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Linear Alkylbenzene Sulfonate concentration varies Daily, Weekly and Seasonaly in Pittsburg's Wastewater, Kansas

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Key words: Linear alkylbenzene sulfonate, LAS, Wastewater, Wastewater treatment plant.

Abstract

Linear Alkyl Benzene Sulfonate (LAS) is a biodegradable surfactant, which is commonly utilized industrially and domestically. Concentrations of LAS 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 (10:00, 14:30, and 19:00), every day for 12 weeks February 24, 2014 - May 18, 2014). Concentrations were determined by the Methylene Blue Active Substances method. There was very little variation in the effluent concentrations which were much lower (one tenth) and more consistent (0.27 – 0.53mg/L) than 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 (17 March – 22 April – 5 May). These three weeks were significantly different from almost all the other weeks but were not significantly different from each other. They coincided with certain university activities. College students may have a large impact on influent LAS concentrations because they make up 25% of Pittsburg's population. The week of March 17 was spring break when many of the students were out of town, and the other two weeks were during final exams. During these two weeks, students probably are not doing much laundry. The effectiveness of Pittsburg's Wastewater Treatment Plant was 90%, which is similar to others reported in the literature.

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Introduction

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).

The chemical structure of LAS is $CH_3(CH_2)_mCH\text{-}(CH_2)_n$ $-CH_3SO_3Na$ (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 (Fig. 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).

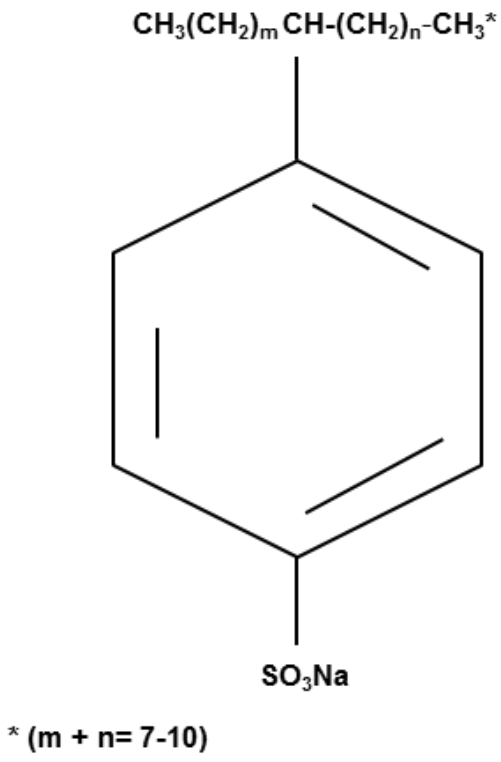

Fig. 1. Structure of LAS.

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.

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 199.7mg/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).

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.

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 8.22mg/L.

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 – 299mg/L) for the fresh water algae and (0.024 – 9.9)mg/L for the marine groups.

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 3.5mg/L to 5.0mg/L. The secondary lamellae in gill epithelium was destroyed when LAS concentration reached 2.5mg/L. The LC_{50} value was 3.28mg/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 28mg/L at 24 hours. The LC_{50} was 25.5mg/L. Thus, the overall LAS toxicity data concerning the aquatic organisms fluctuate between 1 and 10mg per liter in brief durability experiments.

LAS shows slight acute toxicity in Mammals. The oral the median lethal dose (LD_{50}) values for rats range from 1,080 to 1,980mg/kg body weight (bw) (UNEP, 2005). While the oral LD_{50} values for mice are 2,160mg/kg bw for males and 2,250mg/kg bw for females. The dermal LD_{50} value for rat was greater than 2,000mg/kg bw. Mortality occurring at respirable particle concentrations of 310mg/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 750mg/kg bw/day. While no-observed adverse effect level (NOAELs) ranged between 40 and 250mg/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 2.5mg/kg and 5.0mg/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) (4mg/kg), LAS (12mg/kg) and PPD (4mg/kg) plus LAS (12mg/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.

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).

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. 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.

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)

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).

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.

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).

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).

Methods and materials

Influent and effluent samples were collected within five minutes at the bar screen and cascade respectively (Fig. 2). Samples were collected three times each day (10:00, 14:30, 19:00) (Fig. 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).

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 (Fig. 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.

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

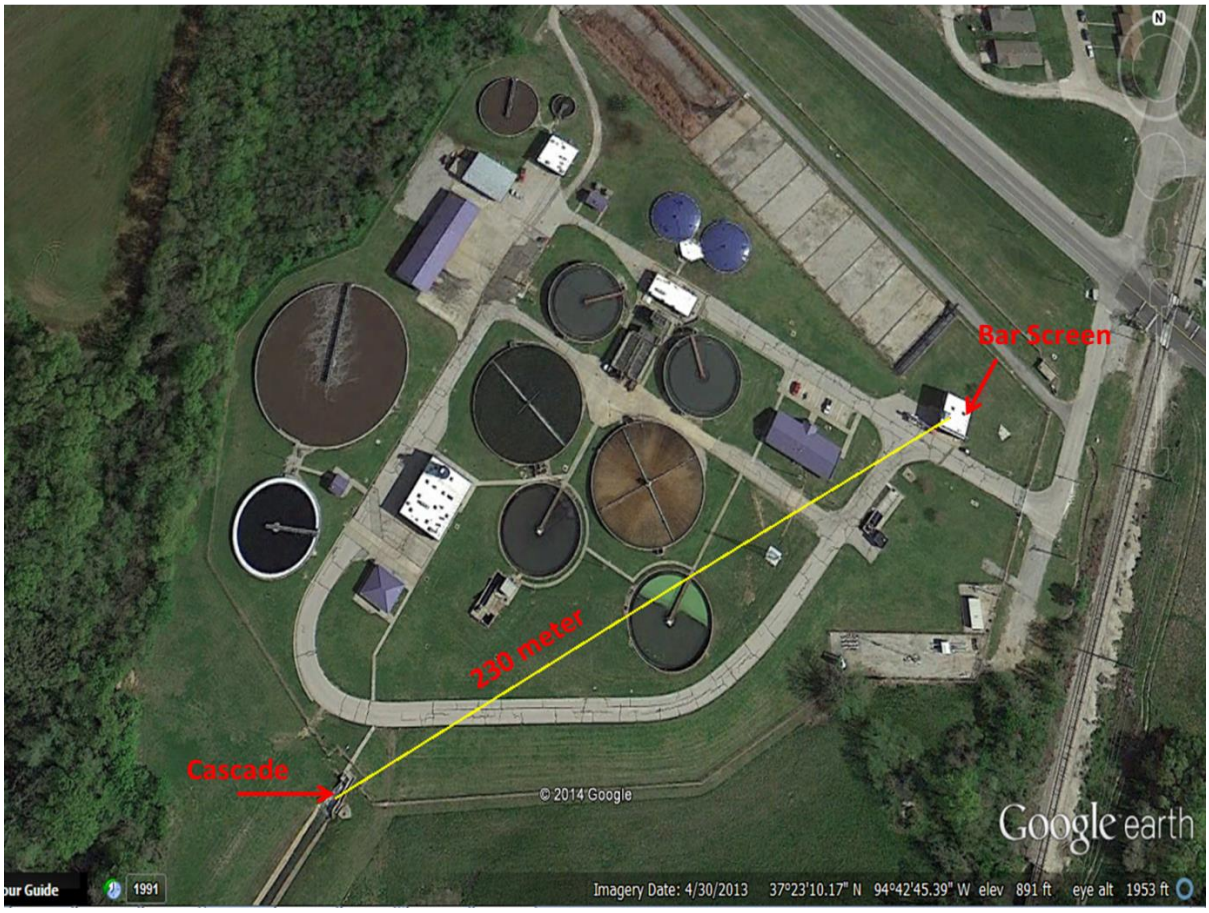

Fig. 2. Pittsburg's Wastewater Treatment Plant illustration for Bar Screen and Cascade.

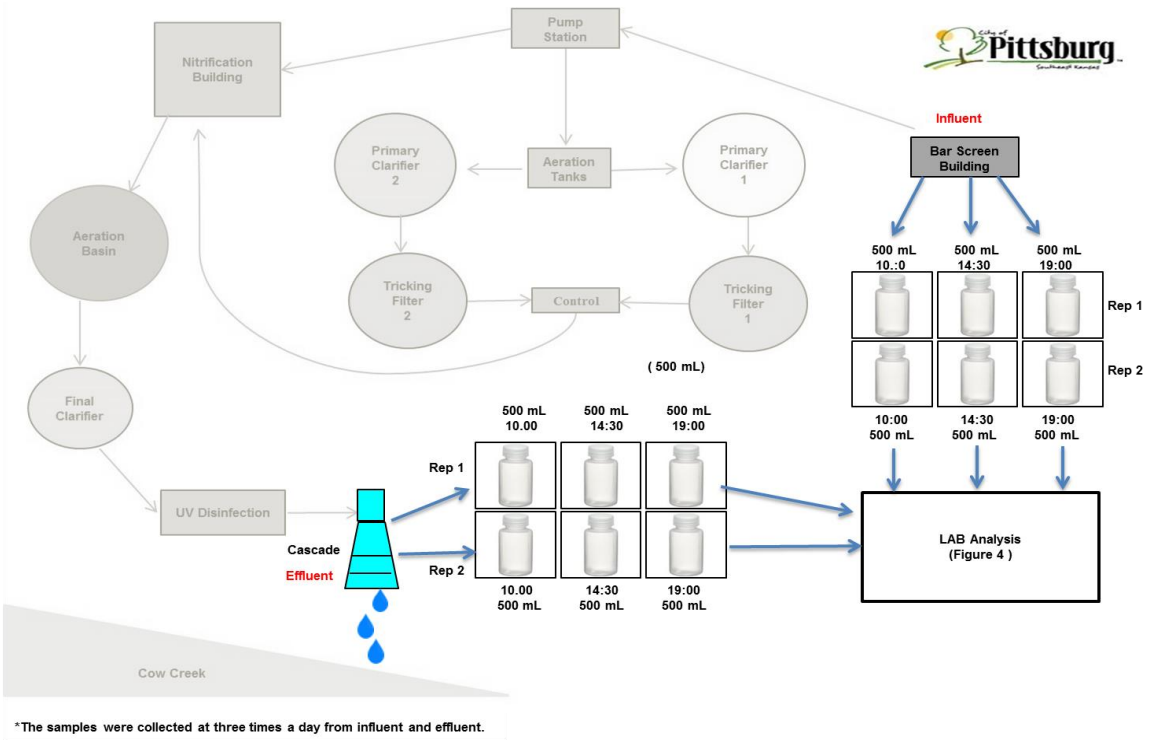

Fig. 3. Collecting Wastewater Samples* at Pittsburg's wastewater Treatment Plant.

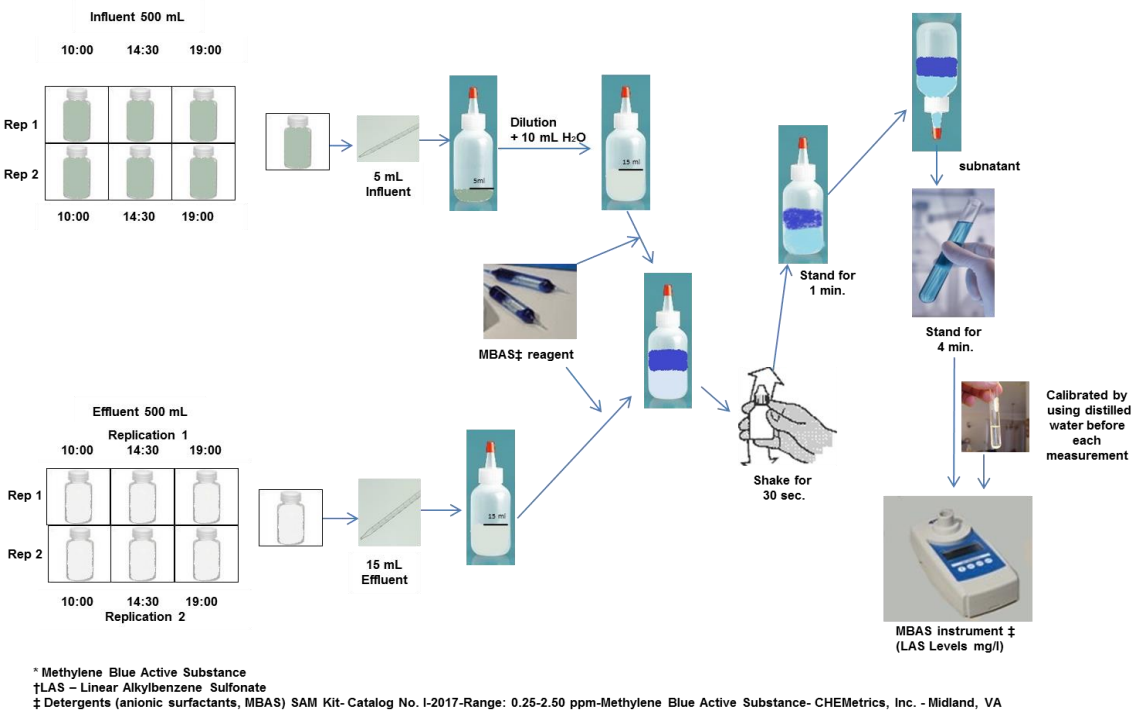

Fig. 4. Lab Analysis* of LASt concentration.

Results and discussion

The weekly means (pooling each weekday and its three sample times) of influent LAS concentration (Fig. 5) were very high and varied throughout the study period (2.4 – 3.9mg/L). The standard deviation was also high and varied throughout the study period (0.5 – 1.4mg/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 – 0.53mg/L) than the influent. The effluent standard deviation was also lower and more consistent (0.1 – 0.26mg/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 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.

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).

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 0.62mg/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}).

Examining the time of day for each week throughout the study period, Fig. 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 – 4.4mg/L). The mean LAS concentration at 14:30 for the entire study period ($\bar{x}$ =3.5 ± 1mg/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 ± 0.56mg/L). However, examining each week, 10:00 was lowest only eight out of twelve weeks (ranging from 1.9 – 3.7mg/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). Examining each day within each week throughout the study period, Fig. 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.

However, Sunday was lowest only three out of twelve weeks (ranging from 1.7 – 3.3mg/L) and Tuesday was highest only two out of twelve weeks (ranging from 1.9 – 4.7mg/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 ± 1.1mg/L), and lowest on Sunday ($\bar{x}$ =2.57 ± 0.70mg/L). There was no significant difference in influent LAS by day (ANOVA f=0.82, df=72 , and P=0.8060, Tukey's HSD).

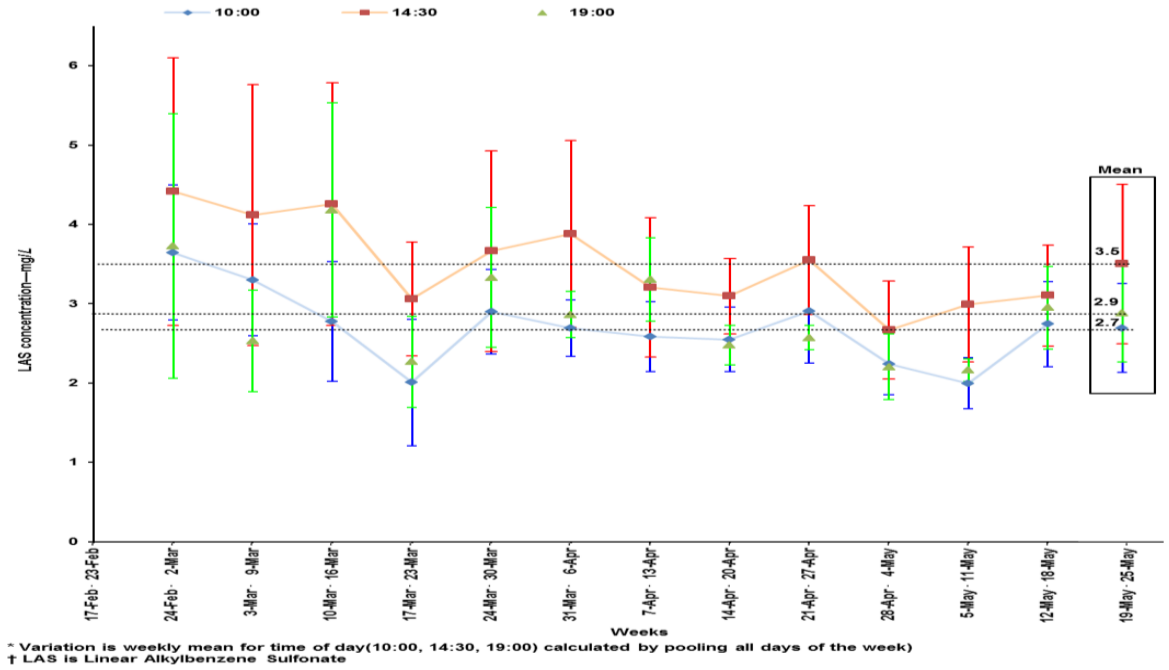

Fig. 6. Variation * in influent Last concentration through the day.

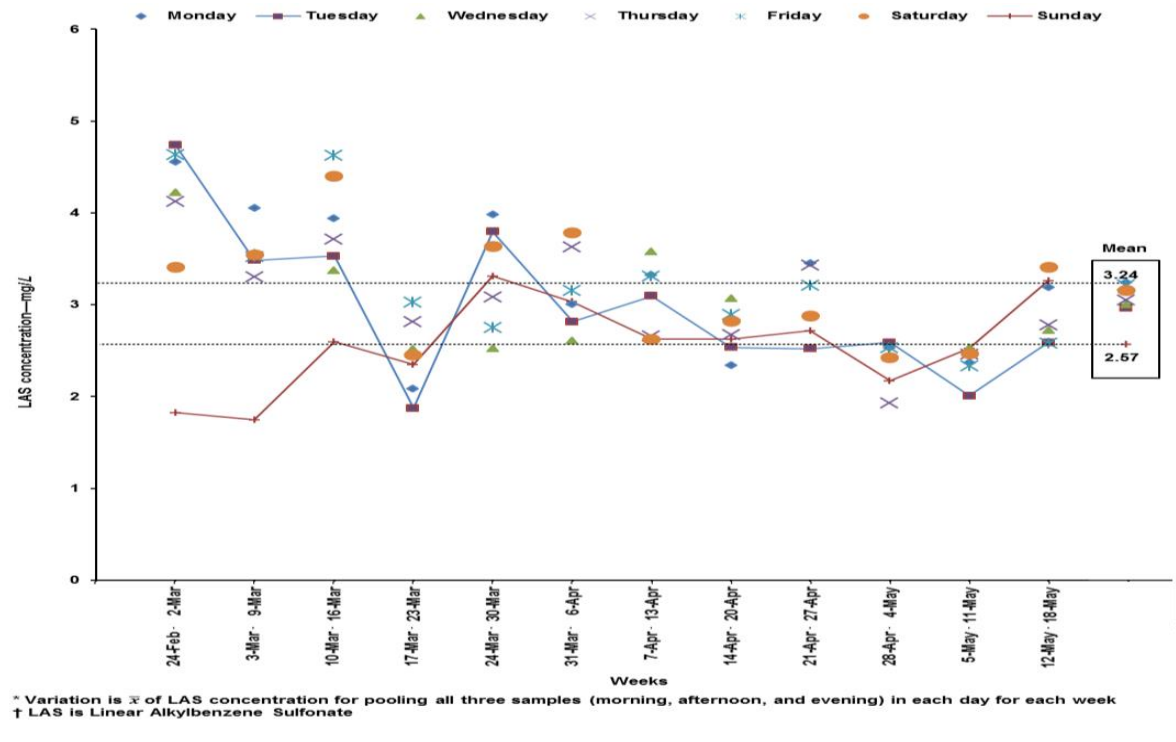

Fig. 7. Variation * in influent Last concentration through the week.

Conclusion

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).

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.

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.

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.

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.

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