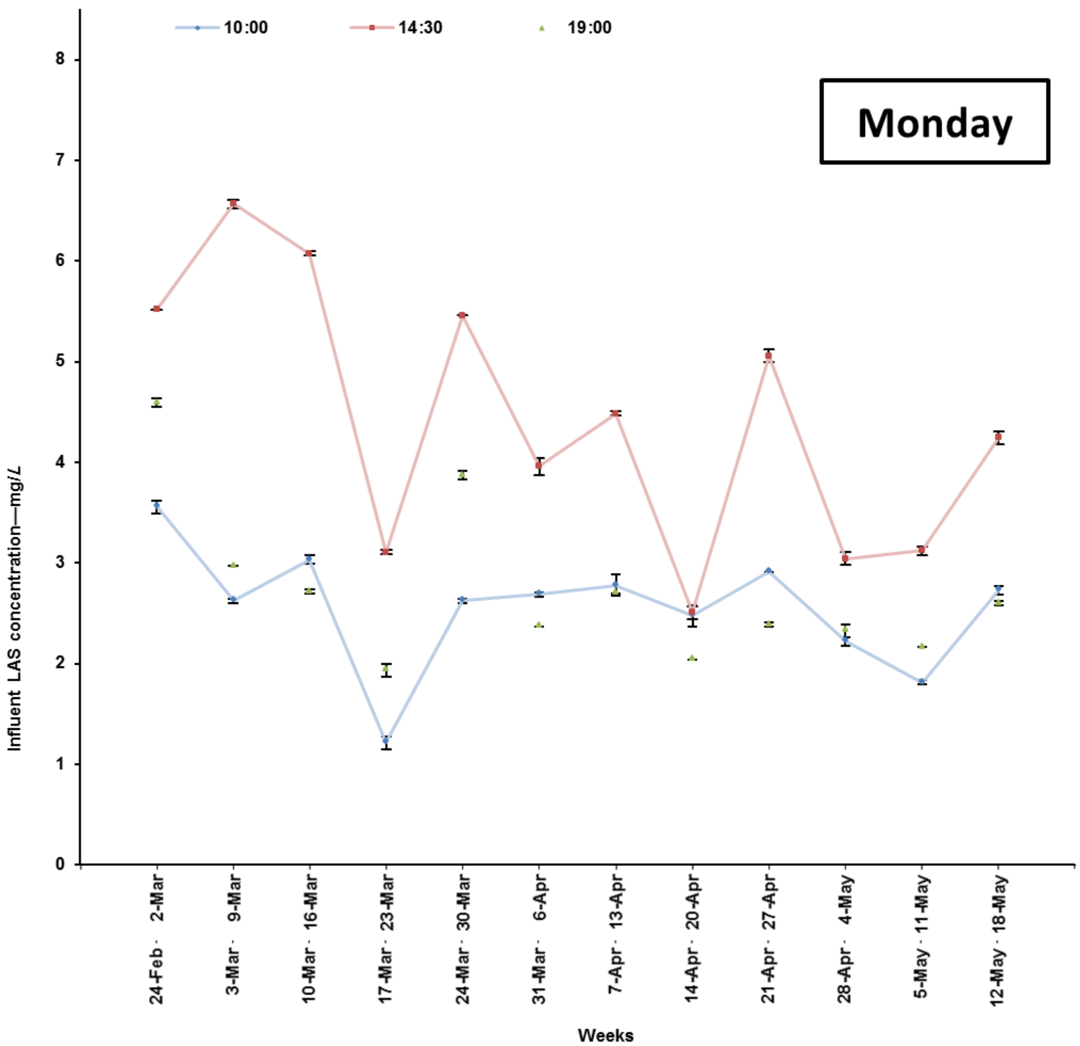


Figure A1.1. Influent LAS concentration* at three times of the day for Monday through study period. The highest mean of influent LAS concentration was at 14:30 ($\bar{x}$ = 4.43 ± 1.32 mg/L). Each week was highest at 14:30 was higher. The lowest mean was at 10:00 ($\bar{x}$ =2.56 ± 0.60 mg/L). However, only 6 out of 12 weeks were lowest at 10:00.

* Influent LAS concentration is the mean ±SD of two replicates/sample.

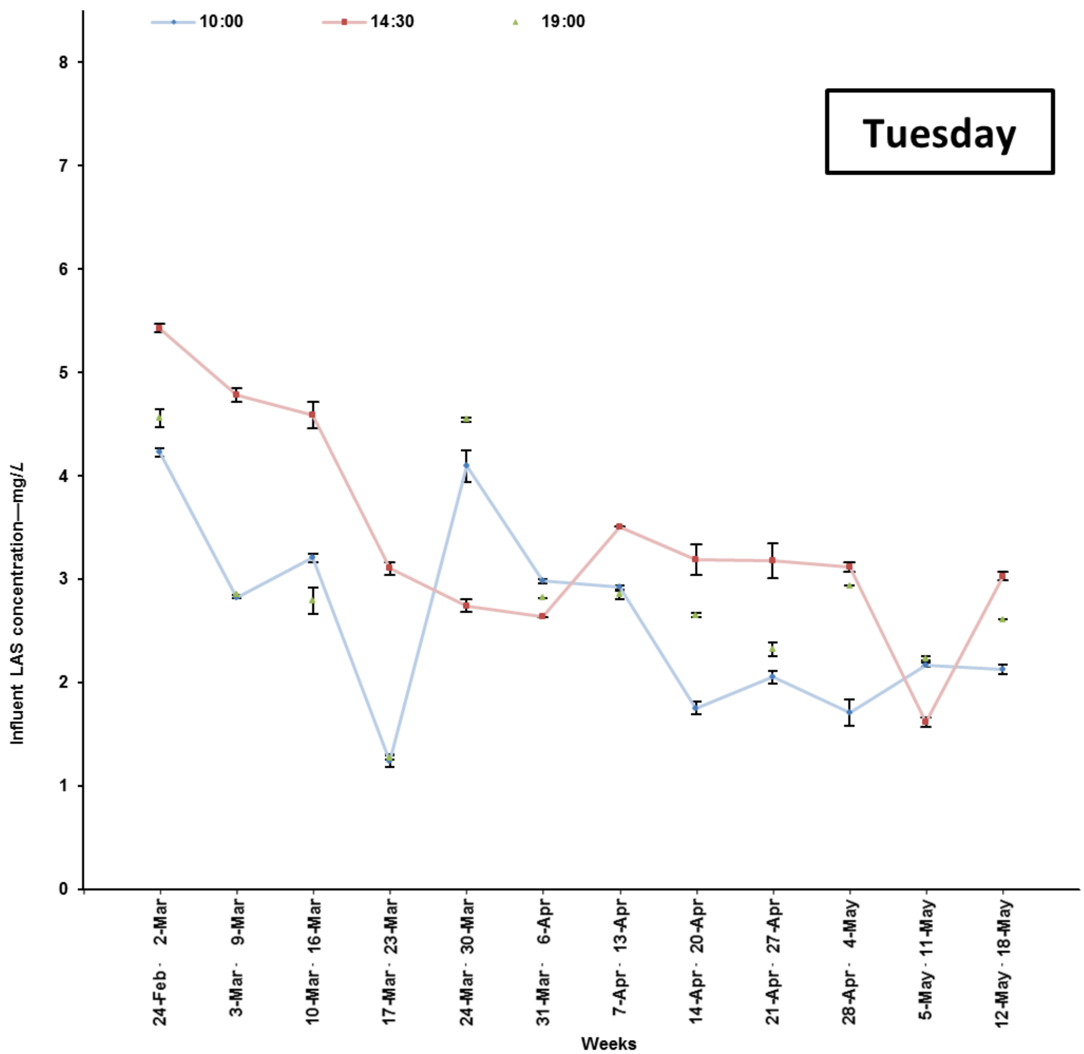


Figure A1.2. Influent LAS concentration* at three times of the day for Tuesday through study period. The highest mean of influent LAS concentration was at 14:30 ($\bar{x}$ = 3.41 ± 1.05 mg/L). However, only 9 out of 12 weeks were highest at 14:30. The lowest mean was at 10:00 ($\bar{x}$ =2.61 ± 0.93 mg/L). However, only 7 out of 12 weeks were lowest at 10:00.

* Influent LAS concentration is the mean ±SD of two replicates/sample.

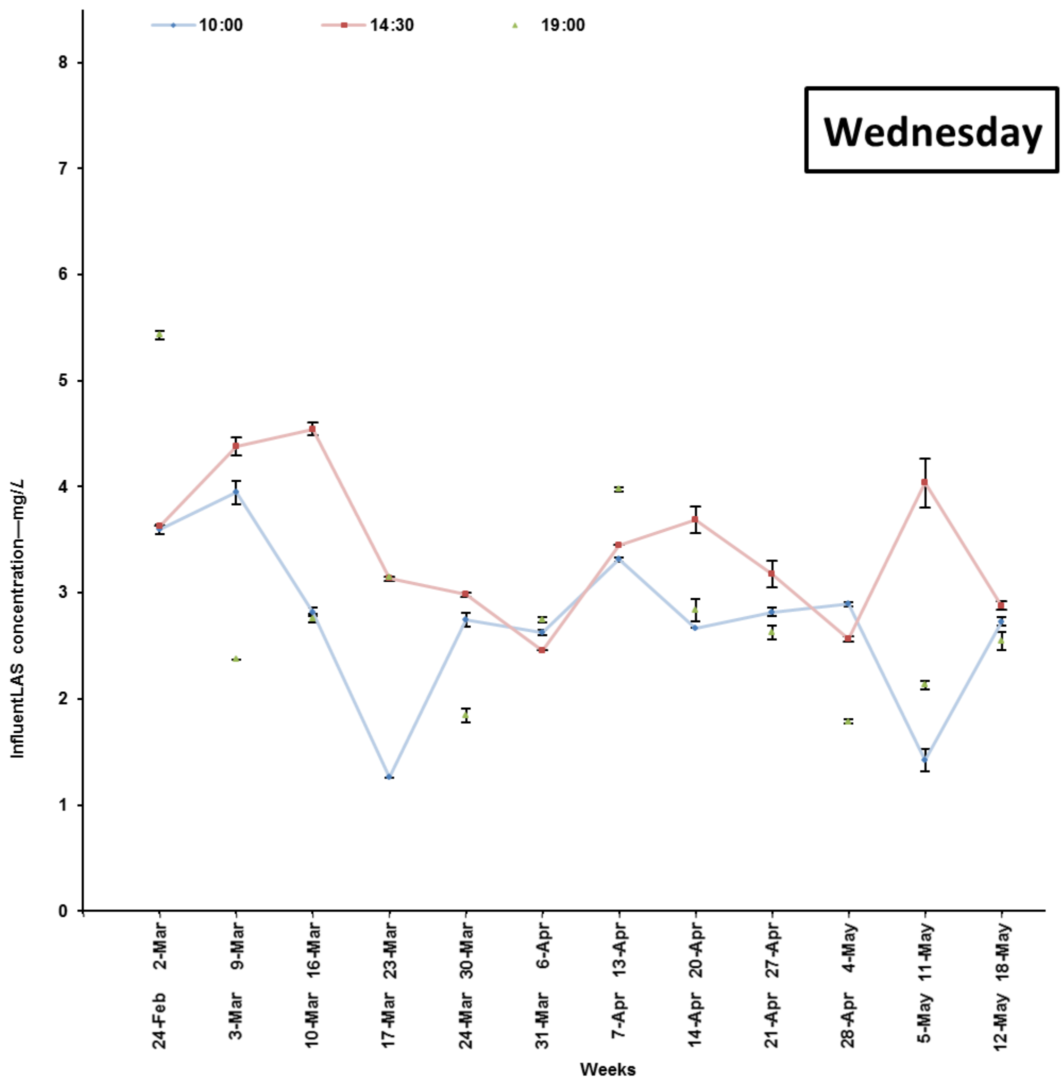


Figure A1.3. Influent LAS concentration* at three times of the day for Wednesday through study period. The highest mean of influent LAS concentration was at 14:30 ($\bar{x}$ = 3.41 ± 0.67 mg/L). However, only 9 out of 12 weeks were highest at 14:30. The lowest mean was at 10:00 ($\bar{x}$ = 2.74 ± 0.77 mg/L). However, only 5 out of 12 weeks were lowest at 10:00.

* Influent LAS concentration is the mean ±SD of two replicates/sample.

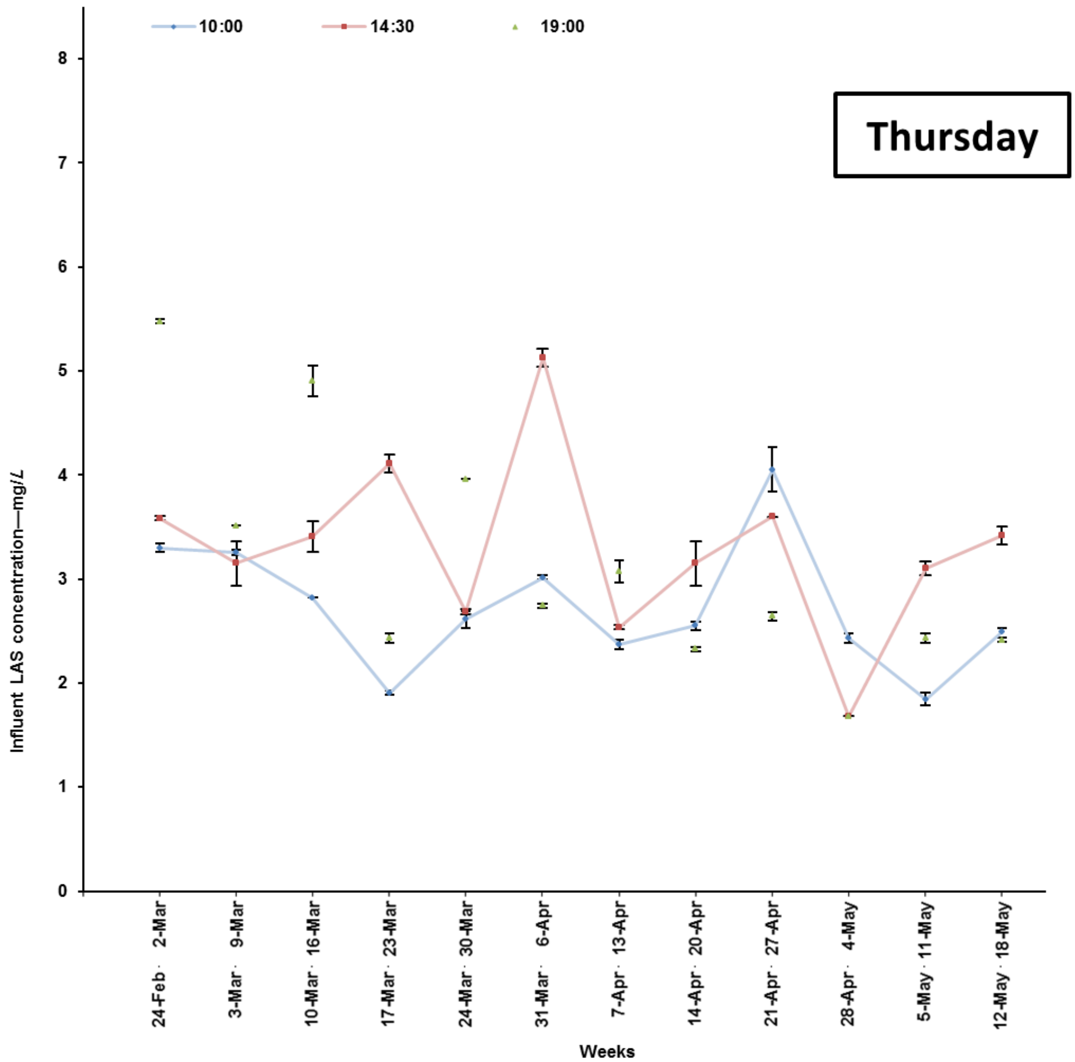


Figure A1.4. Influent LAS concentration* at three times of the day for Thursday through study period. The highest mean of influent LAS concentration was at 14:30 ($\bar{x}$ = 3.3 ± 0.85 mg/L). However, only 5 out of 12 weeks were highest at 14:30. The lowest mean was at 10:00 ($\bar{x}$ = 2.72 ± 0.62 mg/L). However, only 6 of 12 weeks were lowest at 10:00.

* Influent LAS concentration is the mean ±SD of two replicates/sample.

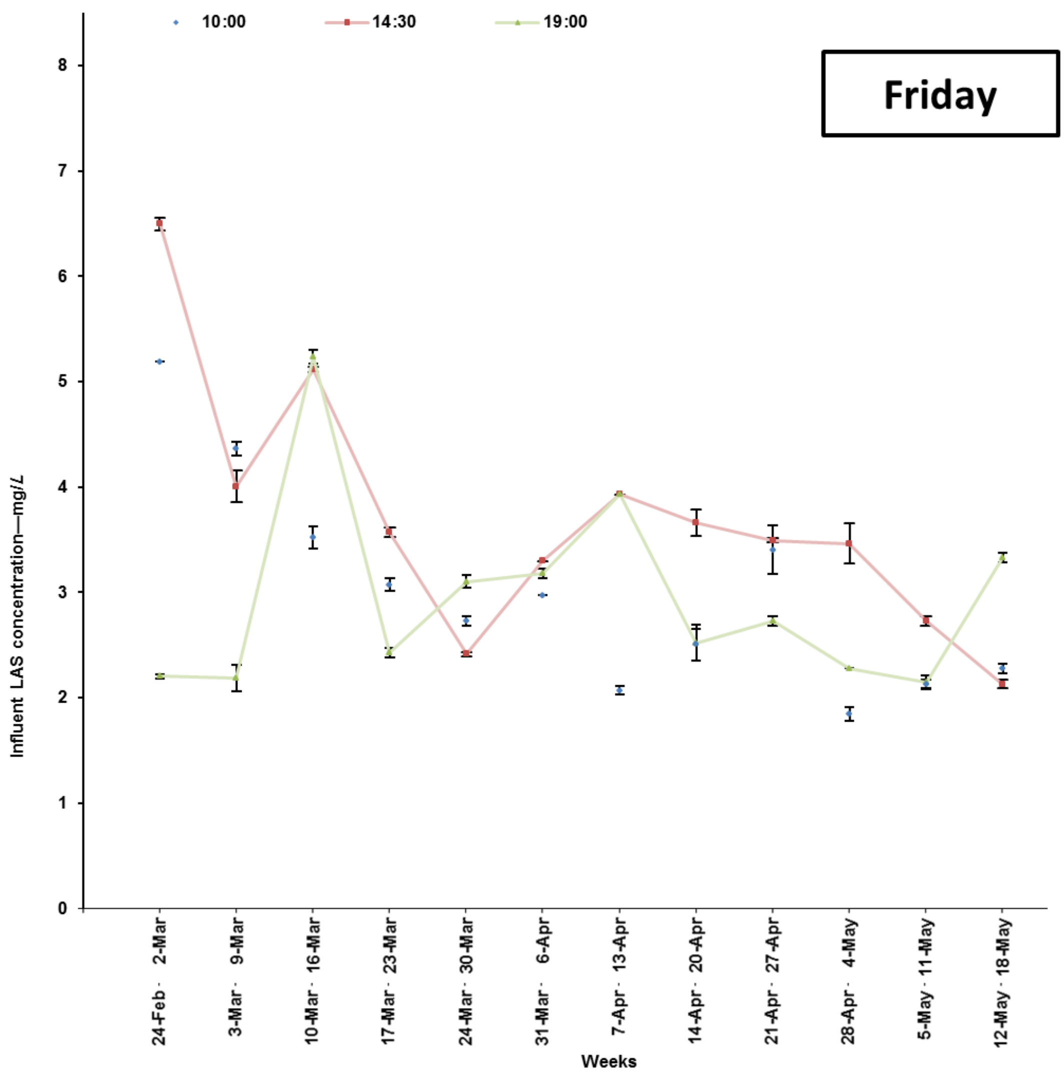


Figure A1.5. Influent LAS concentration* at three times of the day for Friday through study period. The highest mean of influent LAS concentration was at 14:30 ($\bar{x}$ = 3.7 ± 1.18 mg/L). However,only 8 out of 12 weeks were highest at 14:30. The lowest mean was at 19:00 ($\bar{x}$ = 2.94 ± 0.91 mg/L). However, only 4 out of 12 weeks were lowest 19:00.

* Influent LAS concentration is the mean ±SD of two replicates/sample.

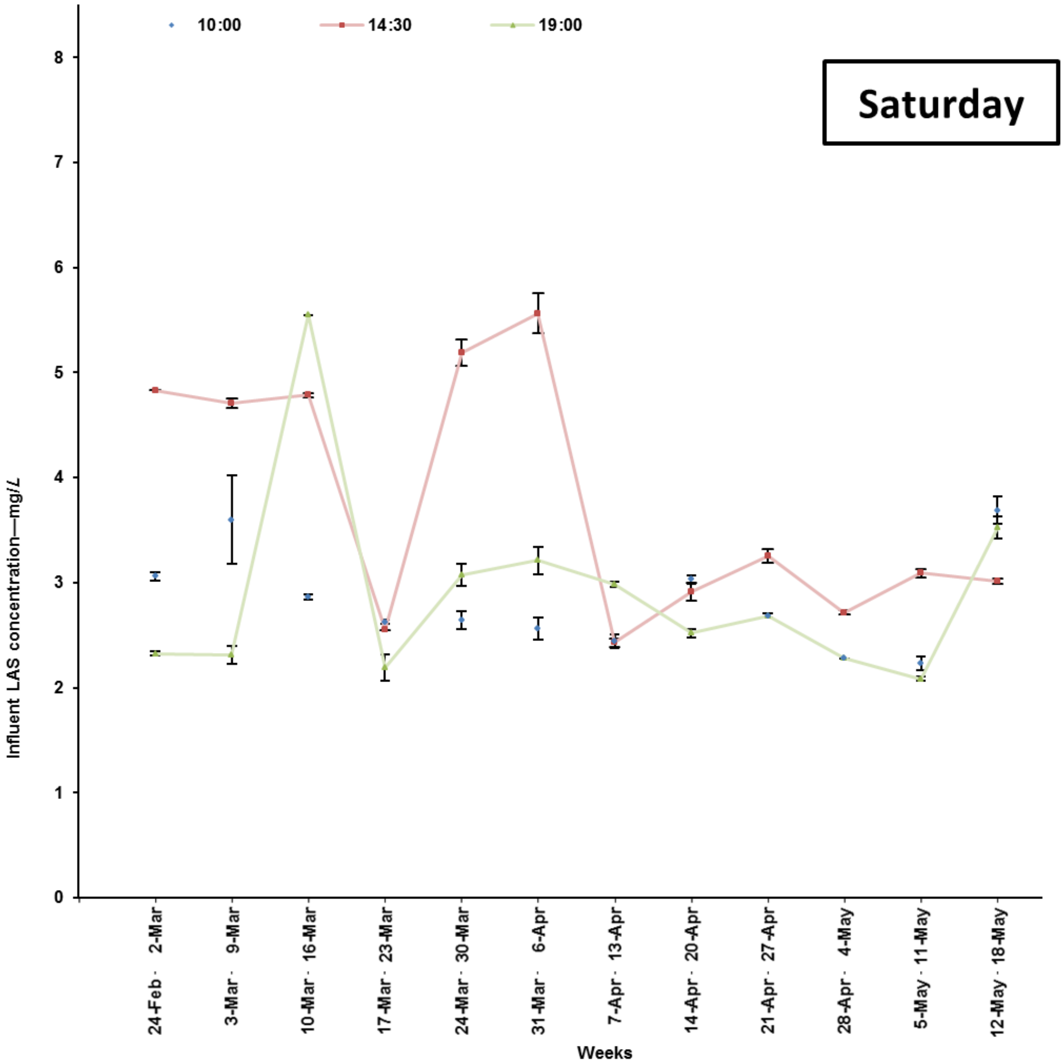


Figure A1.6. Influent LAS concentration* at three times of the day for Saturday through study period. The highest mean of influent LAS concentration was at 14:30 ($\bar{x}$ = 3.75 ± 1.16 mg/L). However, only 7 out of 12 weeks were highest at 14:30. The lowest mean was at 19:00 ($\bar{x}$ = 2.9 ± 0.95 mg/L). However, only 7 out of 12 weeks were lowest at 19:00.

* Influent LAS concentration is the mean ± SD of two replicates/sample.

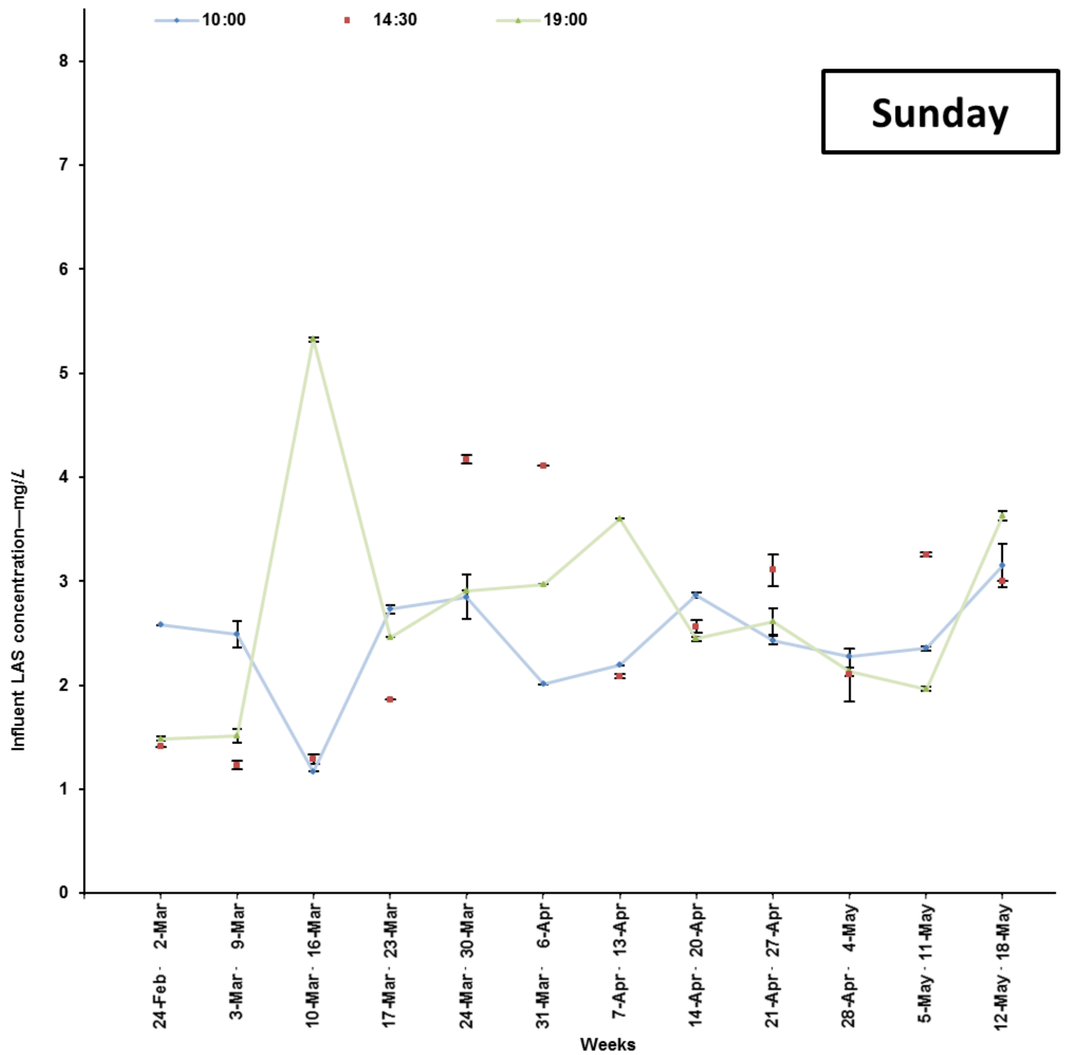


Figure A1.7. Influent LAS concentration* at three times of the day for Sunday through study period. The highest mean of influent LAS concentration was at 19:00 ($\bar{x}$ = 2.75 ± 1.07 mg/L). However,only 3 out of 12 weeks were highest at 19:00. The lowest mean was at 10:00 ($\bar{x}$ = 2.43 ± 0.51 mg/L). However, only 3 out of 12 weeks were lowest at 10:00 .

* Influent LAS concentration is the mean ±SD of two replicates/sample.

Appendix 2. The Variation of influent LAS concentration through the day.

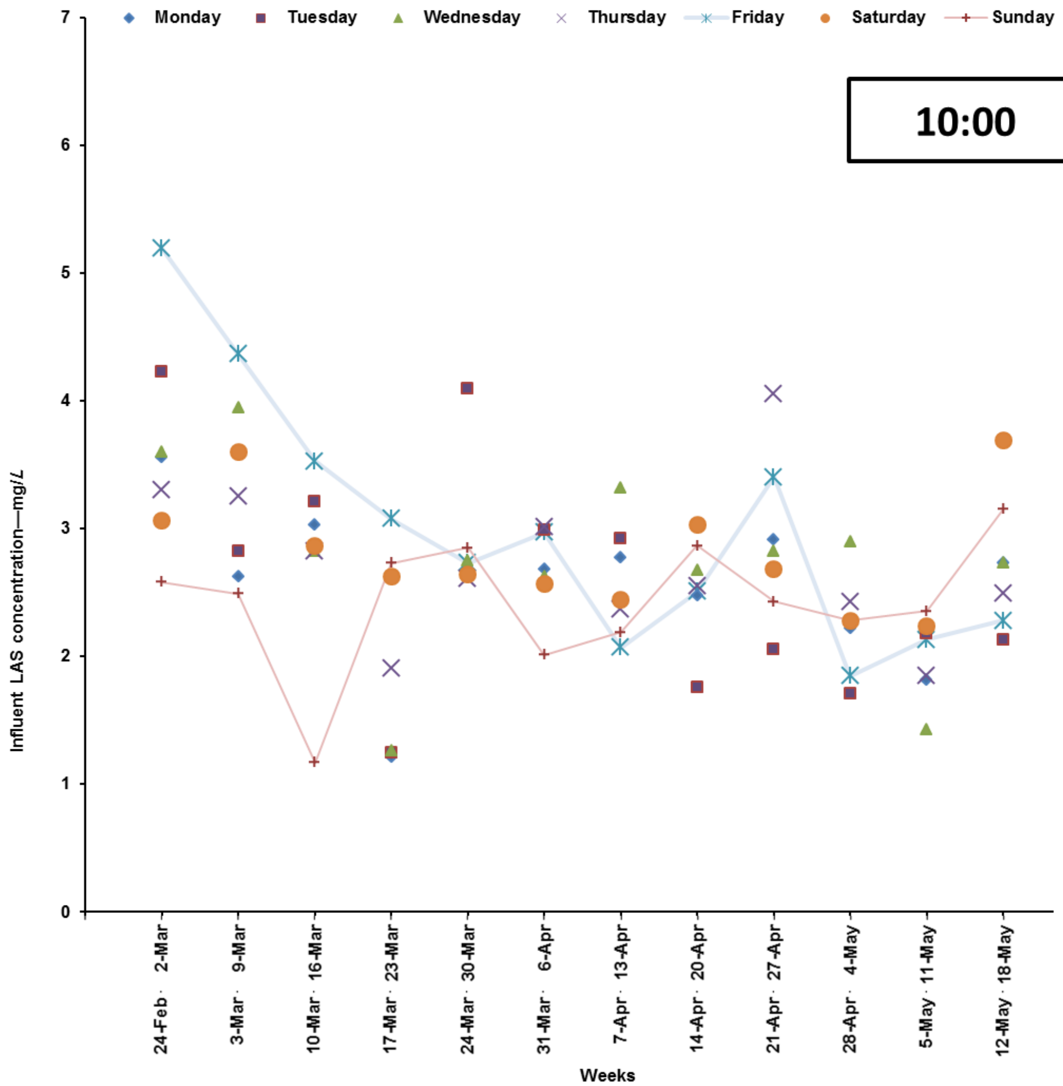


Figure A2.1. Influent LAS concentration at 10:00 each day of the week through study period. The highest mean of influent LAS concentration was on Friday ($\bar{x}$ = 3.01 ± 1 mg/L). However,only 4 out of 12 weeks were highest on Friday. The lowest mean was on Sunday ($\bar{x}$ = 2.43 ± 0.51 mg/L). However, only 4 out of 12 weeks were lowest on Sunday.

* Influent LAS concentration is the mean ± SD of two replicates/sample.

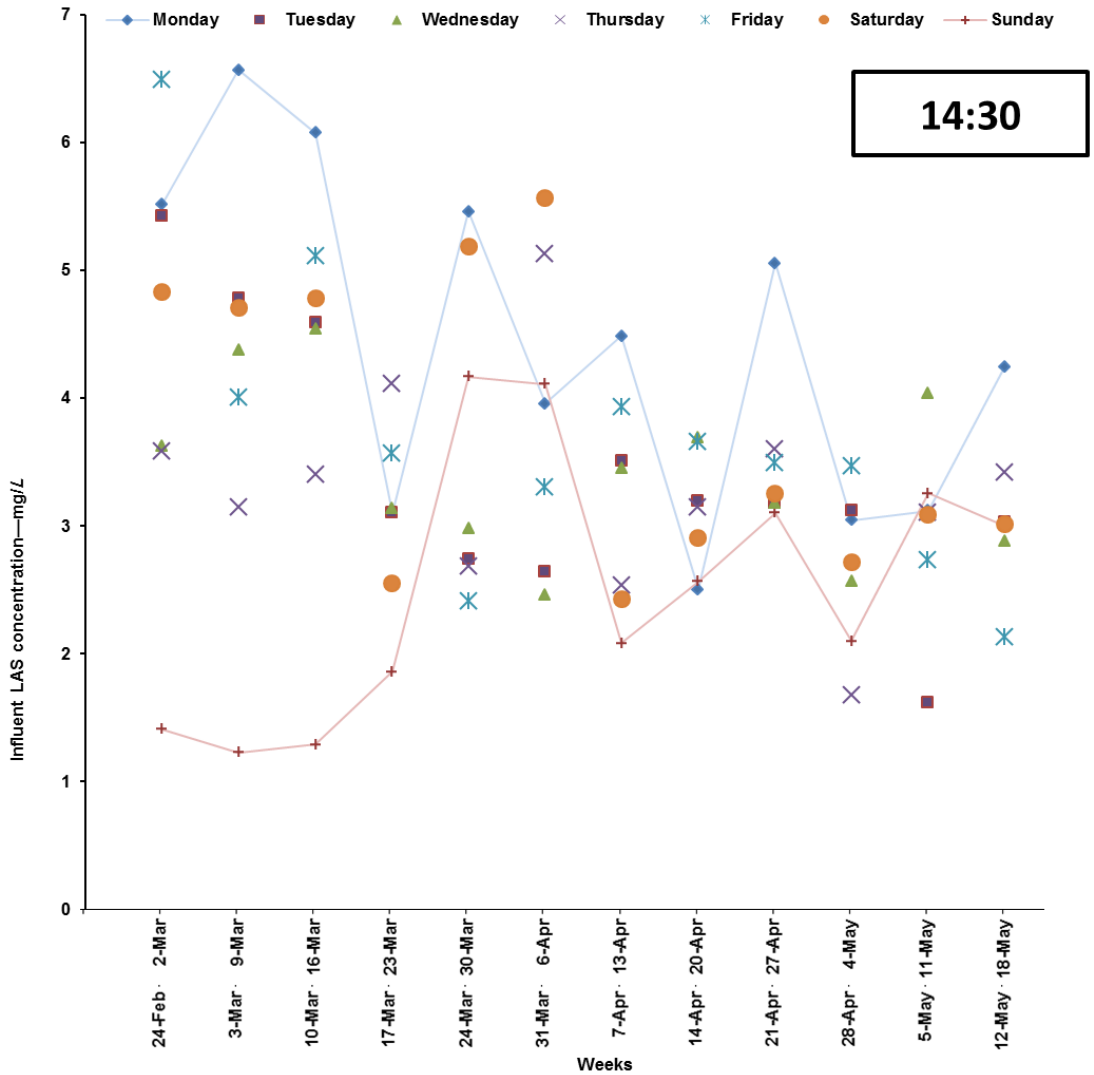


Figure A2.1. Influent LAS concentration at 14:30 each day of the week through study period. The highest mean of influent LAS concentration was on Monday($\bar{x}$ = 4.43 ± 1.32 mg/L). However, only 6 out of 12 weeks were highest on Monday. The lowest mean was on Sunday ($\bar{x}$ = 2.52 ± 1.03 mg/L). However, only 5 out of 12 were lowest on Sunday.

* Influent LAS concentration is the mean ± SD of two replicates/sample.

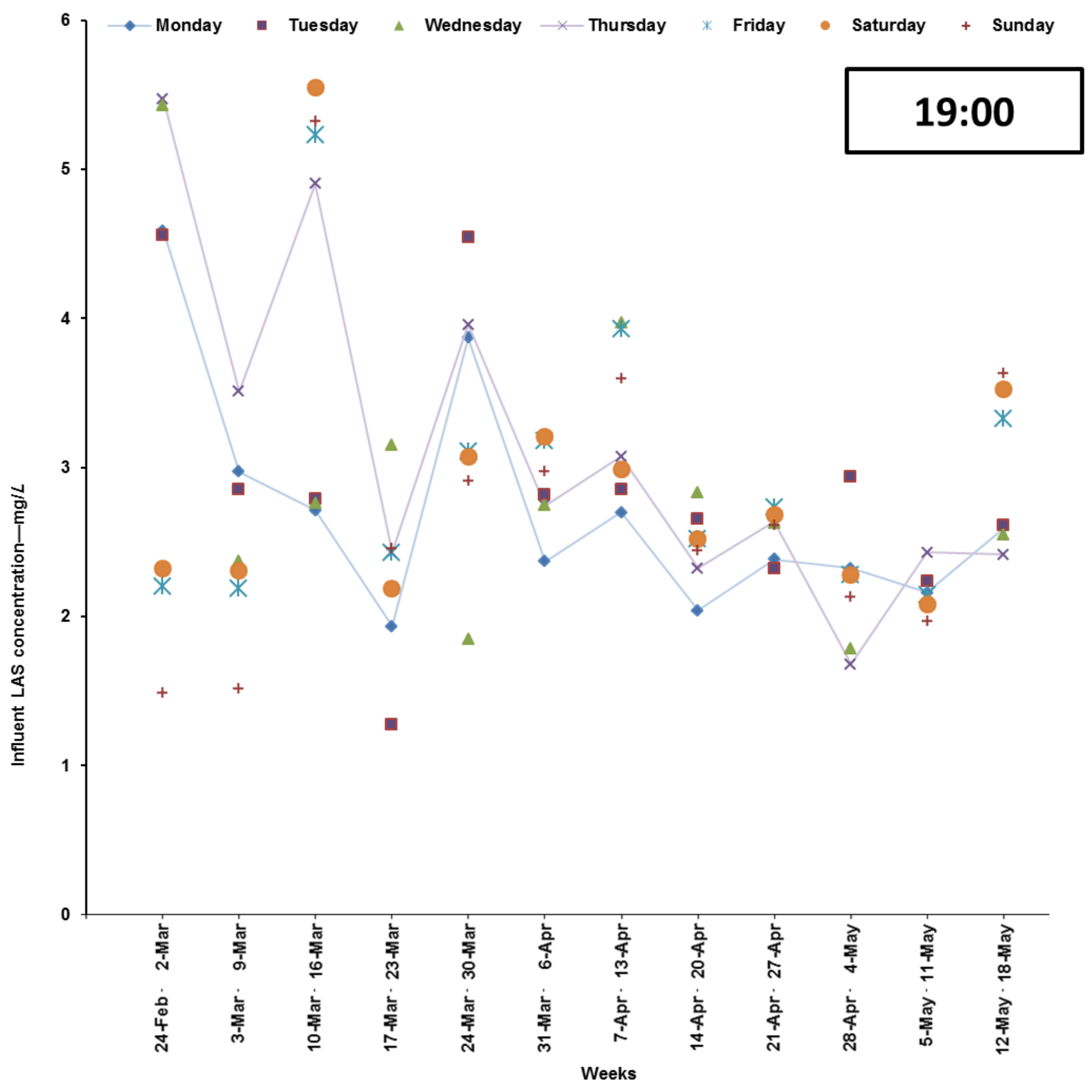


Figure A2.1. Influent LAS concentration at 19:00 each day of the week through study period. The highest mean of influent LAS concentration was on Thursday ($\bar{x}$ = 3.31 ± 1.13mg/L). However, only 3 out of 12weeks were highest on Thursday. The lowest mean was on Monday ($\bar{x}$ = 2.72 ± 0.78 mg/L). However, only 3 out of 12 weeks were lowest on Monday.

* Influent LAS concentration is the mean ± SD of two replicates/sample.

Appendix 3. The Correlation Between Temperature and LAS Concentration.

Introduction (A3.)

Degradation of LAS in water is largely affected by temperature. Prats et al. (2006) investigated the effect of temperature on the biodegradation of LAS by carrying out experiments using a 10 mg/L initial surfactant concentration and setting up temperatures at 48, 59, and 77°F. They found greater than 90% biodegradation of LAS regardless of temperature that had been set up in the test. However, low temperature require longer time period.

Methods and Materials (A3.)

The temperature for each replicate was recorded immediately after sampling.

Results and Discussion (A3.)

The initial intent was to examine the effect of temperature on degradation. On analyzing the data, there was significant correlation between temperature and LAS concentration in influent and effluent samples through the study period, but R^2 are so small that they are meaningless (Figure A3.1).

On reflection, it became evident that to determine the effect temperature on LAS degradation. Temperature had to be controlled in a laboratory to determine the time that LAS takes to degrade at each temperature.

In the treatment plant, the concentration in stages of the process had to be examined at different times of the year because water temperature will vary. Thus, the stages and time that LAS takes to degrade will be determined. In summer, LAS will be degraded more than winter as shown on pervious study. Moreover, it was reported that

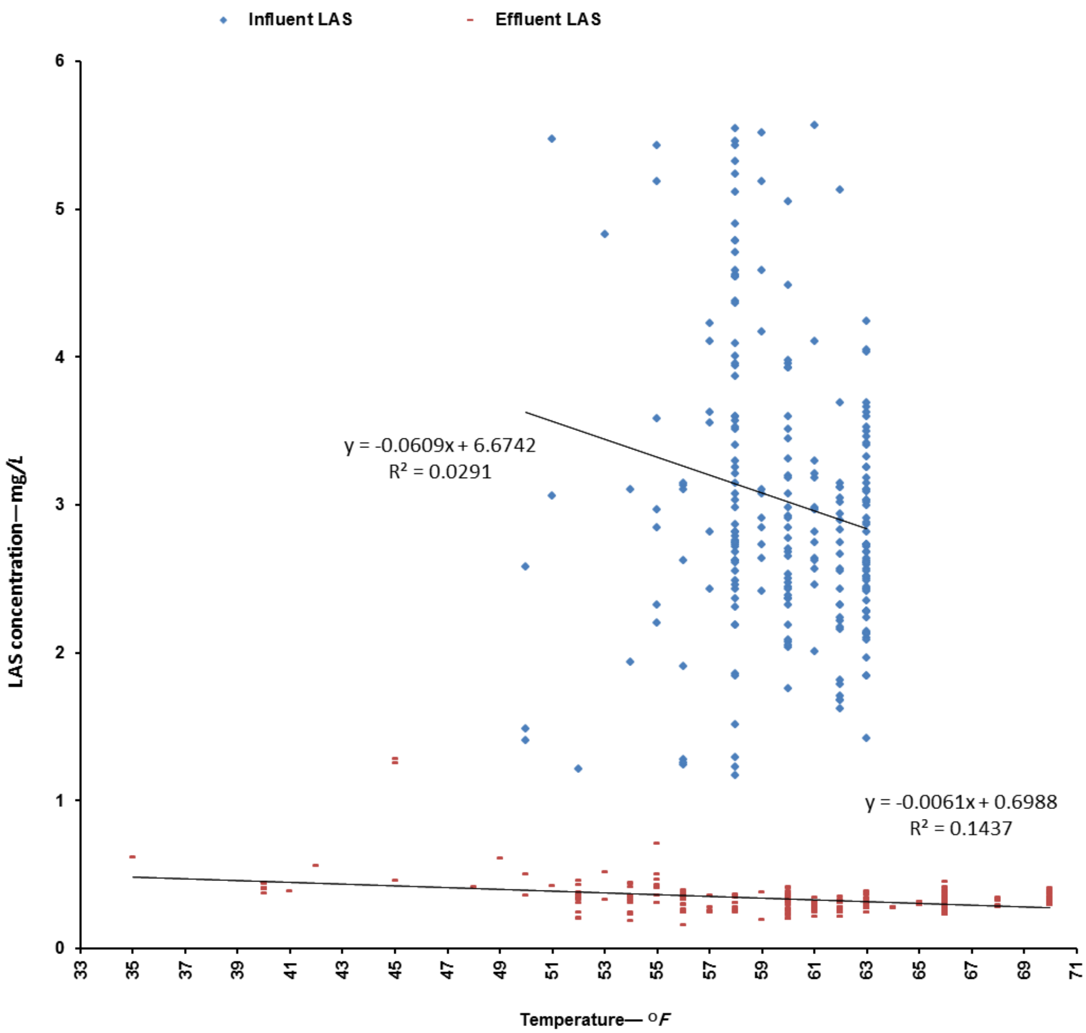


Figure A3.1 The correlation between temperature and LAS concentration

microorganisms, which help in LAS degradation, in water required longer periods for acclimation in lower temperatures (Prats et al,2006).

References (A3)

Prats, D., Lopez, C., Vallejo, D., Varo, P. & Leon, V. (2006). 'Effect of temperature on the biodegradation of LAS and alcohol ethoxylates'. Journal of Surfactants and Detergents, 9(1): 69-75.

Appendix 4. Statistics

Table A4.1. Nested Random Effects Analysis of Variance for Variable Influent LAS

Variance Source	DF	Sum of Squares	F Value	Pr > F	Error Term	Mean Square	Variance Component	Percent of total
Total	335	337.83				1.01	1.11	100.00
Week	11	67.78	3.99	0.0001	Day	6.16	0.16	14.88
Day	72	111.07	0.82	0.8060	Time	1.54	-0.08	0.00
Time	84	158.06	342.85	<.0001	Error	1.88	0.93	84.62
Error	168	0.922050				0.01	0.01	0.49

LAS Mean	2.99
Standard Error of LAS Mean	0.14

Table A4.2. Nested Random Effects Analysis of Variance for Variable Effluent LAS

Variance Source	DF	Sum of Squares	F Value	Pr > F	Error Term	Mean Square	Variance Component	Percent of total
Total	335	5.26				0.02	0.02	100.00
Week	11	2.36	12.46	<.0001	Day	0.21	0.01	41.96
Day	72	1.24	0.89	0.6912	Time	0.02	-0.0005	0.00
Time	84	1.62	110.59	<.0001	Error	0.02	0.01	56.99
Error	168	0.02				0.0001	0.0002	1.04

LAS Mean	0.34
Standard Error of LAS Mean	0.03

Table A4.3. Tukey's Studentized Range (HSD) Test for Influent LAS

Alpha	0.05
Error Degree of Freedom	252
Error Mean Square	0.630896
Critical Value of Studentized Range	4.66566
Minimum Significant Difference	0.7003

Mean with the same letter are not significantly different

Tukey Grouping				**Mean**	**N**	**week**
		A		3.824	28	1
B		A		3.792	28	3
B		A	C	3.256	28	2
B		D	C	3.121	28	9
B	E	D	C	3.096	28	6
	E	D	C	2.974	28	5
	E	D	C	2.93	28	7
	E	D	C	2.85	28	12
	E	D	C	2.75	28	8
	E	D		2.50	28	4
	E			2.41	28	10
	E			2.41	28	11

Appendix 5. Data tables

Table A5.1. Highest and Lowest Mean of Influent LAS Concentration Through the Week (Figure 7)

Weeks	Days	Highest mean of influent LAS	Days	Lowest mean of influent LAS
1	Tuesday	4.740	Sunday	1.825
2	Monday	4.055	Sunday	1.745
3	Friday	4.625	Sunday	2.595
4	Friday	3.025	Tuesday	1.875
5	Monday	3.985	Wednesday	2.525
6	Saturday	3.78	Wednesday	2.61
7	Wednesday	3.58	Saturday	2.62
8	Wednesday	3.065	Monday	2.34
9	Monday	3.45	Tuesday	2.52
10	Tuesday	2.59	Thursday	1.93
11	Wednesday	2.53	Tuesday	2.01
12	Saturday	3.41	Friday	2.58

Table A5.2. The LAS Concentration Data Through the Study Period

Week	Date	Day	Time	Influent LAS Sample1	Influent LAS Sample2	Effluent LAS Sample1	Effluent LAS Sample2
Week1	24-Feb	Monday	10:00	3.51	3.6	0.23	0.23
Week1	24-Feb	Monday	14:30	5.52	5.52	0.43	0.4
Week1	24-Feb	Monday	19:00	4.62	4.56	0.47	0.47
Week1	25-Feb	Tuesday	10:00	4.2	4.26	0.72	0.71
Week1	25-Feb	Tuesday	14:30	5.46	5.4	0.41	0.43
Week1	25-Feb	Tuesday	19:00	4.62	4.5	0.45	0.45
Week1	26-Feb	Wednesday	10:00	3.63	3.57	0.42	0.43
Week1	26-Feb	Wednesday	14:30	3.63	3.63	0.43	0.43
Week1	26-Feb	Wednesday	19:00	5.4	5.46	1.27	1.3
Week1	27-Feb	Thursday	10:00	3.27	3.33	0.38	0.38
Week1	27-Feb	Thursday	14:30	3.6	3.57	0.5	0.51
Week1	27-Feb	Thursday	19:00	5.46	5.49	1.26	1.25
Week1	28-Feb	Friday	10:00	5.19	5.19	0.51	0.5
Week1	28-Feb	Friday	14:30	6.54	6.45	0.52	0.52
Week1	28-Feb	Friday	19:00	2.22	2.19	0.4	0.41
Week1	1-Mar	Saturday	10:00	3.03	3.09	0.39	0.39
Week1	1-Mar	Saturday	14:30	4.83	4.83	0.56	0.57
Week1	1-Mar	Saturday	19:00	2.31	2.34	0.44	0.44
Week1	2-Mar	Sunday	10:00	2.58	2.58	0.45	0.44
Week1	2-Mar	Sunday	14:30	1.41	1.41	0.38	0.37
Week1	2-Mar	Sunday	19:00	1.47	1.5	0.61	0.63
Week2	3-Mar	Monday	10:00	2.61	2.64	0.42	0.4
Week2	3-Mar	Monday	14:30	6.6	6.54	0.42	0.42
Week2	3-Mar	Monday	19:00	2.97	2.97	0.42	0.4
Week2	4-Mar	Tuesday	10:00	2.82	2.82	0.43	0.4
Week2	4-Mar	Tuesday	14:30	4.83	4.74	0.6	0.63
Week2	4-Mar	Tuesday	19:00	2.85	2.85	0.41	0.4
Week2	5-Mar	Wednesday	10:00	3.87	4.02	0.46	0.46
Week2	5-Mar	Wednesday	14:30	4.44	4.32	0.46	0.46
Week2	5-Mar	Wednesday	19:00	2.37	2.37	0.38	0.35
Week2	6-Mar	Thursday	10:00	3.27	3.24	0.38	0.36
Week2	6-Mar	Thursday	14:30	3.3	3	0.38	0.38
Week2	6-Mar	Thursday	19:00	3.51	3.51	0.43	0.45
Week2	7-Mar	Friday	10:00	4.32	4.41	0.37	0.32
Week2	7-Mar	Friday	14:30	4.11	3.9	0.38	0.36
Week2	7-Mar	Friday	19:00	2.1	2.28	0.41	0.45
Week2	8-Mar	Saturday	10:00	3.3	3.9	0.34	0.38
Week2	8-Mar	Saturday	14:30	4.74	4.68	0.32	0.38

Week	Date	Day	Time	Influent LAS Sample1	Influent LAS Sample2	Effluent LAS Sample1	Effluent LAS Sample2
Week2	8-Mar	Saturday	19:00	2.25	2.37	0.32	0.35
Week2	9-Mar	Sunday	10:00	2.4	2.58	0.41	0.43
Week2	9-Mar	Sunday	14:30	1.2	1.26	0.4	0.4
Week2	9-Mar	Sunday	19:00	1.47	1.56	0.25	0.24
Week3	10-Mar	Monday	10:00	3.06	3	0.39	0.38
Week3	10-Mar	Monday	14:30	6.06	6.09	0.41	0.31
Week3	10-Mar	Monday	19:00	2.73	2.7	0.33	0.29
Week3	11-Mar	Tuesday	10:00	3.18	3.24	0.29	0.38
Week3	11-Mar	Tuesday	14:30	4.68	4.5	0.34	0.34
Week3	11-Mar	Tuesday	19:00	2.7	2.88	0.35	0.34
Week3	12-Mar	Wednesday	10:00	2.85	2.79	0.33	0.34
Week3	12-Mar	Wednesday	14:30	4.5	4.59	0.37	0.36
Week3	12-Mar	Wednesday	19:00	2.73	2.79	0.36	0.36
Week3	13-Mar	Thursday	10:00	2.82	2.82	0.37	0.37
Week3	13-Mar	Thursday	14:30	3.51	3.3	0.34	0.35
Week3	13-Mar	Thursday	19:00	5.01	4.8	0.36	0.36
Week3	14-Mar	Friday	10:00	3.45	3.6	0.35	0.34
Week3	14-Mar	Friday	14:30	5.13	5.1	0.41	0.42
Week3	14-Mar	Friday	19:00	5.28	5.19	0.36	0.34
Week3	15-Mar	Saturday	10:00	2.88	2.85	0.38	0.35
Week3	15-Mar	Saturday	14:30	4.77	4.8	0.41	0.43
Week3	15-Mar	Saturday	19:00	5.55	5.55	0.39	0.39
Week3	16-Mar	Sunday	10:00	1.17	1.17	0.34	0.33
Week3	16-Mar	Sunday	14:30	1.32	1.26	0.31	0.32
Week3	16-Mar	Sunday	19:00	5.34	5.31	0.35	0.35
Week4	16-Mar	Monday	10:00	1.17	1.26	0.25	0.24
Week4	16-Mar	Monday	14:30	3.12	3.09	0.2	0.21
Week4	16-Mar	Monday	19:00	1.89	1.98	0.21	0.21
Week4	17-Mar	Tuesday	10:00	1.29	1.2	0.19	0.19
Week4	17-Mar	Tuesday	14:30	3.06	3.15	0.16	0.16
Week4	17-Mar	Tuesday	19:00	1.26	1.29	0.25	0.25
Week4	18-Mar	Wednesday	10:00	1.26	1.26	0.3	0.24
Week4	18-Mar	Wednesday	14:30	3.15	3.12	0.26	0.25
Week4	18-Mar	Wednesday	19:00	3.15	3.15	0.25	0.25
Week4	19-Mar	Thursday	10:00	1.92	1.89	0.28	0.28
Week4	19-Mar	Thursday	14:30	4.17	4.05	0.26	0.25
Week4	19-Mar	Thursday	19:00	2.4	2.46	0.26	0.26
Week4	20-Mar	Friday	10:00	3.03	3.12	0.25	0.24
Week4	20-Mar	Friday	14:30	3.54	3.6	0.37	0.35
Week4	20-Mar	Friday	19:00	2.46	2.4	0.34	0.33

Week	Date	Day	Time	Influent LAS Sample1	Influent LAS Sample2	Effluent LAS Sample1	Effluent LAS Sample2
Week4	21-Mar	Saturday	10:00	2.64	2.61	0.34	0.33
Week4	21-Mar	Saturday	14:30	2.55	2.55	0.37	0.36
Week4	21-Mar	Saturday	19:00	2.1	2.28	0.34	0.34
Week4	22-Mar	Sunday	10:00	2.7	2.76	0.35	0.35
Week4	22-Mar	Sunday	14:30	1.86	1.86	0.33	0.34
Week4	22-Mar	Sunday	19:00	2.46	2.46	0.32	0.3
Week5	24-Mar	Monday	10:00	2.61	2.64	0.32	0.3
Week5	24-Mar	Monday	14:30	5.46	5.46	0.37	0.35
Week5	24-Mar	Monday	19:00	3.84	3.9	0.36	0.36
Week5	25-Mar	Tuesday	10:00	3.99	4.2	0.32	0.33
Week5	25-Mar	Tuesday	14:30	2.79	2.7	0.3	0.3
Week5	25-Mar	Tuesday	19:00	4.53	4.56	0.3	0.32
Week5	26-Mar	Wednesday	10:00	2.7	2.79	0.32	0.31
Week5	26-Mar	Wednesday	14:30	2.97	3	0.26	0.25
Week5	26-Mar	Wednesday	19:00	1.89	1.8	0.3	0.31
Week5	27-Mar	Thursday	10:00	2.67	2.55	0.25	0.22
Week5	27-Mar	Thursday	14:30	2.67	2.7	0.23	0.22
Week5	27-Mar	Thursday	19:00	3.96	3.96	0.26	0.26
Week5	28-Mar	Friday	10:00	2.7	2.76	0.2	0.19
Week5	28-Mar	Friday	14:30	2.4	2.43	0.2	0.21
Week5	28-Mar	Friday	19:00	3.15	3.06	0.25	0.23
Week5	29-Mar	Saturday	10:00	2.58	2.7	0.27	0.26
Week5	29-Mar	Saturday	14:30	5.28	5.1	0.26	0.26
Week5	29-Mar	Saturday	19:00	3.15	3	0.2	0.21
Week5	30-Mar	Sunday	10:00	2.7	3	0.27	0.27
Week5	30-Mar	Sunday	14:30	4.14	4.2	0.26	0.25
Week5	30-Mar	Sunday	19:00	2.91	2.91	0.2	0.21
Week6	31-Mar	Monday	10:00	2.67	2.7	0.26	0.28
Week6	31-Mar	Monday	14:30	4.02	3.9	0.25	0.26
Week6	31-Mar	Monday	19:00	2.37	2.37	0.27	0.27
Week6	1-Apr	Tuesday	10:00	2.97	3	0.31	0.31
Week6	1-Apr	Tuesday	14:30	2.64	2.64	0.27	0.26
Week6	1-Apr	Tuesday	19:00	2.82	2.82	0.27	0.27
Week6	2-Apr	Wednesday	10:00	2.61	2.64	0.29	0.29
Week6	2-Apr	Wednesday	14:30	2.46	2.46	0.26	0.27
Week6	2-Apr	Wednesday	19:00	2.76	2.73	0.27	0.27
Week6	3-Apr	Thursday	10:00	3	3.03	0.28	0.28
Week6	3-Apr	Thursday	14:30	5.19	5.07	0.29	0.28
Week6	3-Apr	Thursday	19:00	2.76	2.73	0.28	0.27
Week6	4-Apr	Friday	10:00	2.97	2.97	0.27	0.27

Week	Date	Day	Time	Influent LAS Sample1	Influent LAS Sample2	Effluent LAS Sample1	Effluent LAS Sample2
Week6	4-Apr	Friday	14:30	3.3	3.3	0.28	0.27
Week6	4-Apr	Friday	19:00	3.21	3.15	0.4	0.35
Week6	5-Apr	Saturday	10:00	2.49	2.64	0.37	0.36
Week6	5-Apr	Saturday	14:30	5.43	5.7	0.37	0.37
Week6	5-Apr	Saturday	19:00	3.3	3.12	0.32	0.32
Week6	6-Apr	Sunday	10:00	2.01	2.01	0.32	0.32
Week6	6-Apr	Sunday	14:30	4.11	4.11	0.3	0.3
Week6	6-Apr	Sunday	19:00	2.97	2.97	0.35	0.35
Week7	7-Apr	Monday	10:00	2.7	2.85	0.22	0.22
Week7	7-Apr	Monday	14:30	4.5	4.47	0.31	0.3
Week7	7-Apr	Monday	19:00	2.7	2.7	0.3	0.3
Week7	8-Apr	Tuesday	10:00	2.91	2.94	0.29	0.3
Week7	8-Apr	Tuesday	14:30	3.51	3.51	0.28	0.28
Week7	8-Apr	Tuesday	19:00	2.82	2.88	0.3	0.3
Week7	9-Apr	Wednesday	10:00	3.3	3.33	0.28	0.28
Week7	9-Apr	Wednesday	14:30	3.45	3.45	0.27	0.27
Week7	9-Apr	Wednesday	19:00	3.99	3.96	0.26	0.26
Week7	10-Apr	Thursday	10:00	2.34	2.4	0.29	0.28
Week7	10-Apr	Thursday	14:30	2.52	2.55	0.28	0.28
Week7	10-Apr	Thursday	19:00	3	3.15	0.33	0.33
Week7	11-Apr	Friday	10:00	2.04	2.1	0.31	0.31
Week7	11-Apr	Friday	14:30	3.93	3.93	0.3	0.32
Week7	11-Apr	Friday	19:00	3.93	3.93	0.25	0.24
Week7	12-Apr	Saturday	10:00	2.4	2.49	0.21	0.22
Week7	12-Apr	Saturday	14:30	2.46	2.4	0.22	0.21
Week7	12-Apr	Saturday	19:00	2.97	3	0.32	0.3
Week7	13-Apr	Sunday	10:00	2.19	2.19	0.24	0.26
Week7	13-Apr	Sunday	14:30	2.07	2.1	0.26	0.24
Week7	13-Apr	Sunday	19:00	3.6	3.6	0.33	0.32
Week8	14-Apr	Monday	10:00	2.55	2.4	0.24	0.25
Week8	14-Apr	Monday	14:30	2.55	2.46	0.26	0.26
Week8	14-Apr	Monday	19:00	2.04	2.04	0.27	0.27
Week8	15-Apr	Tuesday	10:00	1.71	1.8	0.3	0.32
Week8	15-Apr	Tuesday	14:30	3.09	3.3	0.28	0.28
Week8	15-Apr	Tuesday	19:00	2.67	2.64	0.27	0.26
Week8	16-Apr	Wednesday	10:00	2.67	2.67	0.3	0.32
Week8	16-Apr	Wednesday	14:30	3.78	3.6	0.35	0.35
Week8	16-Apr	Wednesday	19:00	2.76	2.91	0.25	0.25
Week8	17-Apr	Thursday	10:00	2.52	2.58	0.29	0.28
Week8	17-Apr	Thursday	14:30	3.3	3	0.31	0.31

Week	Date	Day	Time	Influent LAS Sample1	Influent LAS Sample2	Effluent LAS Sample1	Effluent LAS Sample2
Week8	17-Apr	Thursday	19:00	2.31	2.34	0.32	0.32
Week8	18-Apr	Friday	10:00	2.61	2.4	0.39	0.39
Week8	18-Apr	Friday	14:30	3.57	3.75	0.37	0.39
Week8	18-Apr	Friday	19:00	2.64	2.4	0.33	0.33
Week8	19-Apr	Saturday	10:00	3.06	3	0.25	0.25
Week8	19-Apr	Saturday	14:30	2.85	2.97	0.25	0.25
Week8	19-Apr	Saturday	19:00	2.55	2.49	0.3	0.3
Week8	20-Apr	Sunday	10:00	2.88	2.85	0.28	0.27
Week8	20-Apr	Sunday	14:30	2.61	2.52	0.29	0.29
Week8	20-Apr	Sunday	19:00	2.46	2.43	0.31	0.3
Week9	21-Apr	Monday	10:00	2.91	2.91	0.31	0.31
Week9	21-Apr	Monday	14:30	5.01	5.1	0.3	0.3
Week9	21-Apr	Monday	19:00	2.37	2.4	0.32	0.32
Week9	22-Apr	Tuesday	10:00	2.01	2.1	0.3	0.3
Week9	22-Apr	Tuesday	14:30	3.3	3.06	0.31	0.3
Week9	22-Apr	Tuesday	19:00	2.28	2.37	0.32	0.32
Week9	23-Apr	Wednesday	10:00	2.79	2.85	0.29	0.28
Week9	23-Apr	Wednesday	14:30	3.27	3.09	0.28	0.28
Week9	23-Apr	Wednesday	19:00	2.67	2.58	0.3	0.3
Week9	24-Apr	Thursday	10:00	3.9	4.2	0.3	0.3
Week9	24-Apr	Thursday	14:30	3.6	3.6	0.28	0.27
Week9	24-Apr	Thursday	19:00	2.67	2.61	0.29	0.29
Week9	25-Apr	Friday	10:00	3.24	3.57	0.36	0.28
Week9	25-Apr	Friday	14:30	3.48	3.51	0.36	0.3
Week9	25-Apr	Friday	19:00	2.7	2.76	0.28	0.28
Week9	26-Apr	Saturday	10:00	2.67	2.7	0.35	0.34
Week9	26-Apr	Saturday	14:30	3.3	3.21	0.32	0.3
Week9	26-Apr	Saturday	19:00	2.7	2.67	0.28	0.29
Week9	27-Apr	Sunday	10:00	2.4	2.46	0.35	0.37
Week9	27-Apr	Sunday	14:30	3	3.21	0.27	0.26
Week9	27-Apr	Sunday	19:00	2.52	2.7	0.25	0.25
Week10	28-Apr	Monday	10:00	2.25	2.19	0.27	0.25
Week10	28-Apr	Monday	14:30	3.09	3	0.23	0.23
Week10	28-Apr	Monday	19:00	2.28	2.37	0.23	0.23
Week10	29-Apr	Tuesday	10:00	1.62	1.8	0.24	0.25
Week10	29-Apr	Tuesday	14:30	3.09	3.15	0.26	0.27
Week10	29-Apr	Tuesday	19:00	2.94	2.94	0.29	0.3
Week10	30-Apr	Wednesday	10:00	2.91	2.88	0.24	0.25
Week10	30-Apr	Wednesday	14:30	2.58	2.55	0.26	0.26
Week10	30-Apr	Wednesday	19:00	1.8	1.77	0.36	0.35

Week	Date	Day	Time	Influent LAS Sample1	Influent LAS Sample2	Effluent LAS Sample1	Effluent LAS Sample2
Week10	1-May	Thursday	10:00	2.46	2.4	0.32	0.32
Week10	1-May	Thursday	14:30	1.68	1.68	0.31	0.31
Week10	1-May	Thursday	19:00	1.68	1.68	0.33	0.33
Week10	2-May	Friday	10:00	1.8	1.89	0.34	0.34
Week10	2-May	Friday	14:30	3.33	3.6	0.32	0.32
Week10	2-May	Friday	19:00	2.28	2.28	0.29	0.3
Week10	3-May	Saturday	10:00	2.28	2.28	0.27	0.28
Week10	3-May	Saturday	14:30	2.73	2.7	0.27	0.28
Week10	3-May	Saturday	19:00	2.28	2.28	0.34	0.35
Week10	4-May	Sunday	10:00	2.28	2.28	0.29	0.3
Week10	4-May	Sunday	14:30	1.92	2.28	0.37	0.37
Week10	4-May	Sunday	19:00	2.1	2.16	0.37	0.37
Week11	5-May	Monday	10:00	1.83	1.8	0.33	0.33
Week11	5-May	Monday	14:30	3.09	3.15	0.3	0.3
Week11	5-May	Monday	19:00	2.16	2.16	0.35	0.34
Week11	6-May	Tuesday	10:00	2.19	2.16	0.34	0.34
Week11	6-May	Tuesday	14:30	1.59	1.65	0.27	0.3
Week11	6-May	Tuesday	19:00	2.25	2.22	0.32	0.33
Week11	7-May	Wednesday	10:00	1.35	1.5	0.3	0.3
Week11	7-May	Wednesday	14:30	3.87	4.2	0.33	0.32
Week11	7-May	Wednesday	19:00	2.1	2.16	0.3	0.3
Week11	8-May	Thursday	10:00	1.89	1.8	0.32	0.32
Week11	8-May	Thursday	14:30	3.15	3.06	0.31	0.31
Week11	8-May	Thursday	19:00	2.4	2.46	0.32	0.33
Week11	9-May	Friday	10:00	2.1	2.16	0.34	0.34
Week11	9-May	Friday	14:30	2.7	2.76	0.32	0.32
Week11	9-May	Friday	19:00	2.1	2.19	0.31	0.31
Week11	10-May	Saturday	10:00	2.28	2.19	0.3	0.31
Week11	10-May	Saturday	14:30	3.12	3.06	0.32	0.32
Week11	10-May	Saturday	19:00	2.07	2.1	0.33	0.33
Week11	11-May	Sunday	10:00	2.34	2.37	0.32	0.32
Week11	11-May	Sunday	14:30	3.27	3.24	0.31	0.31
Week11	11-May	Sunday	19:00	1.98	1.95	0.33	0.34
Week12	12-May	Monday	10:00	2.76	2.7	0.4	0.37
Week12	12-May	Monday	14:30	4.29	4.2	0.35	0.35
Week12	12-May	Monday	19:00	2.61	2.58	0.37	0.37
Week12	13-May	Tuesday	10:00	2.16	2.1	0.4	0.4
Week12	13-May	Tuesday	14:30	3	3.06	0.35	0.36
Week12	13-May	Tuesday	19:00	2.61	2.61	0.32	0.32
Week12	14-May	Wednesday	10:00	2.7	2.76	0.37	0.39

Week	Date	Day	Time	Influent LAS Sample1	Influent LAS Sample2	Effluent LAS Sample1	Effluent LAS Sample2
Week12	14-May	Wednesday	14:30	2.91	2.85	0.4	0.42
Week12	14-May	Wednesday	19:00	2.49	2.61	0.33	0.33
Week12	15-May	Thursday	10:00	2.52	2.46	0.35	0.35
Week12	15-May	Thursday	14:30	3.48	3.36	0.42	0.42
Week12	15-May	Thursday	19:00	2.43	2.4	0.38	0.38
Week12	16-May	Friday	10:00	2.31	2.25	0.41	0.41
Week12	16-May	Friday	14:30	2.16	2.1	0.39	0.39
Week12	16-May	Friday	19:00	3.3	3.36	0.48	0.43
Week12	17-May	Saturday	10:00	3.78	3.6	0.41	0.39
Week12	17-May	Saturday	14:30	3.03	3	0.33	0.32
Week12	17-May	Saturday	19:00	3.6	3.45	0.35	0.35
Week12	18-May	Sunday	10:00	3.3	3	0.39	0.36
Week12	18-May	Sunday	14:30	3	3	0.35	0.35
Week12	18-May	Sunday	19:00	3.66	3.6	0.36	0.36