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Analysis of Bacteriological Content of Wastewater in Some Selected Locations in Maiduguri, Borno State, Nigeria

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Abstract

In this study, analysis of bacteriological content of wastewater in Maiduguri was carried out. One sample each, that is, Abattoir, Nigerian Bottling Company (NBC) and Monday Market was made from domestic, commercial and the industrial sites in the city respectively. Samples were collected from twenty (20) samples points along each of the wastewater channels at every 5meters distance, making a total of 1km distance in each channel. About 25 mls of the wastewater was aseptically collected using sterile universal containers. Seven organisms (Escherichia Coli, Klebsiella sp, Protens sp, Alromonas sp, Enterobacter sp, Salmonella sp and Shigella sp), were screened for in each of the three sampled locations, but only four (Escherichia Coli, Klebsiella sp, Enterobacter sp and Shigella sp) were positive. Monday market and Abattoir were found to have high prevalence of Escherichia coli. Salmonella sp was also found to be higher in Monday Market than the other two locations. The result of the study also shows that the three locations are not significantly different in terms of the organisms they generate which reflected in the similarity of microbiological contaminants in all the three sample locations. However, the result of the variance among the organisms revealed that there is significant difference among the organisms in the three sites, that is, some organisms are more suitable to some sites than the others. Finally, it was also revealed that some essential commodities as satchet water, milk, meat among others have already been contaminated by some of the idenified bacteria in sampled sites. It was recommended that there should be implementation and compliance with standard policy guidelines on wastewater disposal in order to reduce the outbreak of diseases. Since it has been established that some essential items such as satchet water, milk, meat and food have been contaminated by these bacteria, it is necessary to have functional quality control of these items before they reach the consumers.

Keywords: Bacteriological content; microbiological contaminants; wastewater; *Escherichia coli; Klebsiella sp; Enterobacter sp; Salmonella sp.*

Introduction:

Wastewater is generally refered to as used water, that is, water that has changed its natural stage due to human use. Therefore, wastewater can be derived from domestic, industrial, commercial or agricultural activities (United States Environmental Protection Agency (USEPA) 2018). Among all these, municipal wastewater are mainly domestic wastewater from households such as flush toilets, sinks, dishwater, washing machines or showers and also municipal wastewater from communities which is also known as sewage and industrial wastewater from industrial activities (USEPA2018). Abdullahi, Humuani and Musa (2013) reported that wastewater that comes from human waste (faeces, urine or other body fluids), also known as blackwater, includes water from lavatories, septic tanks or soak away, and washing water; while greywater is wastewater that comes from urban rainfall runoff from roads, roofs, and sidewalks. Wastewater may contain pollutants which can be physical, chemical or biological. In the report of Institute of Agriculture and Natural Resources (2019), wastewater consists of 99.9% water and 0.1% pollutants which includes: Nutrients (Phosphorous and Nitrogen), Fats, oils, grease: (cooking oils, body lotions), Pathogens; such as disease-causing bacteria and viruses, Biochemical Oxygen Demand (BOD).

Environment Canada (2018) report some examples of pollutants that can be found in wastewater and the potentially harmful effects these substances can have on ecosystems and human health: decaying organic matter can use up the dissolved oxygen in a lake thus, affecting survival of aquatic life forms; excessive nutrients, such as phosphorus and nitrogen (including ammonia), can cause eutrophication, or overfertilization of receiving waters, which can be toxic to aquatic organisms, promote excessive plant growth, reduce available oxygen, harm spawning grounds, alter habitat and lead to a decline in certain species; chlorine compounds and inorganic chloramines can be toxic to aquatic invertebrates, algae and fish; bacteria, viruses and disease-causing pathogens can pollute beaches and contaminate shellfish populations, leading to restrictions on human recreation, drinking water consumption and shellfish consumption; metals, such as mercury, lead, cadmium, chromium and arsenic can have acute and chronic toxic effects on species. Finally, other substances such as some pharmaceutical and personal care products, primarily entering the environment in wastewater effluents, may also pose threats to human health, aquatic life and wildlife.

Wastewater contains many constituents and contaminants arising from diffuse and point sources. Point sources could be large point sources which are easily quantifiable and result from specific activities in the area that are connected to the wastewater collecting systems (Environmental Protection Authority (EPA 2019). On the other hand, small point sources such as households and small businesses are much more difficult to identify and quantify (EPA 2019). Diffuse sources include atmospheric deposition and road runoff (EPA 2019). Wastewater contains contaminants that may be of natural or anthropogenic sources (Thornton, Butler, Docx, Hession, Makropoulos, et al., 2001). The contaminants in wastewater are grouped mainly into two: chemical and microbiological contaminants). The microbiological components of wastewater contaminants constitute the living microorganisms

and may include bacteria such as Staphylococcus, Salmonella and Shigella species. Others are viruses such as Hepatitis A and B virus), protozoan (Giardia, Amoeba. and Cryptosporidium), fungi and helminthes (Ancyclostoma), cestodes and trematodes (Ellis, 2011). The microbiological components could also lead to outbreak of infectious diseases such as typhoid fever, shigellosis, cholera and poliomyelitis (Tortora, Funke and Case 2007). One of the effects of a wastewater source draining into the soil is making the soil oxygen to become less available as an electron acceptor, prompting denitrifying bacteria to reduce available nitrate to gaseous nitrogen which enters the atmosphere with resultant negative effect (Rabah, Oyeleke, Manga, Hassan, and Ijah, 2010).

Maiduguri city has been the headquarters of the north-east region since 1976, and the Borno State headquarters since 1987 till date. With this administrative previledges, several growth and developemntal projects such as industrial, commercial, agricultural, educational among others have taken place which have directly or indirectly lead to urbanization and population growth. To this end, lots of water are used for human survival and in that process a lot of waste water are also generated. One of the most practical ways of the management of wastewater is wastewater reuse (USEPA, 2018), but wastewater resuse in Nigeria has not been put into practice (Ibrahim 2014). Therefore, wastewater in most part of the country including Maiduguri metropoly is left open and in some places flow through the densely populated and urbanized parts of the city without proper treatment and disposal sites. If wastewater is not properly managed it could become a point source of pollution which could be a hazard for the health of human populations and the environment. Diseases caused by bacteria, viruses and protozoa are the most common health hazards associated with untreated wastewater. The quality of domestic wastewater effluents is one of the main causes of degradation of the receiving water bodies such as rivers, lakes and streams. Wastewater that is directed to the environment is the prime breeding sites for mosquitoes, houseflies, rodents, and other vectors of communicable diseases such as dysentery, diarrhea among others. Though, several studies exist on sources and effects of wastewater in Maiduguri (Fabian and Abubakar 2015, Adam, Isah, Bello and

Ismail 2016), however, most of the studies dwelt only on one source and comparative analysis among the different sources are very scanty. Therefore, comparative analysis among commercial (Monday market), industrial (Cocacola plant) and domestic (Abattoir) of bacteriological content of wastewater in the city was carried out in this study.

Materials and Methods Study Area

Abdulrasheed (2019) reported that Maiduguri was founded in 1907 on a ridge about 305 meters above sea level on the left bank of the River Alau by Shehu Garbai. Maiduguri, the capital of Borno State, North-Eastern part of Nigeria, with a population of 504,016 (National Population Commission, 2006), covers an average area of 158km². This means that the population density of Maiduguri city is about 7,190 persons per square kilometers.



Figure1: The Study Area

Maiduguri lies in the Sahel savannah zone, located between latitude 11.772'N and 11.917'N and longitude 13°.031'E and 13°.108°E. Mean annual rainfall of Maiduguri is about 500mm. The rainy season is normally from June to September with temperature range from 35°c to 40°c and with prolong period of dry season and dusty winds which blows from the Sahara desert. The land is characterized by a sandy flat plain land with scanty vegetation. The study area is drained by River Ngadda and the Ngaddabul, which flow North East in to the Ngadda delta, that is, Jere Bowl They have their peak flow during the rainy season in the month of August and September (Nyanganji, 2002). Since 1907 when the city was founded, the study area has been witnessing the large influx of people as it serve as the administrative and commercial nerve center of the state. The inhabitants engage themselves in different administrative and commercial and agricultural activities.

The following materials were used for this study: sterile universal container, sterile hand gloves, sterile cotton wool, bijou bottles, methylated spirit, disinfectant (Tepol), double strain macConkey broth, conical flask, autoclave paper, autoclave tape, autoclave machine, pasteur pipette, hospital flat bottle, peptone water (media), kovac reagent, ethyl alcohol, citrate media, klegler iron agar (KIA), and test tubes.

Samples and Sampling Techniques

One sample location was purposively selected from domestic, commercial and industrial wastewater in Maiduguri city. The adoption of purposive sampling was based on accessible sites due to the current security challenges in the city. Therefore, sample locations include: Abattoir for domestic wastewater, Monday market for commercial wastewater and Nigeria Bottling Company (NBC) Maiduguri for industrial wastewater.

Twenty sampling points were identified along wastewater channel in each sample location. Each of the twenty sampling points was five (5) meters apart, making a total of 1km distance in each channel during the dry season in March, 2018. About 25 mls of the wastewater was aseptically collected using sterile universal containers from each sampling point. The universal containers were lowered into the wastewater reservoir and filled to the volume of 25 ml in each of the sterile containers. The sterile universal containers were screw-caped and transported to the diagnostic microbiology laboratory of the University of Maiduguri for analysis. The bacteriological examination was conducted as described by the methods of Ochei and Kolhatkar (2007). Seven organisms were screened for in each of the three sampled locations, that is, Escherichia Coli, Klebsiella. Protens. Alromonas. Enterobacter. Salmonella and Shigella.

Inoculation and Incubation

The presumptive coliform counts of wastewater samples were analyzed in the laboratory of the Department of Microbiology, University of Maiduguri using the technique of Shariq, Singh, Farooq, Dhariyal Singh et al (2015). Estimate of the number of coliform organisms was determined by adding 15 ml of water to double strength MacConkey broth and single strength MacConkey broth containing bromocresol blue sterilized in bottles containing Durham's tubes for indication of gas production. A loopful of culture of a positive tube from the presumptive test was transferred into a tube of brilliant green lactose bile broth with Durham's tube. The tubes were incubated at 37 °C for 24-48 hours for total coliforms and 44.5 for 24-48 hours for fecal coliform and observed for gas production. Coliform count was determined by matching the number of positive test with acid (yellow coloration) and gas production with McGrandy statistical table and most probable of coliform present in the samples was determined.

Biochemical tests for bacteriology

Other confirmatory diagnoses were carried out to identify the suspected organism down to specific level. These tests included:

Indole test:

The suspected samples were further inoculated into 5mls of peptone water (bacteriological peptone) containing growth nutrients and incubated at 37° C for 18 - 24 hours, following incubation about 5 drops of the Kovacs, a reagent product of ethyl alcohol was added as an electron indicator which produces the red junction indicating end product of metabolic reaction produced by *Escherichia coli* while other gram negative bacilli remained non-productive.

Kliegler Iron Agar Test

Kliegler Iron agar is a biochemical test used for enterobacteria that are gram negative bacteria and aerobic. This test is carried out by inoculating suspected colonies into a Kliegler iron agar tube. The test is incubated aerobically at 37° C for 18 - 24 hours. Results show a black precipitate with gas production indicating coliform bacteria like *Salmonella* sp and other gram negative aerobic organisms.

Citrate Utilization Test.

Citrate test is one of the biochemical analysis used to identify enterobacteria that utilizes citrate as metabolic end product particularly, *Klebsiella sp.* A gram negative aerobic sporing bacteria utilizes citrate by changing deep green to blue background indicating positive. Young colony of fresh inoculums was inoculated inside a test tube of citrate agar base aseptically. The preparation was aerobically incubated at 37°C for 18-24 hours. The young culture produced the blue color after exhibiting phases of growth.

Statistical Analysis

Minitab 17 statistical software was used to run a Two-Way Analysis of Variance (Anova) of the three selected sites and the four identified organisms in each of the sites. The P-Values from the ANOVA output were used to interpret the significance of the variables at 0.05, that is, at 95% confidence level.

Results and Discussion

Out of the seven organisms (Escherichia coli, Klebsiella sp, Protens sp, Alromona sps,

Table 1: Number of isolated organisms in the three sampled sites

Enterobacter sp, Salmonella sp and Shigella sp), that were screened for in each of the three sampled locations, only four (*Escherichia Coli, Klebsiella sp, Enterobacter sp* and *Shigella sp*) were positive, while the remaining three were not found in any of the wastewater channels as presented in Table 1.

SPP	Number Isolated	Percentage		
Escherichia coli	18	51.4		
Klebsiella sp	8	22.9		
Protens sp	0	0.0		
Alromonas sp	0	0.0		
Enterobacter sp	5	14.3		
Salmonella sp	4	11.4		
Shigella sp	0	0.0		
Total	35	100		

Source: Fieldwork, 2018

The numbers of points that are positive in each of the four sampled organisms in the twenty sampled points and in the three locations are presented in Table 2.

Table 2: Microbiological constituents of wastewater

S/N	Organisms	Abattoir	battoir Monday		Ikt NBC Plant			Total	
		No	%	No	%	No	%	Positive/%	
1	E.coli	6	33.3	8	44.4	4	22.2	18 (100)	
2	Klebsiella sp	2	25	2	25	4	50	8 (100)	
3	Enterobacter sp	3	60	1	20	1	20	5 (100)	
4	Salmonella sp	1	25	2	50	1	25	4 (100)	
Total		12 (34.3%)		13(37.1%)		10 (28.6%)		35	

Source: Fieldwork (2018)

It was revealed in Table 2 that contaminations are larger in Monday market (37.1%), which is a commercial nerve of the city and from which assorted wastes are generated. The Abattoir which housed wastewater from the city's abattoir and most of the domestic wastewater around the densely populated custom areas in the metropolis was second with 34.3%. NBC with mainly industrial wastes, and which environment is not urbanized, are third with 28.6%. In Nigeria, majority of the urban and rural populace do not have access to portable water and therefore depend on wells, streams and rivers for domestic water supply, (Ibrahim, 2014). Some of these water sources are prone to contamination from wastewater. The number and percentages of the isolated organisms is presented in Table3, followed by the discussion of each of the isolated organisms.

	Organisms	Number	Percentage	
1	E.coli	18	51.4	
2	Klebsiella sp	8	22.9	
3	Enterobacter sp	5	14.3	
4	Salmonella sp	4	11.4	
	Total	35	100	

Table 3: Number and percentages of isolated organisms

Source: Fieldwork (2018).

Escherichia coli

Table 3 shows the four isolated organisms in the sampled locations. Among these four, more than half (51.4%) was Escherichia coli (Table 3) and which was found to be more prevalent especially in Monday market (44.4%) and Abattoir (33.3%) as shown in Table 2. The two locations were found to have high prevalence of Escherichia coli due to the excessive discharged of faecal wastes from humans and animals, contaminating water, (River Nggadda) which runs through the two locations) soil and the surrounding environment. The prevalence of Escherichia coli and other bacilli organisms in the wastewater in these locations may be associated to the high load of animal excreta and other decomposing matter in the urban and commercial wastewater in Monday market and Abattoir. This finding is in agreement with similar studies that reported bacterial contaminants in wastewater effluents from abattoir; Ezeronye and Ubalua (2005) reported high prevalence of Escherichia coli in Aba River as a result of contamination with abattoir effluents. Rabah et al (2010) also reported bacterial contaminants in wastewater effluents from abattoir in Sokoto Metropolis.

The effect of *Escherichia coli* has been deeply reported in various studies, such as Christian (2017), that some types of *Escherichia coli* can cause illness in humans, including; diarrhea, abdominal pain, fever, and sometimes vomiting. Some other types of *E. coli* infection can lead to urinary tract infections (diarrhea, abdominal pain, and fever), respiratory illness, pneumonia, and other illnesses like meningitis and intestinal infections. Some types of *E. coli*, particularly *E. coli* O157:H7, can cause intestinal infection called Shiga toxin. Healthline (2017) also opined that more severe cases can lead to bloody diarrhea, dehydration, or even kidney failure

and that people with weakened immune systems, pregnant women, young children, and older adults are at increased risk for developing these complications. Most intestinal infections are caused by contaminated food or water. Adam, Isah, Bello, and Ismail (2016) worked on the resistance profiles of bacteria isolated from wastewater in the University of Maiduguri Teaching Hospital, Maiduguri and discovered the presence of e.coli among others in the wastewater. Of all the antibiotics, e-coli showed 100% resistance to all the ten sampled commonly used antibiotics (Tarivid, Reflacine, Ciprofloxacin, Augmentin, Gentamycin, Streptomycin, Ceporex, Nalidixic Acid, Septrin and Ampicillin).

Klebsiella sp

Klebsiella sp was also found to be present especially in the NBC wastewater channel. Klebsiella sp is a type of bacteria commonly found in nature. In humans, the bacteria are often present in parts of the digestive tract where they do not generally cause problems (Ibrahim (2014). Klebsiella pneumoniae and Klebsiella oxytoca are the two members of this genus responsible for most human infections. These infections can be more aggressive and difficult to treat. Klebsiella can cause infection in different parts of the body including the lungs, urinary tract, or the bloodstream (Wen-Liang and Chuang 2017. Quereshi, 2018, Bush and Perez, 2018, Bush 2018). In a study on microbiological assessment of Abattoir effluent on water quality of River Katsina-Ala, Nigeria, Vershima, Terkimbi, Haruna, and Ibrahim (2015) found out that Klebsiella spp. can lead to wide range of disease states, notably pneumonia, urinary tract infection, septicemia, spondylitis, and soft tissue infection.

Entrobactor sp

Entrobactor sp were found in five locations in the entire twenty locations of study. Out of these five, three of them were in Abattoir locations. . This findings confirmed the report of Emmanuel, Bawo and Lawrence (2016) in their work on the evaluation of bacterial profile and biodegradation potential of abattoir wastewater in Benin city, Nigeria, that the bacteria occurrence frequency revealed that Escherichia sp. was dominant (P>0.05) in both abattoir samples. The order of dominancy was Escherichia sp.> Pseudomonas sp.> Enterobacter sp.> Klebsiella sp.> Staphylococcus sp.> Salmonella sp.>Serratia sp.> Streptococcus sp. which have some of the discovered ones in this study. Nishijima (1999) described Enterobacter cloacae as rodshaped, gram-negative bacteria from the Enterobacteriaceae family. The size of this bacteria ranges from 0.3-0.6 x 0.8-2.0 µm. Enterobacter cloacae live in the mesophilic environment with its optimal temperature at 37 °C and use its peritrichous flagella for movement. This organism is oxidase negative but catalase positive and is facultative anaerobic. In other words, this organism can make ATP by aerobic respiration when oxygen is present but can switch to fermentation in the absence of oxygen. Frazer (2019) reported that Enterobacter infections can include bacteremia, lower respiratory tract infections, skin and soft-tissue infections, urinary tract infections (UTIs), endocarditis, intraabdominal infections, septic arthritis, osteomyelitis, CNS infections. and ophthalmic infections. Enterobacter infections can necessitate prolonged hospitalization, multiple and varied imaging studies and laboratory tests, various surgical and nonsurgical powerful procedures. and and expensive antimicrobial agents. Frazer and Christian (2019) concluded that Enterobacter infections do not have a clinical presentation that is specific enough to differentiate them from other acute bacterial infections. Enterobacter was one of the pathogenic bacteria that have contaminated satchet water in Maiduguri as reported by Bukar, Isah, Mustapha, Kyari, and Ibrahim, (2014) in their work on the bacteriological analysis of sachet water in Maiduguri Metropolis.

Salmonella sp

Monday market locations were found to be more positive to Salmonella sp than the other two locations (Table 1). Since it has been revealed that Salmonella may be introduced through animal or human fecal and sewage pollution, the suitability of Monday market for its occurrence is not surprising because Monday market environs especially the river Ngadda valley are always full of human fecal and sewage pollution (Ibrahim 2014). According to Abdellah, Rachida, Hasna, Hamadi and Nouredine (2017), wastewater is known to be a common vehicle for the transport and transmission of Salmonella serovars and is able to pollute environment and infect humans, and that several outbreaks and contaminations have been related to Salmonella in wastewater through irrigation of crops, infiltration and transport in soil to groundwater or to its discharge into the marine environment. They concluded that Salmonella may be introduced through animal or human fecal and sewage pollution. Numerous research have also found the existence of these organisms in different locations in Maiduguri. Jasini, Kwoji, Fati, Dauda, Maina and Solomon (2017), in their work on prevalence and antibiotic sensitivity pattern of salmonella isolates from milk products and water reservoirs in Maiduguri, North-Eastern Nigeria stated that the overall prevalence of Salmonella in milk samples was found to be 10.00%, while the total prevalence of Salmonella from water sample was 40.00%. In related work, Musa, Onvilokwu,, Jauro, Yakubu and Musa(2017) who also worked on occurrence of Salmonella in ruminants and camel meat and their antibiotic resistant pattern in Maiduguri, Nigeria showed that Salmonella species are present in fresh meat sold in abattoir, retail markets and shops. Moreover, it is unfortunate to discover that some of the satchet water that are produced in the city have been contaminated by some of these organisms as reported by Bukar, et al, (2015) that some sachet water have been contaminated by pathogenic bacteria such as Klebsiella species, Escherichia coli, Pseudomonas species and Enterobacter species in their work on the bacteriological analysis of sachet water in Maiduguri Metropolis. Meat which are also widely consumed by people has also been reported to have been contaminated especially by salmollena species as reported by Musa, et al (2017) that Salmonella species are present in fresh meat sold in abattoir, retail markets and shops

Comparative analysis of the presence of bactoriological content in industrial, commercial and domestic wastewater in Maiduguri was also examined using a two-way analysis of variance in Minitab 17 Statistical package. The result of the analysis (APPENDIX) revealed that the P-value of the three sampled locations is 0.770 which is higher than the significant level of 0.05. Therefore it was concluded that since the p-value is greater than the rejection level, it means that the three locations are not significantly different in terms of the bacteria that were generated, which reflected in the similarity of microbiological contaminants in all the three sample locations. However, the major concern in this study is the level of significance among the four isolated organisms in the three locations (Table 2). The pvalue of the organisms is 0.027 which is less than the rejection level of 0.05. Therefore, it was concluded that there is significant difference among the organisms in the three sites. This finding means that the organisms are more suitable in some locations than the others. Monday market and Abattoir for instance, were found with high prevalence of Escherichia coli due to the excessive discharged of wastes from humans and animals, faecal contaminating water, soil and the surrounding environment, and have the potential to cause urinary and wound infections, and diarrhea in children (under three years) and the very elderly (Joanne et al., 2011). Salmonella sp was also found to be higher in Monday Market than the other two sites. Since it has been confirmed in this study that Salmonella may be introduced through animal or human fecal and sewage pollution, among the three sampled sites, none is as exposed to animal or human fecal and sewage pollution like that of the Monday Market where human feacals are indiscriminately dumped in open places such as along River Ngadda, several and large open waste dump sites among others which are not as numerous in either Abattoir or NBC locations (Fieldwork, 2019).

Conclusion

The analysis of bacteriological properties of wastewater in some selected locations in Maiduguri, Borno State, Nigeria has been carried out in this

study. Three locations; Monday Market, Nigeria Bottling Company (NBC) and Abattoir each representing commercial, industrial and domestic sites respectively, were sampled for the study. Out of the seven organisms (Escherichia Coli, Klebsiella, Protens, Alromonas, Enterobacter, Salmonella and Shigella), that were tested in each of the three sampled sites, only four (Escherichia Coli, Klebsiella, Enterobacter and Shigella) were positive, while the remaining three were not found in any of the wastewater channels. The bacteriological properties of wastewater from Abattoir, Monday Market and NBC Plc, showed that the wastewater could pose public and environmental health challenges, that is, the wastewater are unsafe and serve of could as sources environmental contamination. Finally, it was also revealed that some essential commodities as satchet water, milk, meat among others have already been contaminated by some of the idenified bacteria in sampled sites.

Recommendations

- 1 There should be implementation and compliance with standard policy guidelines on wastewater disposal in order to reduce the outbreak of diseases.
- 2 There should be collaboration of the organizations with the State Environmental Sanitation Board to have a specific well-secured area far away from settlement for the discharge of wastewater before or after treatment.
- 3 It is important to have a monitoring team that will periodically monitor the proper disposal of the wastewater from residential and industrial areas.
- 4 Since it has been established that some essential items such as satchet water, milk, meat and food have been contaminated by these bacteria, it is necessary to have functional quality control of these items before they reach the consumers.

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APPENDIX Two-way ANOVA: Number versus Location, Isolated Organisms

Source Location Organisms Error Total	3 4 6 1	1.1667 40.9167 12.8333	0.5833		0.770		
S = 1.462	R-Sc	A = 76.63	% R−Sq	(adj)	= 57.16%		
Location 1 2 3	3.00 3.25	Pooled S + (+))) +	
-	6.000	Pool ean+	ed StDev	-+	+	n Based on)	

1	6.00000			(*)	
2	2.66667	(*)		
3	1.66667	(*	·)		
4	1.33333	(*)			
		+	+	+	+	
		0.0	2.5	5.0	7.5	