



## STUDY OF BACTERIAL POLLUTION IN THE SURFACE MICROLAYER COMPARED WITH SUBSURFACE LAYER IN AL- HILLA RIVER WATER, IRAQ

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### Abstract

This study was conducted on the Hilla River water which specialized in the study of bacterial pollution and some pollutants in the surface microlayer layer (SML) and its comparison with pollution in the subsurface layer (SSL). The samples were collected monthly in five locations in the Babylon province in Iraq. The first location included the north of Bita Bridge before entering the river the residential regions, the second location in the health district region near the police academy, the third location near the doctors bridge, the fourth location in the center of densely populated residential regions and the fifth location near AL-Farsy region south of AL-Hilla city in this location found water treatment plant, places for breeding cattle and slaughterhouses, the collection of samples began from July 2018 to the middle of the third month from March for the year 2019. We used in our study Rotating Glass Drum Apparatus. This study aims to detect the total microbial pollution (Total Count) in general and Fecal Pollution in particular which is an indicator of bacterial water pollution which included the isolation and diagnosis of some bacterial species which are: (*Enterococcus faecalis*)(*Klebsiella pneumoniae*) (*Proteus mirabilis*) (*E. coli*) (*Staphylococcus aureus*) the study also measuring PH, TDS, temperature. The results of our study showed for total microbial count and bacterial pollution in the surface microlayer (SML) is much higher than pollution in the subsurface layer (SSL).

**Keywords:** SML, SSL, physicochemical and biological parameter, Rotating Glass Drum apparatus, Al-Hilla River.

### Introduction

Water pollution is the study of pollution water resources (rivers, lakes, reservoirs, seas, oceans and groundwater) as result for human actions on these resources, where there are many human interventions in the polluting of these water resources such as the discharge of untreated wastewater directly to water leading to the deterioration of ecosystems for these resources and caused health problems for people living along the riverbed through using in drinking, swimming, irrigation and so on. because of pollution of water millions of people die every year around the world (Pink and West, 2006). Most of water organisms are extremely sensitive to change in physicochemical characteristics of water bodies such as pH, alkalinity, hardness, nutrients, dissolved gases (O<sub>2</sub> and CO<sub>2</sub>), temperatures and others (Hassan *et al.*, 2010). The surface microlayer SML is the dividing border between the air and water surface, the SML possess physical, chemical and biological features which are distinctly from subsurface layer (SSL) waters as its unique place between the air- water the SML has remained in a distinct research corner (Wurl *et al.*, 2017). as well as SML is characterized by the capacity to accumulate of organic chemical compounds specially proteins, carbohydrates and lipid (Plusquellec *et al.*, 1991). the presence of such as energy-rich organic matters in the surface microlayer works to find distinct condition for the accumulation and development of aerobic mainly heterotrophic Bacteria forming bacterioneuston (Antonowicz *et al.*, 2015) Bacterioneuston also bacterioplankton bacteria that live deep in the layers of water play a key role in the processes of transformation of the organic substances accumulated in aquatic life (Maki 1993, Maki and Hermansson, 1994). Aim of this study is the study of some bacteriological agents in SML and SSL, study of some Physical and Chemical Pollutants in SML and SSL and comparison between the SML and SSL Pollution, the density of bacterial numbers in the SML where there are study was conducted in 1979 on fresh river water which was intended to measure the number

of bacteria in SML and the subsurface layer SSL where isolates were randomly examined and there were between 130 and 5000 times higher in the SML layer than the subsurface layer SSL per ml. some bacterial species were also studied there was a difference in the species found in SML for species in the subsurface layer where *Pseudomonas* were dominant in the SML layer at about two-thirds while one third of *Pseudomonas* was found in the subsurface layer, various studies indicate that the bacterial abundance in SML exceeds those in the sub-surface water while the dominance of species *Alcaligenes* was in the subsurface layer (Fehon and Oliver, 1979).

### Material and Methods

#### Study Area

River of Euphrates is one of the main deliberate sources of drinking water, Watering plants and other uses Al-Hilla river is branch exit from River of Euphrates, five locations were selected from River of Al-Hilla which has length about 102 km, L1: north of Bita bridge region near Babylon Archaeological region it exhibits sometimes few effluent enter the river in this location, L 2 : near the police academy ( health district region) where found in this location Swimming pool L 3: bridge of the doctors region found in this location discharge for some Medical waste, L4 : near Babylon city center river flow increases and the effluent to the river relatively high, L5: Al-Farisy region south of Al-Hilla city there was a water treatment plant, Places for breeding cattle and slaughterhouses Figure (1)

#### Sample Collection

Collection of samples took eight months were collected from the target study site from July of 2018 to the March of 2019, the sampling process began by sampling areas designated as potential sources of contamination emphasis was placed on the collection of samples from places likely to be human and animal fecal contamination.

**Subsurface layer (SSL) :** Subsurface water (30cm) collected using pre-cleaned dark glass bottle (1L) in metallic holder around the bottle connected with a rope that lowered into water and allows rest briefly to ensure that it filled with water and then transferred to labeled dark bottle (with volume 2.5L) containing (50 ml) of ( $\text{CCl}_4$ ) carbon tetrachloride, sample bottles then finally closed with aluminum foiled lined cap, then stored at  $4\text{ }^\circ\text{C}$  in freezing box till return to the laboratory and analyzed it (UNEP,1989)

**Surface microlayer (SML):** The (SML) samples collected using a rotating glass drum sampler (Harvey, 1966) with some modulations. The SML sampler consisting of a rotating glass drum (R.G.D), vacuum and a sample collect container

was connect with boat by a 3 m long iron bar fixed Surface that holds the device, before collection of sample the sampler was operated in the river water for 15 minute to wash the tubing container and for purpose clean the glass drum with river water. The SML sample on the surface of the (R.G.D) The number of cycles of the device is controlled by a switch installed near the control unit of the boat where diameter 20cm, length 30 cm, rpm 10-12 in minute was collected into the container using by blade from rubber then pumped into the big plastic container. samples kept in polypropylene container and stored in the box of ice before to laboratory work. Figure (2)

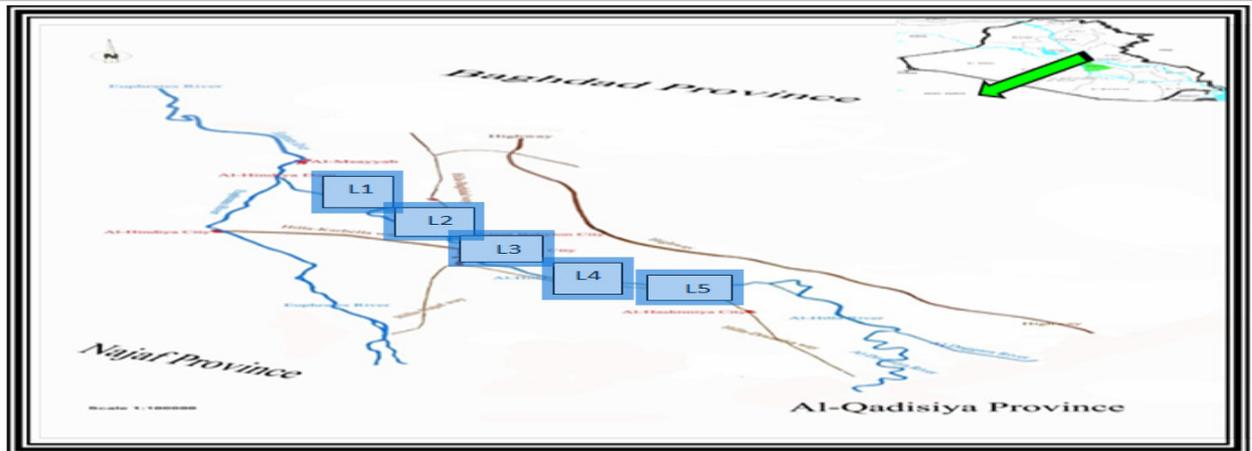


Figure (1) Map shows the studied Locations in Al-Hilla River



Fig. 2 : Rotating glass drum apparatus

### Field work

Water temperature, PH (Hydrogen ions concentration), Total dissolved solids (TDS) all parameters measured by Lovi bond Apparatus after immersing the probe under water and waiting for some minutes .

### Laboratory work

**Total count :** 0.1 ml of river sample was cultured on nutrient agar after a series of dilutions with three duplicates and incubated with  $37\text{ }^\circ\text{C}$  for 24 h or 48 hours after that

counting the developing colonies and multiplying them in the inverted coefficient of dilution and extraction of the average of numbers were also studied and recorded the general specifications of these bacteria.

**Diagnostic of bacterial isolates:** Analyzed by VITEK (2)

## Result and Discussion

### Physical and chemical properties

Different physicochemical parameters (temperature, pH and T.D.S) of water samples were measured only in subsurface layer SSL while others measured in both layers (SSL and SML).

**Temperature of water :** The variation in water temperature always depends on the year seasons, Time at which the sample was taken and the heat of the material thrown into the river (Ahipathy and Puttaiah, 2006). temperature showed high monthly changes where ranged between 33 °C as a highest value in (L 5) in August and the lowest value 13.9 °C in (L 2) in December during the study period Figure (3 ) the maximum limit during the study period (33 °C) was exceeds permissible limit of WHO standards , As this difference in temperature may return to the time of sampling or increase solar radiation on the surface of water, (Ismail, 2001). temperature showed a high significant positive correlation  $** (P \geq 0.01)$  with *Klebsiella pneumonia II* and Total Count II, while showed a slight significant positive correlation  $*(P \geq 0.05)$  with *Enterococcus faecalis II*, *E. coli II* and *Staphylococcus aureus II* and a slight significant negative correlation  $*(P \leq 0.05)$  with *Proteus mirabilis II* Table (1)

**pH Value :** Generally seasonal differences in pH due to many factors such as removal of carbon dioxide by photosynthesis during bicarbonate degradation, low primary production, degradation of organic materials and decrease of salinity and temperature (Bragadeeswaran *et al.*, 2007), pH values ranged between the highest (8.1) in (L 2) and (L 4) in February and the lowest (6.2) in (L 1) in November Figure (4). The maximum limit during the study period was (8.1) it was consistent with the permissible limits of WHO standards, pH showed slight significant positive correlation  $*(P \geq 0.05)$  with T.D.S Table (1)

**Total Dissolved solids (TDS Values) :** T.D.S are the total summation of mobile charged ions which include salts and minerals it is related with water quality and purity of water purification systems, municipal as well as industrial wastes can have the effect of raising the total dissolved solids of rivers water (Hammer, 1971), T.D.S values was between the highest (900 mg / l ) in (L 5) in March and lowest (730 mg /l) in (L 1) in December Figure (5) the maximum limit during study periods (900 mg/l ) was permissible for standards of WHO, the high concentrations of the T.D.S of AL-Hilla river in the study region were explained in terms of the effect of the sewage water on the river water quality.

### Biological Parameter

Different biological parameters of water samples listed in Figures from figure (6) to figure (11).

**Total count (T.C) :** T.C give us a quantitative valuation of the density of microorganisms such as bacteria, yeasts or moulds spores in the sample, the Total Count stand for the numbers of colony forming units (CFUs) (per g or per ml) of samples. This is achieved by plating serial tenfold dilutions of the samples (between 30 and 300 colonies) which are counted on a single dish. The reported count is the number of colonies counted multiplied by the dilution used for the counted dish. a high T.C interpreter a high numbers of microorganisms which may explain for drinking water are poor quality (Biyani *et al.*, 2018). Numbers of total count

varied from higher value was in SML (29900 cells/100 ml) in (L 4) in July and in (L 5) in August, to the lower value (600 cells/100ml) in (L 5) in January, while the highest value of Total Count in SSL (23500 cells/100 ml) in (L 2) in October and the lowest value was (600 cells/100 ml) in (L 1) in January, generally Total Count was higher in SML up to (29900 cells/100 ml) than in SSL was (23500 cells/100 ml), these results as regards to the SSL are consistent with (Jahsani, 2003) Figure (6). The increases in the numbers of bacteria in these locations which are very close to the heavy water discharges where amount of discharges into the river with its enormous bacterial numbers leading to high density of microbial pollution the presence of a source of organic pollutant leads to an increase in the number of bacteria as well as the rise in temperature, which will increase the activity and proliferation of bacteria, other sites recorded an increase in the number of bacteria and exceeded the limits allowed globally as the reports of the World Health Organization allow 50 cells/cm<sup>3</sup> (Talee and AL-Barhawi, 2000). calculating Pearson's correlation coefficient in the Table (1) showed a high positive significant correlation  $** (P \geq 0.01)$  between *Total count I*, *temperature*, and *Klebsiella pneumonia I* as well as found a slight positive significant correlation with *Enterococcus faecalis I*, *E. coli I* and *Staphylococcus aureus I*, moreover there was slight negative correlation  $*(p \leq 0.05)$  with Total Count in surface microlayer and subsurface layer Table (1)

**Enterococcus faecalis :** Former classification is regarded as a part of the group *Streptococcus* system is a gram-positive, commensal bacterium occur in the intestinal tract of humans and mammal. (Ryan and Ray, 2004) like other species in the genus (*Enterococcus*) and (*E. faecalis*) is occur in healthy humans however can cause threatening infection especially in the nosocomial. naturally high levels of antibiotics resistance can be in *E. faecalis* Or participate to its pathogenicity. (Ryan and Ray, 2004). Figure (7) showed the highest value of *Enterococcus faecalis* has been higher value in SML estimated (13600 cells/100 ml) in (L 3) in February, and the lower value in SML was 0.0 cell/100 ml in (L 1), (L 4) and (L 5) in July, as well as was 0.0 cell/100ml in (L 3) in March, higher value in SSL (1600 cells/100 ml) at (L 2) in February while the lowest value was 0.0 cell/100 ml in (L1, L2, L3, L4 and L5) in July, (L5) in August, (L1, L3 and L5) in November, (L1) in December (L1, L2 and L3) in March, In general *Enterococcus faecalis* was in SML (13600 cells / 100 ml) which is higher than SSL was (1600 cells/100 ml) this agreed with (Sieburth, 1971) and others in study of surface microlayer and compared with SSL (SSL). (Williams, 1967; Sieburth, 1971; Crow *et al.*, 1975). The increase in the number of bacteria in these locations is due to that locations are very close to the wastes water estuaries to the discharge of wastes thrown in the river with its enormous bacterial numbers (Al-Sanjari, 2001). Table (1) for both layers (SML and SSL) showed that there was a high positive significant correlation at  $** (P \geq 0.01)$  between *Enterococcus faecalis I* in SML and *Klebsiella pneumonia II*, *E.coli II* and *Staphylococcus aureus II*, and slight positive significant correlation  $*(P \geq 0.05)$  between *temperature* and *total Count II* in SML and SSL respectively.

**Klebsiella pneumoniae :** The bacterium is a gram negative, non-motile, capsulated, facultative anaerobic, and rod shaped). look as a mucoid lactose fermented on the MacConkey agar medium. occur in the normal flora of

the skin, intestines and mouth (Ryan, 2004), *Klebsiella pneumoniae* can cause destructive alterations to human and animal lungs when aspirated specifically to the alveoli results of bloody sputum, recently *Klebsiella* genus has become important pathogenic agents in nosocomial infections, normal presence of bacteria in the soils and about 30% of strains can be fix nitrogen in anaerobic conditions (AL-Tear, 1988). Highest value of *Klebsiella pneumoniae* was in SML up to (16600 cells /100 ml) in (L3) in December and the lowest value was (0.0 cell/100 ml) in (L5) in July and in (L1) in March, while the highest value of *Klebsiella pneumoniae* in SSL was (1300 cells/100 ml) in (L2) in February and the lowest value was 0.0 cell/100 ml in (L2 and L5) in July, (L5) in December, (L1) in February, (L1) in December, (L1, L2 and L3) in March Figure (8), *Klebsiella pneumoniae* generally was higher in SML than SSL this agreed with study of (Williams, 1967; Sieburth, 1971; Crow *et al.*, 1975) study about physicochemical and bacteria in surface microlayer (SML) and subsurface layer (SSL) as well as this study is consistent with the findings (Turki, 2001). Pearson's correlation coefficient in Table (1) for both layers (SML and SSL) showed that there was a high positive strong correlation at\*\*( $P \geq 0.01$ ) between *Klebsiella pneumoniae I* and temperature, *Enterococcus faecalis I*, *E. coli II*, *Staphylococcus aureus II* and total Count II, slight negative significant correlation \*( $p \leq 0.05$ ) with *Proteus mirabilis II*.

***Proteus mirabilis*** : The bacterium is a gram negative, facultative anaerobic, rod shaped, shows swarming motility, *P. mirabilis* causes 90% of all *Proteus* infections in humans. it is found in soil and water (Maleki-Ravasan *et al.*, 2015) this bacteria possess the capacity to produce high levels of urease which hydrolyzes urea to ammonia (NH<sub>3</sub>), so makes the urine more alkaline if untreated then increased alkalinity can lead to the formation of crystals of calcium carbonate, apatite and struvite. which can result in kidney stones. Once the stones develop over time they may grow large enough to cause obstruction and renal failure, *Proteus* genus can also causes pneumonia, wound infections and sepsis [https://en.wikipedia.org/wiki/Proteus\\_mirabilis](https://en.wikipedia.org/wiki/Proteus_mirabilis) - cite\_note-2 (Schaffer and Pearson, 2015). The highest value of *Proteus mirabilis* was in SML (3500 cells/100 ml) in (L2) in January, and the lowest value was 0.0 cell/100 ml in (L1, L2 and L3) in July, (L1, L2 and L4) in August and (L1) in March while the highest value of *Proteus mirabilis* in SSL was (600 cells / 100 ml) in (L3, L5, L2 and L5) in August, October, February and March respectively and the lowest value was (0.0 cell/100 ml) in (L1, L2, L3, L4 and L5) in July, (L1, L2 and L4) in August, (L1 and L2) in October, (L1 and L4) in November, (L1, L3 and L4) in December, (L3) in January, (L1, L3 and L4) in February, (L1, L2 and L3) in March. In general, *Proteus mirabilis* was high in SML up to (3500 cells / 100 ml) while the highest value of in SSL was (600 cells/100 ml) Figure (9). this agreed with study of (Williams, 1967; Sieburth, 1971; Crow *et al.*, 1975) and with (Ohran *et al.*, 2009). Pearson's correlation coefficient were listed in Table (1) for both layers (SML and SSL) showed that there was no correlation between *Proteus mirabilis I* and other bacteria in subsurface layer (SSL) while found a slight negative significant correlation \*( $p \leq 0.05$ ) with temperature and *Klebsiella pneumoniae I* in SML and SSL respectively Table (1).

***Escherichia coli*** : A gram negative, facultative anaerobic, spherical shaped, coliform bacterium of

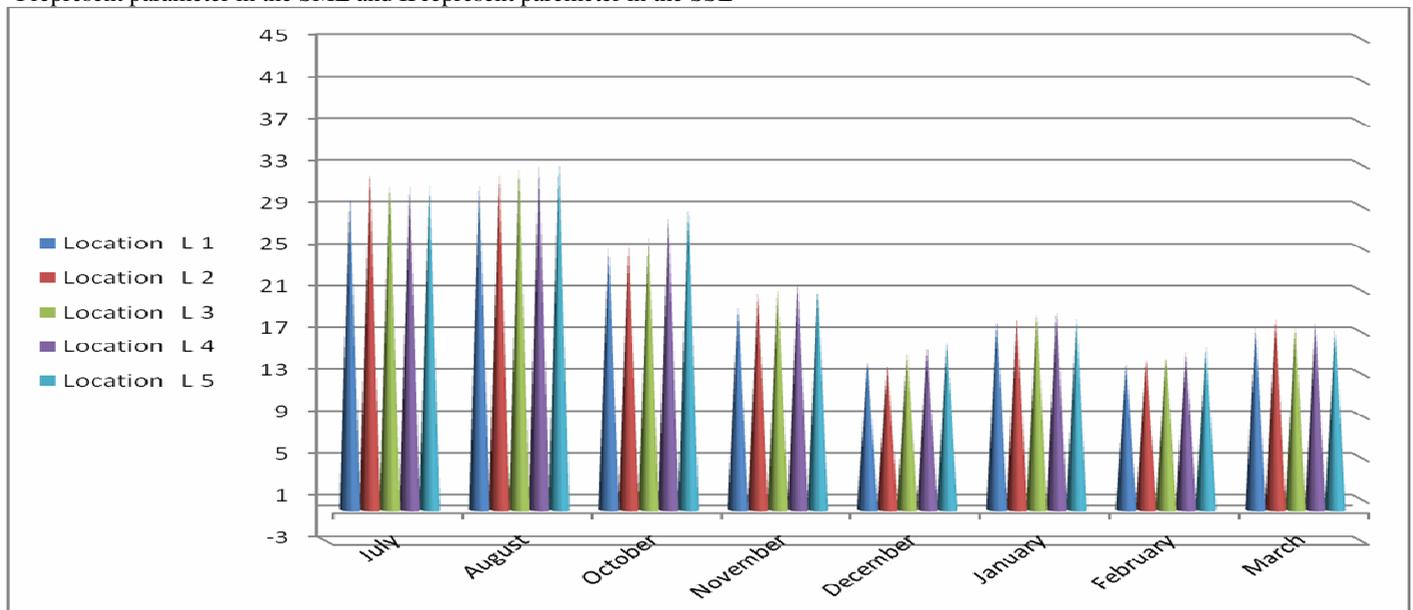
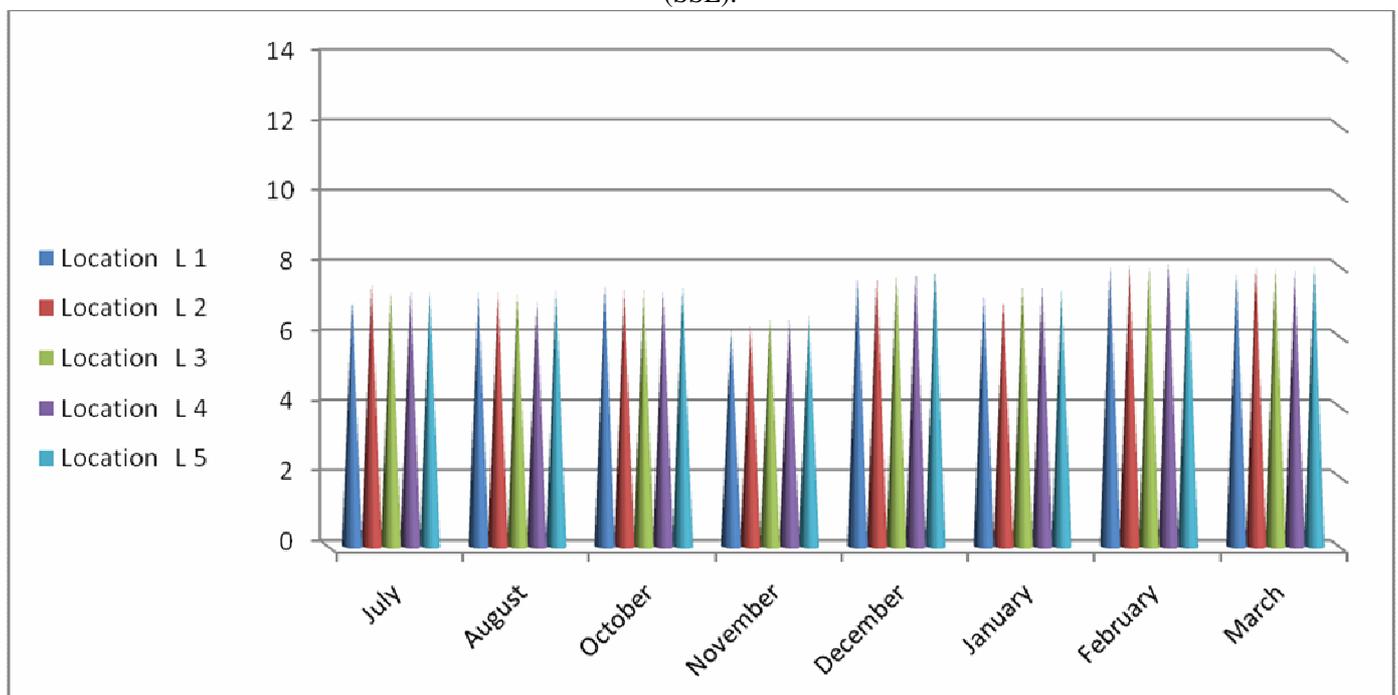
the genus *Escherichia* that is commonly occur in the large intestine of warm blooded organisms (Singleton and Mazliak, 1997; Tenaillon *et al.*, 2010). Some of *E. coli* strains are harmless and other serotypes can cause dangerous food poisoning in their hosts and are responsible for product recalls because of food contamination (Vogt and Dippold, 2005). *E. coli* is discharged into the environment with fecal materials. *E. coli* numbers showed highest values was in SML (42000 cell /100 ml) in (L5) in August, while the lower value of *E. coli* was (0.0 cell/100 ml) in (L4) in July, November and December and (0.0 cell/100 ml) in (L1) and (L3) in March while the highest value of *E. coli* in SSL (6800 cells /100 ml) at (L4) in July and the lowest value in SSL was (0.0 cell/100 ml), (L3) in July, (L2) in August, (L5) in November, (L3, L4 and L5) in December, (L1) in January and February, In general *E. coli* was higher in SML (42000 cells/100 ml) than *E. coli* in SSL (6800 cells/100 ml) this consistent with the study of (Williams, 1967; Sieburth, 1971; Crow *et al.*, 1975; Hardy, 1982; Ohran *et al.*, 2009) Figure (10). a high positive significant correlation at\*\*( $P \geq 0.01$ ) between *E. coli I*, *Enterococcus faecalis I*, *Klebsiella pneumonia I* and *Staphylococcus aureus II* as well as slight positive correlation with temperature and total count II at \*( $p \geq 0.05$ ) Table (1).

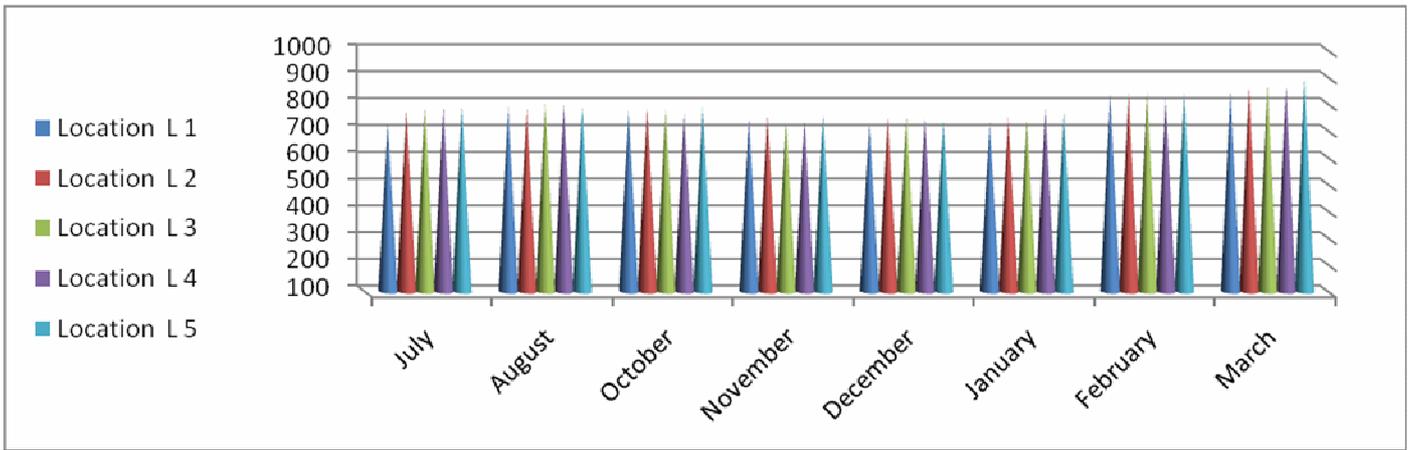
***Staphylococcus aureus*** : The bacterium is a gram positive, spherical shape, occur in the higher respiratory tract and in the skin. this bacterium is often positive for catalase and nitrate reduction and is a facultative anaerobic it is can be grow in the absence oxygen (Masalha *et al.*, 2001), Pathogens often attributed into producing virulence factors such effective protein toxic and the expression of a cell surface protein that connect and inactivates antibody. (Tong *et al.*, 2015). The highest value of *Staphylococcus aureus* was in SML up to (29800 cells/100 ml) in (L4) in August, and the lowest value of *Staphylococcus aureus* in SML was 0.0 cell/100 ml in (L2) in October while the highest value of *Staphylococcus aureus* in SSL 5200 cells/100 ml in (L1) in August as well as the lowest value in SSL was 0.0 cell/100 ml in (L4) in December and (0.0 cell/100 ml) in (L5) Figure (11). Generally *Staphylococcus aureus* was higher in SML up to (29800 cells / 100 ml) than SSL was (5200 cells/100 ml) this study confirmed with (Williams, 1967; Sieburth, 1971; Crow *et al.*, 1975) Figure (10). Results of Pearson's correlation coefficient were showed that there was a high positive significant correlation \*\* $p \geq 0.01$  between *Staphylococcus aureus I*, and *Enterococcus faecalis I*, *Klebsiella pneumonia I* and *E. coli I* also found a slight positive significant correlation \*( $p \geq 0.05$ ) between *Staphylococcus aureus*, temperature and total count II in SML and SSL.

**Table 1 :** Results of calculating Pearson's correlation coefficient between parameters study

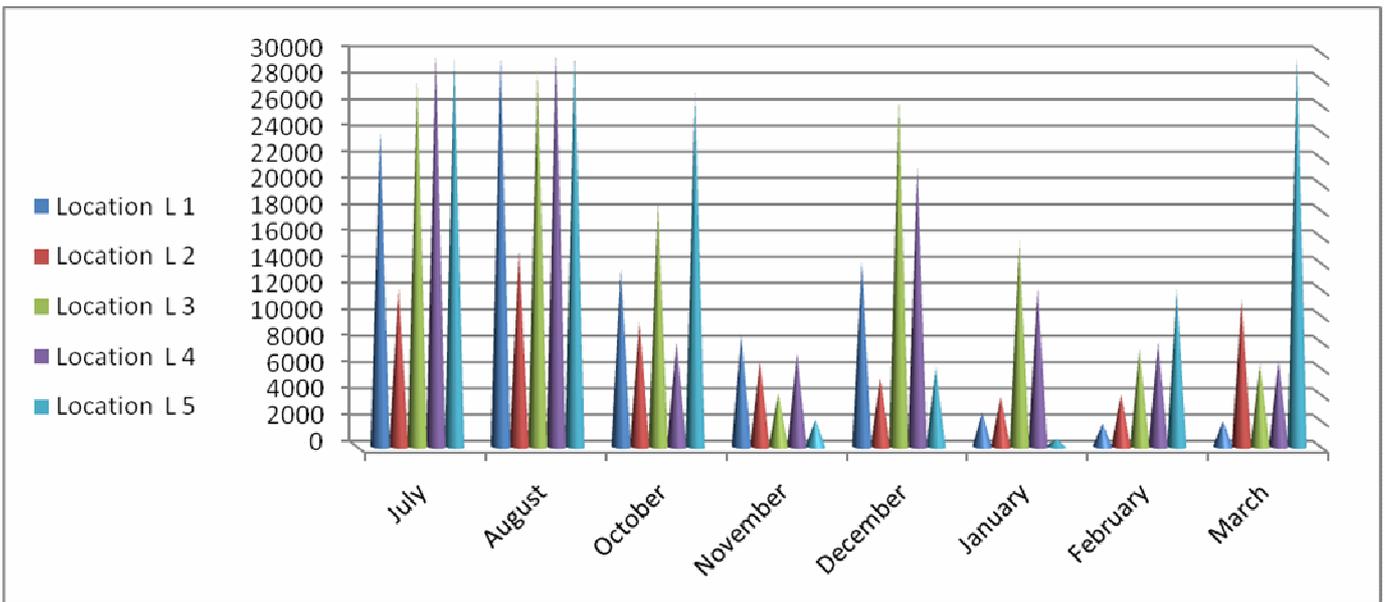
Parameters	PH	TEMP	TDS	Enterococcus faecalis-I	Klebsiellapneumoniae-I	Proteus mirabilis-I	E. coli-I	Staphylococcus aureus-I	Total Count-I
PH	-								
TEMP	-0.35 NS	-							
TDS	0.57 *	-0.19 NS	-						
Enterococcus faecalis II	0.08 NS	0.59 *	0.22 NS	-					
Klebsiella pneumonia II	-0.19 NS	0.89 **	-0.15 NS	0.83 **	-				
Proteus mirabilis II	-0.27 NS	-0.58 *	-0.50 NS	-0.41 NS	-0.53 *	-			
E. coli II	0.01 NS	0.65 *	-0.11	0.96 **	0.87 **	-0.38 NS	-		
Staphylococcus aureus II	0.01 NS	0.61 *	0.13 NS	0.97 **	0.87 **	-0.36 NS	0.97 **	-	
Total Count II	-0.24 NS	0.85 **	-0.34 NS	0.60 *	0.89 **	-0.26 NS	0.72 *	0.70 *	-

\* I represent parameter in the SML and II represent parameter in the SSL

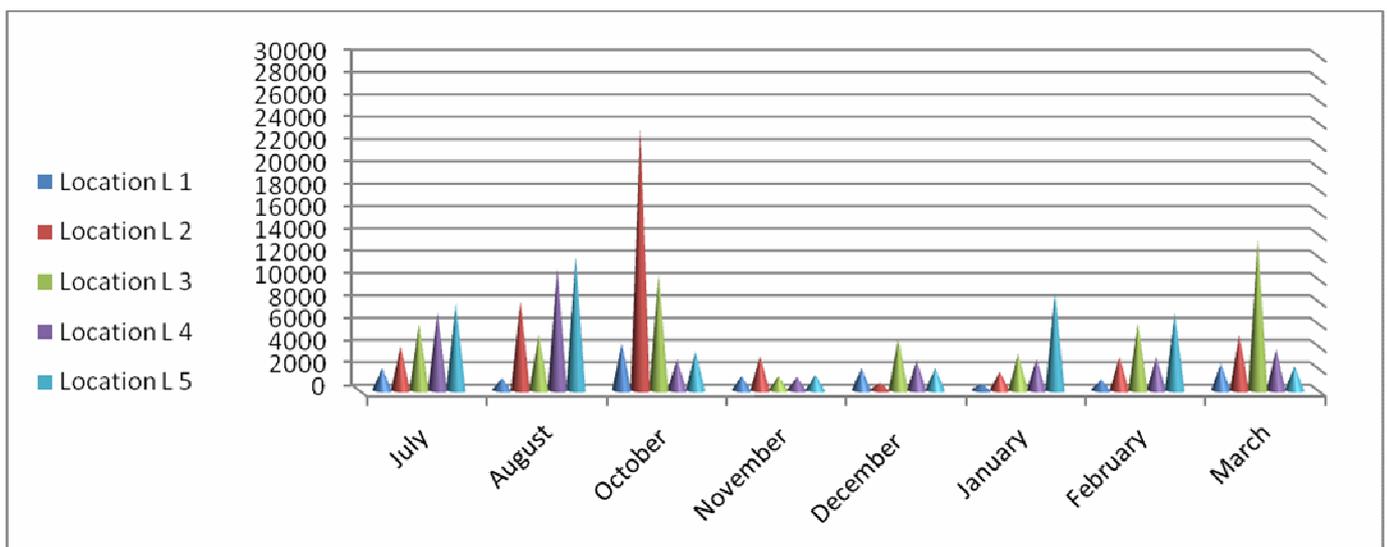
**Fig. 3 :** Monthly variation of Temperature in Al-Hilla River during the study period for five locations in subsurface layer (SSL).**Fig. 4 :** Monthly variation of pH in Al-Hilla River during the study period for five locations in subsurface layer (SSL).



**Fig. 5 :** Monthly variation of T.D.S in Al-Hilla River during the study period for five locations in subsurface layer (SSL) .

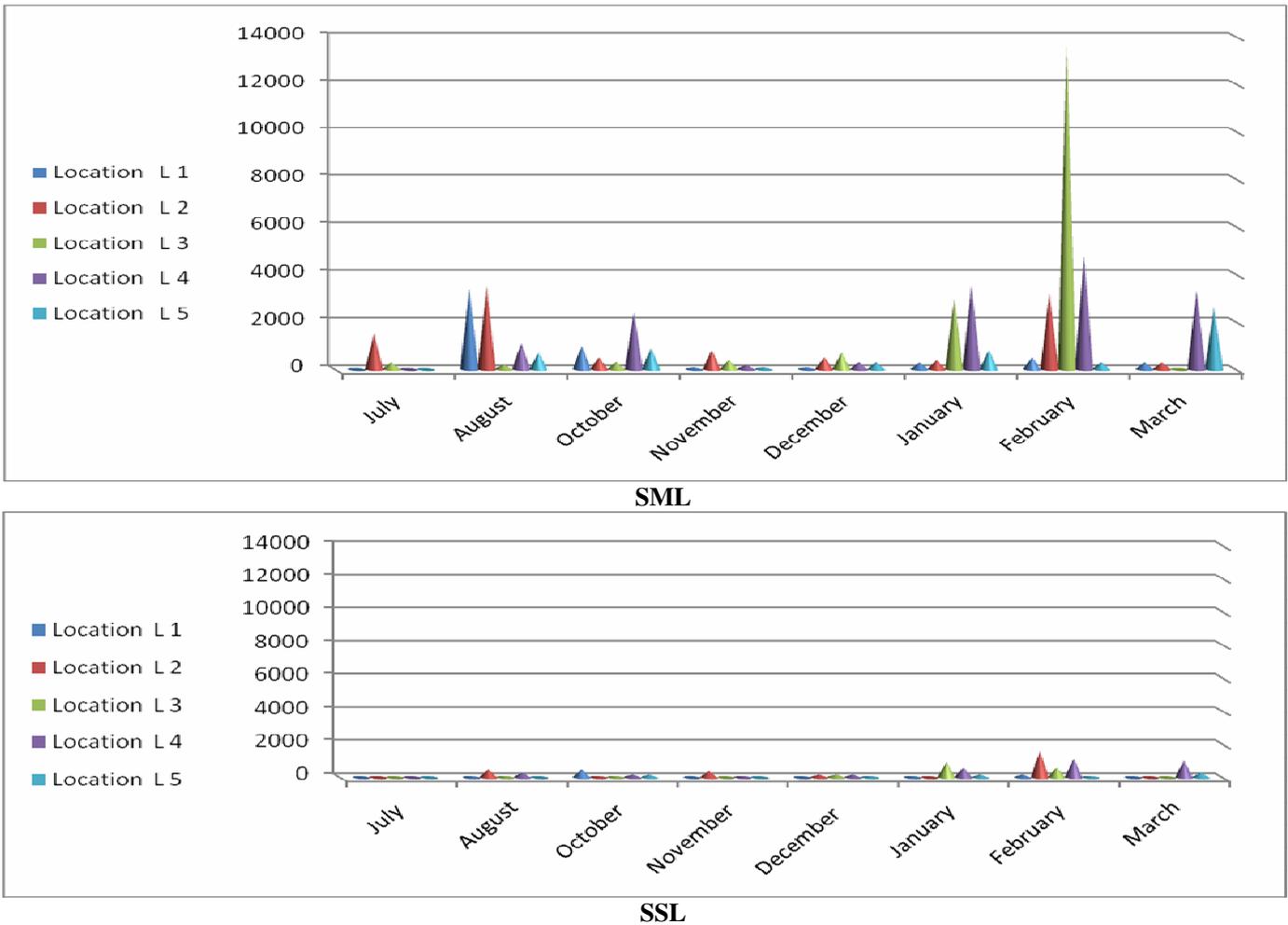


SML

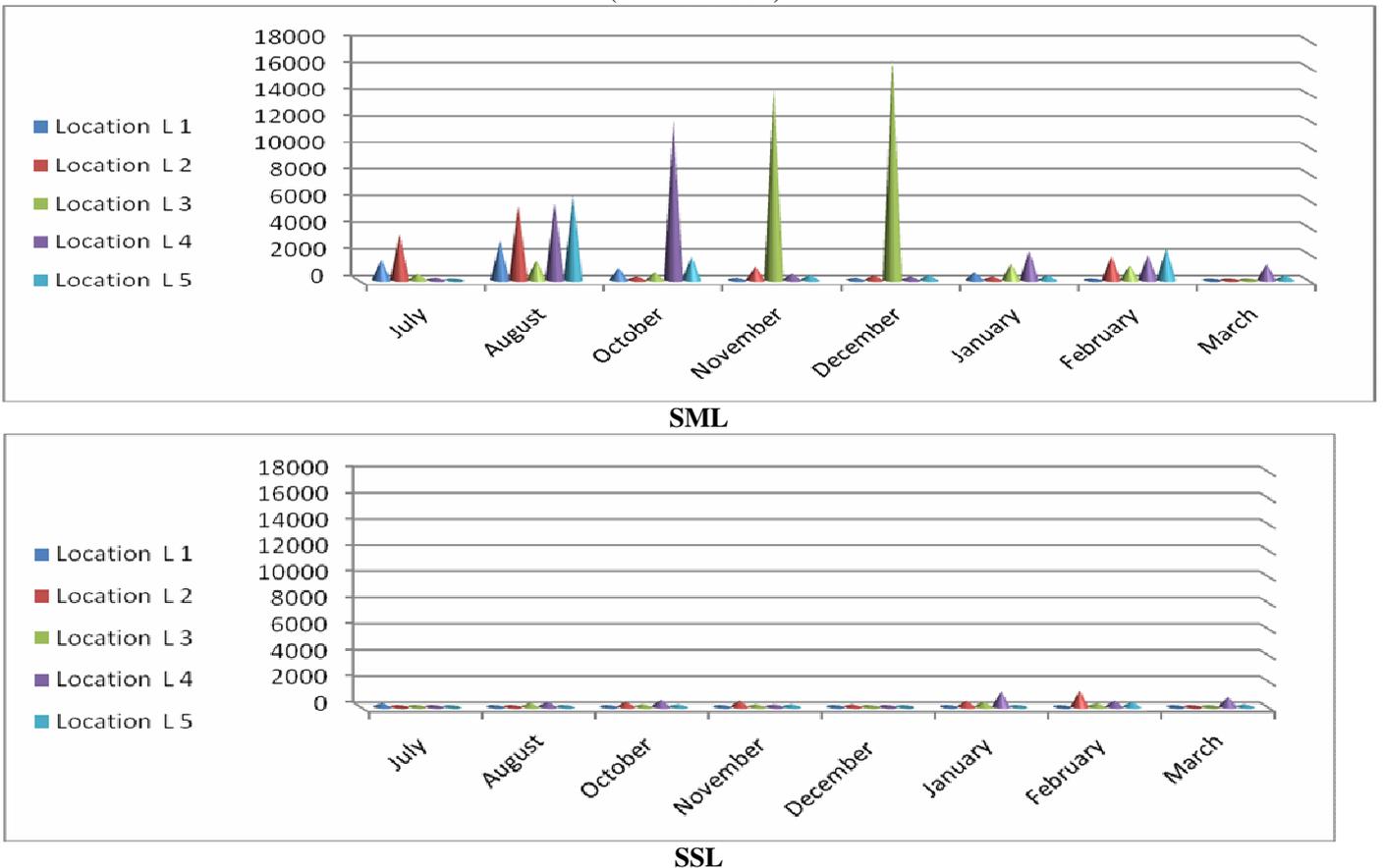


SSL

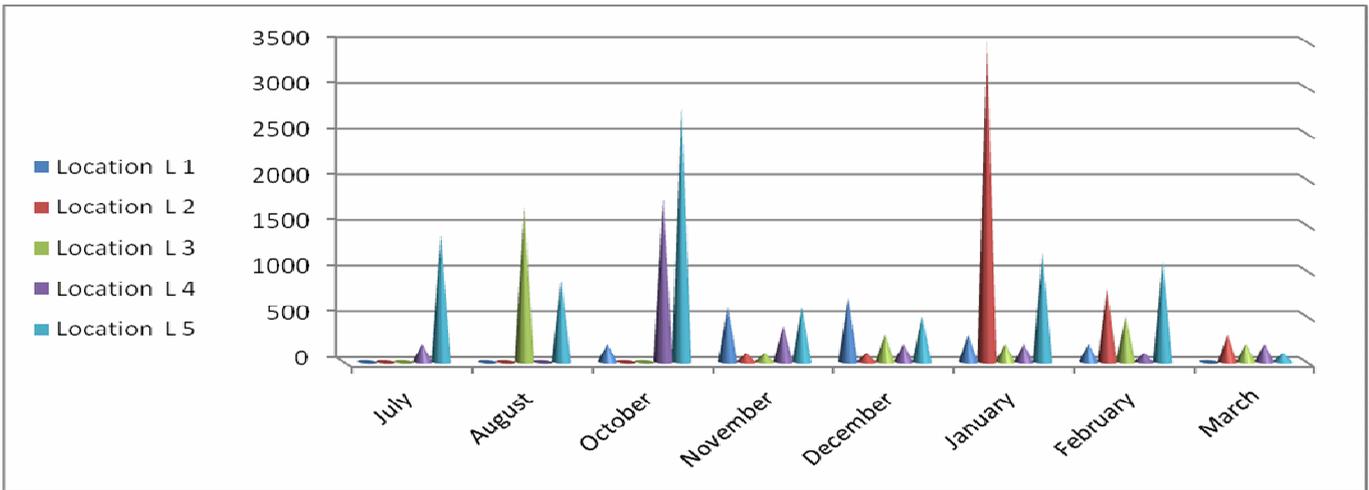
**Fig. 6 :** Monthly variation of Total count in Al Hilla River during the study period (100 CFUs / ml) for both layers (SML and SSL) .



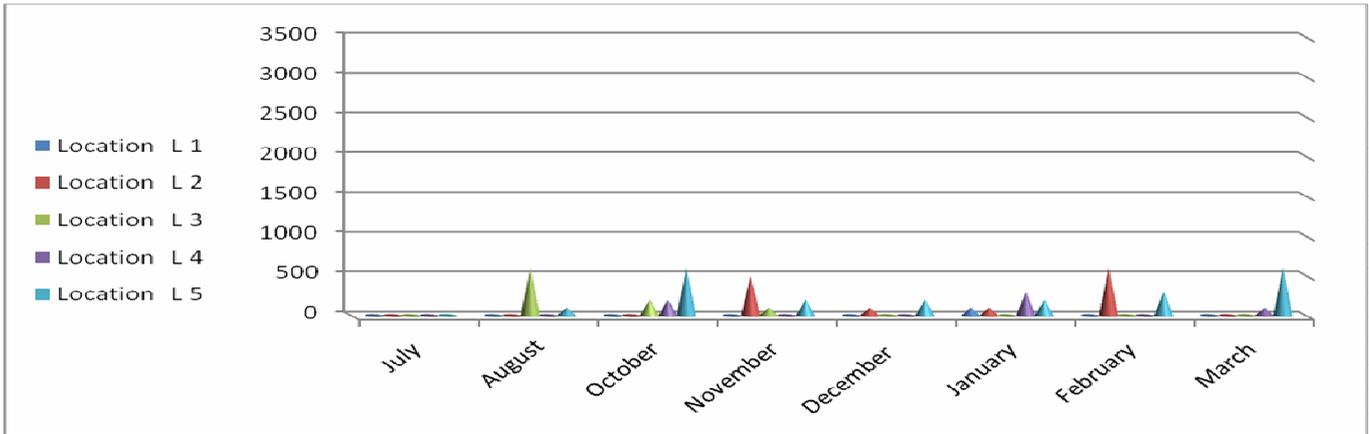
**Fig. 7 :** Monthly variation of *Enterococcus faecalis* in Al Hilla River during the study period (100 CFUs / ml) for both layers (SML and SSL).



**Fig. 8 :** Monthly variation of *Klebsiella pneumoniae* in Al Hilla River during the study period (100 CFUs / ml) for both layers (SML and SSL).

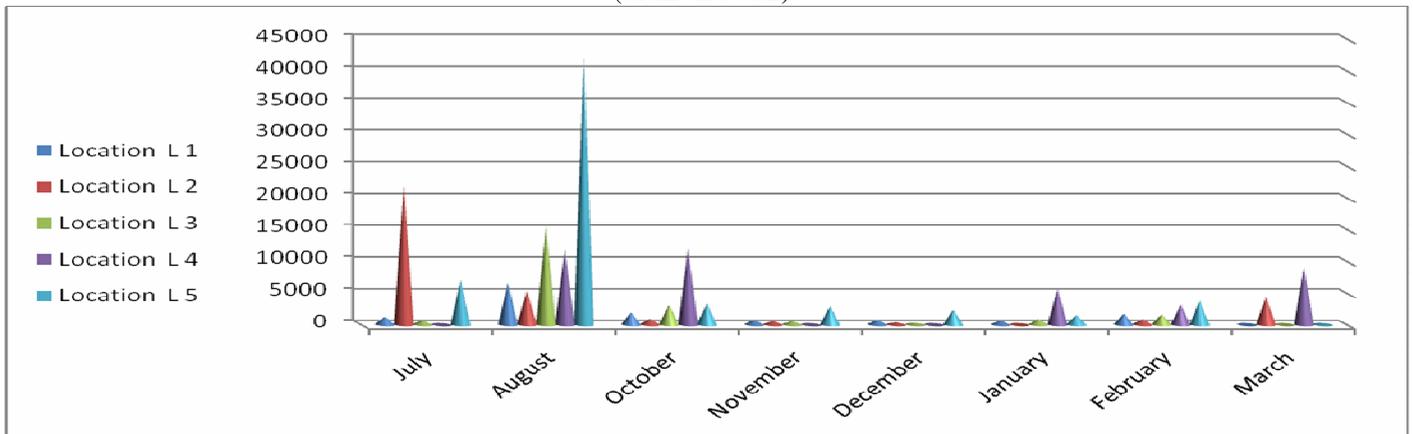


SML

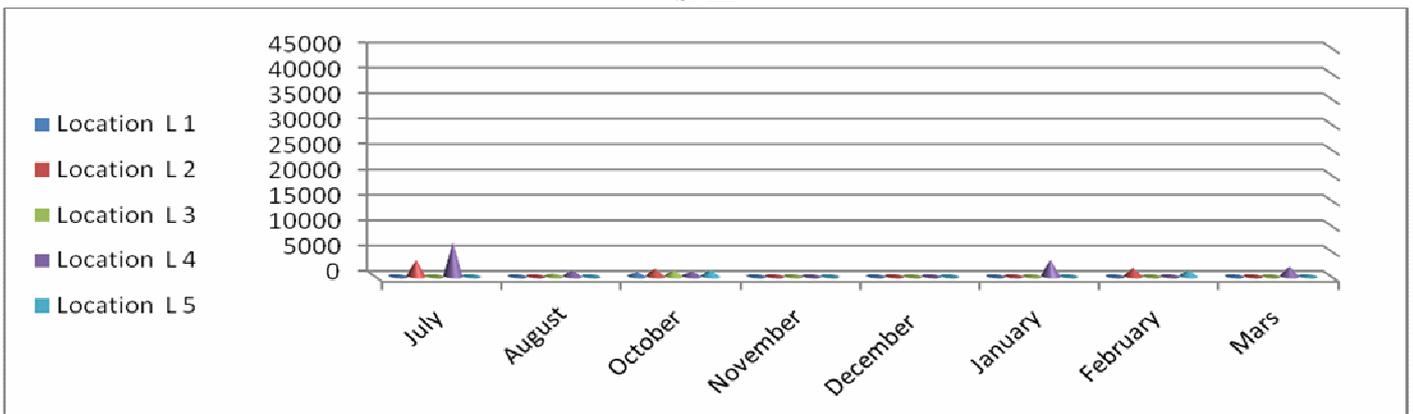


SSL

**Fig. 9 :** Monthly variation of *Proteus mirabilis* in Al Hilla River during the study period (100 CFUs / ml) for both layers (SML and SSL).

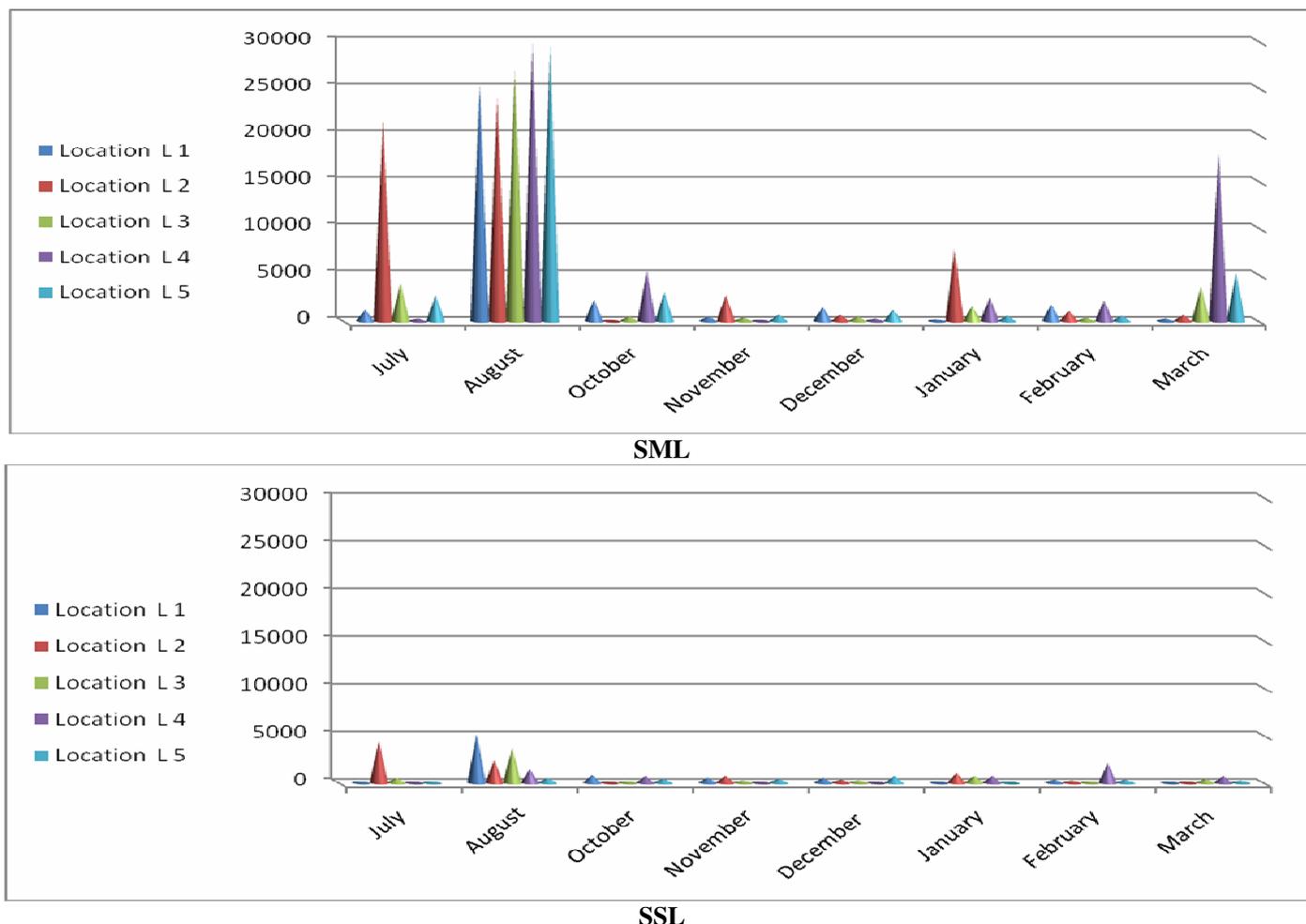


SML



SSL

**Fig. 10 :** Monthly variation of *E. coli* in Al Hilla River during the study period (100 CFUs / ml) for both layers (SML and SSL).



**Fig. 11 :** Monthly variation of *Staphylococcus aureus* in Al Hilla River during the study period (100 CFUs / ml) for both layers (SML and SSL) .

### Conclusions

Increase the pollutants in the SML in contrast in the SSL. SML represent a good indicator for pollution in waters bodies. Organisms and other pollutants thrive and grow in the SML more than SSL. There is an organic pollution resulting from the slaughterhouse along the river in some regions such as throwing dead animals, drainage of sewage without treatment and drainage of agricultural. The results of the study showed that water samples which have obtained from different locations were polluted with T.Hs. where the compounds of Total Hydrocarbons are known to be carcinogenic for Human body and other animal especially essential organs of the body such as kidney, liver, brain and skin are at exposed of being affected by T.Hs

### Recommendations

Continuous the studies about the SML to build idea about the pollution in it. Study other pollutants in the SML and comparison with SSL. Remove any pollution source at and near AL-Hilla river to prevent the pollution. Using another methods to collect the samples and compared it with current methods. Extreme caution against drinking directly from the water of the river because our study confirmed that pollution is concentrated in the upper layer of water (SML). Water should be withdrawal for drinking, irrigation or other uses from a depth of not less than 50 cm for the purpose of minimizing significant pollution in the surface layer of water. Studies like the present one that deals with pollution in the

SML should be given more attention and focus because lack of such studies , so we aspire that there are other intensive studies in Iraq specializing in the study of SML because importance of SML. Following up the qualitative characteristics of the water of AL-Hilla River at high and low water levels, which reflects on the quality of the river water. Preventing the discharge of agricultural water directly into the river.

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