
Preliminary Studies on Pathogenic Bacteria from Al- Baqa'a Sewage Treatment Station

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Abstract

Al- Baqa'a sewage is treated by the activated- sludge process. 36% of the total number of bacteria entered in the raw sewage were removed. Pathogenic bacteria such as *Salmonella typhi* and *Shigella dysentriae* were detected in raw sewage but not in the treated sewage effluent. Also, *Saccharomyces cereviciae* was isolated from sewage samples and about 5% of the yeast number was still present in the treated effluent.

ملخص

تم اجراء دراسة ميكروبيولوجية لمياه المجاري بمنطقة البقعة، وذلك قبل وبعد معاملتها بالطريقة المعتمدة (Activated- Sludge Process). وقد تم عزل خميرة

الساكروميسس من مياه المجاري.

كما وجد ان المياه قبل معاملتها تحتوي على بكتيريا مرضية مثل السلمونيلا التايروفدية، والشيجلا الدوستورية، التي تم عزلها بعد المعاملة.

وقد بينت الدراسة ان ٣٦٪ من المجموع الكلي للبكتيريا قد تم عزلها بعد المعاملة. كما وجد ان ٥٪ من المجموع الكلي للخمائر ما زال موجودا في المياه بعد المعاملة.

Introduction

Various types of pathogenic bacteria which could pose a significant health risk were detected in sewage and treated effluents (Jensen, 1954; Lanyi *et al.*, 1966; Carrington, 1977; Guenzel, 1978; Dudley *et al* 1980; Sallal and Baba'a, 1982). Bacterial survival after aerobic and anaerobic digestion of waste water sludge was studied by Farrah and Bitton (1983).

Waste materials are treated mainly to destroy the causative agents of water related diseases associated with domestic wastes. Furthermore, the waste materials are converted into readily reusable resources and help to prevent the pollution of any body of water (Mara, 1977).

This work presents the enumeration of pathogenic bacteria present in treated and untreated sewage of Al- Baqa'a sewage treatment station.

Materials and Methods

Sewage treatment and sampling

Al- Baqa'a sewage treatment station is located 30 Km north of Amman city in Jordan. This sewage station handles an input volume of 5.000 m³ per day mainly from domestic sewage which

is treated by an activated- sludge process. The raw sewage passes through a grit chamber for separation of some of the floating materials, thereafter it is exposed to aeration in the aeration tank using surface aerators. The aerated sewage (A1) then moves to a settling tank where aerobic decomposition of organic materials takes place. In this tank, effluent (E1) and sludge (S1) are separated. The effluent is then sent to a sedimentation tank to give a treated sewage effluent (E2). Sewage sludge is recycled back to aeration tank. Sewage samples from all stages of sewage processing were collected in 500 ml sterile bottles and transferred to the laboratory for immediate testing. Sewage samples were collected monthly from Jan. 1994 until April 1994. The results are the average of the four collected samples.

Enumeration and identification of bacteria

About 20 ml from each sewage sample was homogenized at high speed in a blender for 5 min. Appropriate dilution of homogenized sewage were made in sterile saline and then duplicate 1.0 ml aliquots of each dilutions were filtered in a sterilized Millipore filtration unit using 0.45 m sterile filters. They were plated on nutrient agar and MacConkey agar. The plates were incubated at 37° C for 24 h.

Different morphological colonies were subcultured on the same media for further physiological biochemical identification according to Cowan and Steel (1974).

The sensitivity of the isolated bacteria to ampicillin, tetracycline, cephradine, chloramphenicol and gentamycin was tested by the disc diffusion assay method after 24 h incubation at 37° C, using the standard procedure given by Matsen and Barry (1974).

Enumeration of *Mycobacterium* spp.

Collected sewage samples were tested for the presence of *Mycobacterium* spp. as follows:

The sewage samples were shaken vigorously to homogenize their content and then 5 ml of each sewage sample were placed in sterile tube and digested with 4% NaOH solution, containing bromothymol blue indicator. Thereafter the samples were incubated at 37° C for 20 minutes, neutralized with 8% HCl, and then centrifuged at 2000 rpm for 20 minutes. The supernatant was discarded and the pellets washed and suspended in physiological saline solution. Staining was performed for fixed smears, using the Ziehl- Neelson technique and the acid alcohol test. Bacilli were microscopically counted according to Bailey and Scott (1974). Sewage samples were then cultured on Lowenstein-Jensen medium with glycerol and pyruvic acid for about 6 weeks.

Enumeration of yeast

Appropriate dilutions of homogenized sewage samples were filtered in duplicate using 0.45 μ m sterile filters and plated on sabouraud dextrose agar. Incubation was at 37 C for 24 h. Colonies were counted and their preliminary identification was done according to Barnett *et al.* (1983).

Results

Domestic sewage of Al- Baqa'a is treated by an activated-sludge process through one treatment stage only. Raw sewage was found to contain a rather high number of bacteria i.e 2×10^{11} colonies/ml, which was not found in any of the treatment stations in Jordan (Unpublished data). About 38% of the total bacterial number was removed as shown from the counts of bacteria in the sewage effluent-1 (Fig.1).

The effluent resulted from the sedimentation tank (E2) still

contained 36% of the bacteria entered with the raw sewage (Fig.1). Total number of Gram negative bacteria was also counted in all sewage samples as presented in Figure 1. 36% of Gram negative bacteria was removed from the sewage as indicated from the counts in effluent-2 (Fig.1).

Salmonella typhi and *Shigella dysenteriae* were present in the raw sewage but they were not detected in sewage effluent-2 as shown in Table 1. However, other Gram negative bacteria species were found in effluent-2. *Shigella dysenteriae* was also isolated from sewage sludge (Table 1).

Gram positive bacteria, *Streptococcus faecalis*, *Micrococcus roseus* and *M. luteus* were also isolated from raw sewage (Table1).

Mycobacterium spp. were isolated from all sewage samples. 33% of the total *Mycobacterium* spp. were removed from sewage as indicated from the number counted in effluent-2 (Fig.2).

A total of 13×10^3 colonies/ml of *Saccharomyces cerevisiae* were counted in raw sewage as presented in Figure 3. 95% of the yeast was removed as indicated from the number of yeast in effluent-2 (Fig.3).

In addition, sensitivity tests done with antibiotics indicated that the isolated bacteria were susceptible to tetracycline, gentamycin, chloramphenicol and cephradine but they were resistant to ampicillin.

Discussion

Waste materials are treated mainly to destroy the causative agents of water related diseases. Most of the bacteria recovered in the sewage samples namely: *Salmonella typhi*, *Shigella dysenteriae*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Streptococcus faecalis* have been reported by other workers for their

ability to cause diseases (Gilardi *et al.*, 1970; Jawetz *et al.* 1976; Carrington, 1977; Dudley *et al.*, 1980). Although the number of bacteria entered the sewage station was high, there was considerable reduction for one stage treatment (Fig.1). Still treated sewage effluent (E2) contained quite a high number which emphasizes the need for another stage of sewage treatment in this station. This is the first attempt to screen the pathogenic bacteria in this sewage treatment station. *Mycobacterium tuberculosis* (Jensen, 1954) and *Mycobacterium* spp. (Dudley, *et al.* 1980, Sallal and Babaa, 1982) were isolated from sewage. In this work *Mycobacterium* spp. was also isolated from all sewage samples. The presence of pathogenic bacteria in raw sewage and long survival of some of them such as *Salmonella* spp. in sewage sludges was reported by others (Hess and Breer, 1975; Farrah and Bitton, 1983). The earlier reports on the survival of *Mycobacterium* spp by Pramer *et al.* (1950) emphasized the need for proper sewage treatment, especially if treated sewage effluent is mainly used for irrigation purposes.

The isolation of *Saccharomyces cereviciae* indicates the possible contamination of the raw sewage with a wastewater from dairy factories in that area. 95% of the yeast entered with raw sewage was removed, which indicates rather an efficient removal of yeast in one stage treatment.

References:

Bailey, W.R. and Scott, E.G. 1974. **Diagnostic Microbiology**. The C.V. Mosby Company, Saint Louis.

Barnett, J.A., Payne, R.W. and Yarrow, D. 1983. **Yeast Characteristics and Identification**. Cambridge University Press. Cambridge, England.

Carrington, E.G. 1977. **The Contribution of Sewage Sludges to the Dissemination of Pathogenic Microorganisms in the Environment**. Water Research Center, Stevenage Laboratory; Hertfordshire, England.

Cowan, S.T. and Steel, K.J. 1974. **Manual for the Identification of Medical Bacteria**. Cambridge University Press, Cambridge, England.

Dudley, D.J., Guentzel, M.N., Ibarra, M.J.H., Moore B.E. and Sagik, B.P. 1980 Enumeration of Potentially Pathogenic Bacteria from Sewage Sludges, *Appl. Environmental Microbiology*. 39 118 - 26.

Farrar, S.R. and Bitton, G. 1983. Bacterial Survival and Association with Sludge Flocs during Aerobic and Anaerobic Digestion of Wastewater Sludge under Laboratory Conditions. *Appl. Microbio.* 45 174- 81.

Girlardy, G.L., Bottone, E. and Birnbaum, M. 1970. Unusual Fermentative Gram Negative Bacilli Isolated from Clinical Specimens. II; Characterization of *Aeromonas* Species. *Appl. Microbio.* 20 156- 59.

Guentzel, M.N. 1978. Potential Impact on Water Sources of Bacterial Pathogenes in Wastewater Applied to Land. In: **Risk Assessment and Health Effects of Land Application of Municipal Waste and Sludges**. Edited by B.P. Sagik and C.A. Sorber PP. 180 - 94 Center for Applied Research and Technology. The University of Texas at San Antonio, San Antonio, Texas, U.S.A.

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- Herkowitz, I. 1988 Life Cycle of Budding Yeast *Sacchomyces Cerevisae*. *Microbiology Rev.* 52 (4), 536- 53.
- Hess, E. and Breer, C. 1975. Epidemiology of *Salmonella* and Fertilizing of Grassland with Sewage Sludge. *Bakt. 1 Orig. Reihe B.* P 161, 54- 60.
- Jawetz, E., Melnick, J.L. and Adelberg, E.A. 1976. Review of Medical Microbiology. Lange Medical Publications; California, U.S.A.
- Jensen, K.E. 1954. Presence and Destruction of Tubercle Bacilli in Sewage. *Bull WHO.* P 10, 171- 79.
- Lanyi, B., Gregacs, M. and Adam, M.M. 1966. Incidence of *Psuedomonas Aeruginosa* in Water and Human Faeces. *Acta Microbiol. Acad. Sci. Hung.* 13 319- 26.
- Mara, D.D. 1977. Wastewater Treatment in Hot Climates. In: *Water, Wastes and Health in Hot Climates.* Edited by: R.E. Feachem, M. McGarry and D. Mara. PP: 264- 83. John Wiley and Sons, New York, U.S.A.
- Masten, J.M. and Barry, A.L. 1974. Suscepibility Testing: Diffusion test procedures. In: *Manual of Clinical Microbiology.* Edited by: E.H. Spaulding and J.P. Truant. Washington. PP: 418- 27.
- Pramer, D., Heukelekian H. and Ragotzkie, R.A. 1950. Survival of Tubercle Bacilli in Various Sewage Treatment Processes. I. Development of a method for quantitive recovery of mycobacteria from sewage. *Public Health Report* 65, 851- 59.
- Sallal, A.K.J. and Babaa, M.M. 1982. Enumeration and Identification of Pathogenic Bacteria from Sewage in Kuwait. *Zbl. Microbiol.* 137 603- 10.

Table 1. Dominant types of bacteria present in treated and concentrated sewage.

Organisms	Raw sewage	Aeration tank 1	Settling tank 1 effluent	Settling tank 1 sludge	Sedimentation tank effluent (E2)
<i>Micrococcus roseus</i>	+	-	-	-	-
<i>Micrococcus luteus</i>	+	+	-	-	-
<i>Streptococcus faecalis</i>	+	-	-	-	-
<i>Escherichia coli</i>	+	+	+	+	+
<i>Enterobacter aerogenes</i>	+	+	+	+	+
<i>Klebsiella pneumoniae</i>	+	+	+	+	+
<i>Pseudomonas fluorescens</i>	+	+	+	+	+
<i>Proteus vulgaris</i>	+	+	+	-	-
<i>Salmonella typhi</i>	+	+	+	+	-
<i>Shigella dysenteriae</i>	+	+	+	+	-

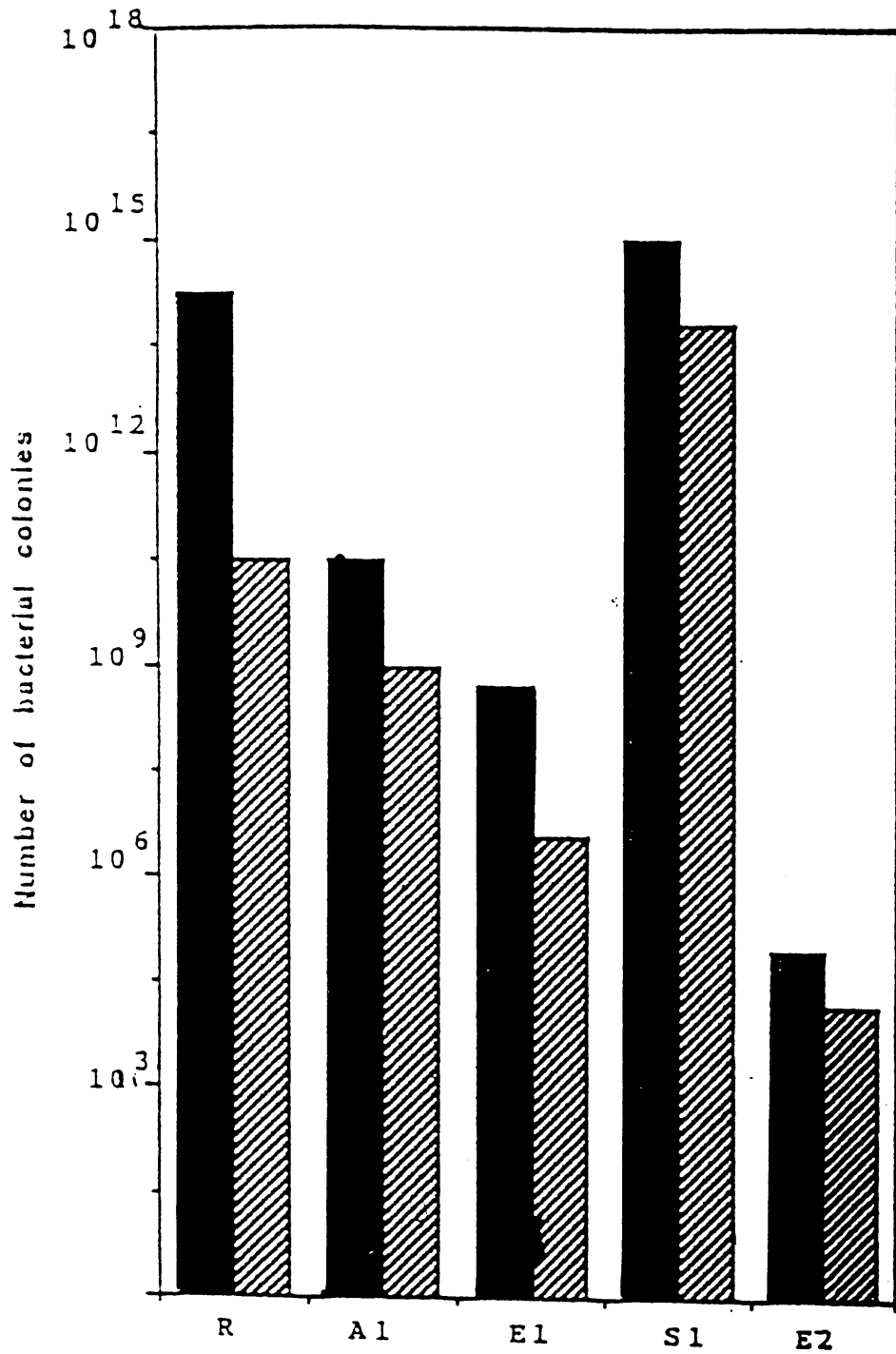


Figure 1. Total number of bacteria ■ and Gram negative bacteria ▨ isolated at the various sampling sites. R, Raw sewage; A1, Aeration tank; E1, Settling tank effluent; E2, Sedimentation tank effluent.

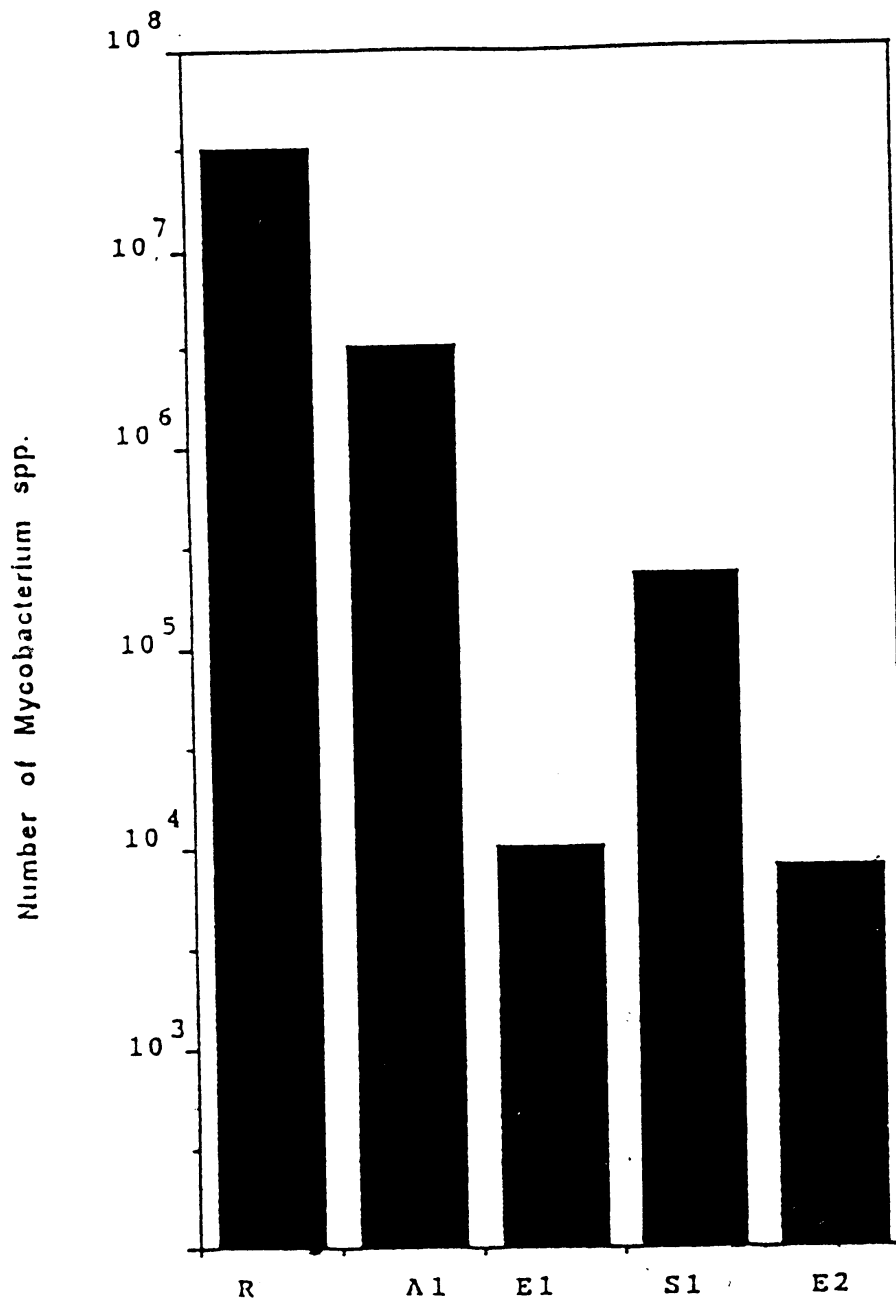


Figure 2. Total number of *Mycobacterium* spp. isolated from sewage samples. R, Raw sewage; A1, Aeration tank; E1, Settling tank effluent; E2, Sedimentation tank effluent.

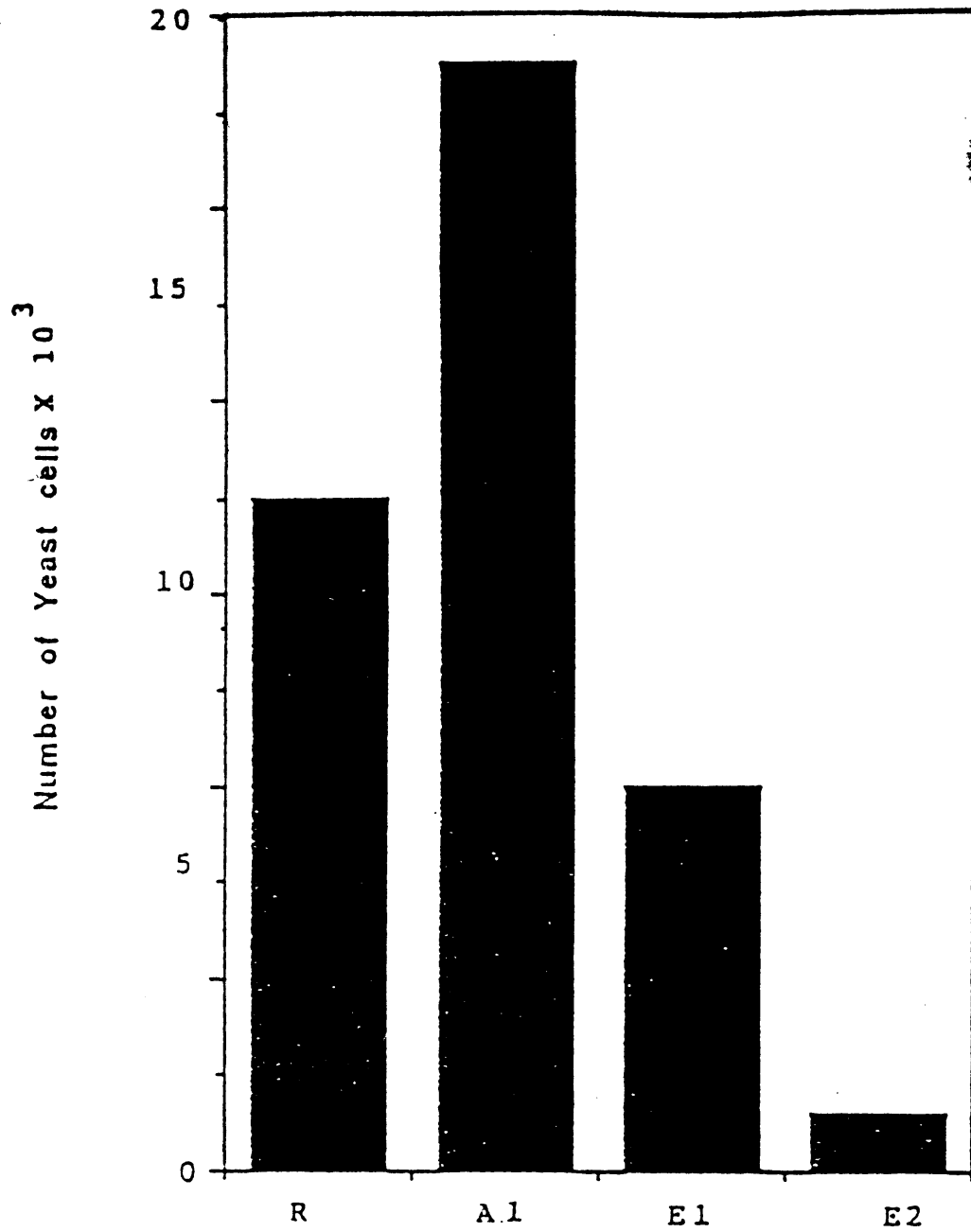


Figure 3. Total number of *Saccharomyces cerevisiae* isolated from sewage samples. R, Raw sewage; A, Aeration tank; E1, Settling tank effluent; E2, Sedimentation tank effluent.