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**HORIZONTAL DISTRIBUTION AND DENSITY OF
YEASTS, FILAMENTOUS FUNGI AND SOME
BACTERIA IN LAKE ST. CLAIR WATER**

by

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Management Perspective

Title: Horizontal distribution and density of yeasts, filamentous fungi and some bacteria in Lake St. Clair water

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Date: October, 1987

Perspective:

This manuscript reports new research results on the occurrence and distribution of yeasts, fungi and some bacteria in Lake St. Clair water, based on samples collected in June 1984 at 67 stations throughout Lake St. Clair and part of the St. Clair delta. The results indicate (i) generally higher yeast densities in the western part of the lake, (ii) predominance of red and white over black yeasts, (iii) presence of black yeasts mostly in the Northern and Middle Channels of the St. Clair River and in the northern and western parts of Lake St. Clair, and (iv) the presence of several common species of bacteria. The observed yeasts and fungi densities ranged from 5 to 1000 colony forming units per 100 mL.

Yeasts, fungi and bacteria are common in all natural waters. Some of the observed yeasts are known to be associated with industrial and/or municipal waste water effluents.

Perspective-gestion

Titre : Distribution horizontale et densité des levures, des champignons filamenteux et de certaines bactéries dans les eaux du lac Sainte-Claire

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Perspective :

Le présent manuscrit indique les résultats de nouvelles recherches sur la présence et la distribution de levures, de champignons et de certaines bactéries dans les eaux du lac Sainte-Claire, d'après les échantillons recueillis en juin 1984 dans 67 stations situées sur le lac Sainte-Claire et une partie de son delta. Les résultats montrent que : i) les densités de levure sont généralement plus élevées dans la partie ouest du lac, ii) les levures rouges et blanches prédominent sur les levures noires, iii) des levures noires se trouvent surtout dans le chenal du nord et le chenal central de la rivière Sainte-Claire ainsi que dans les parties nord et ouest du lac Sainte-Claire, et iv) plusieurs espèces courantes de bactéries y sont présentes. Les densités de levures et de champignons mesurées sont comprises entre 5 et 1000 unités formatrices de colonie par 100 ml.

La présence de levures, de champignons et de bactéries est courante dans les eaux naturelles. Il est reconnu que certaines des levures observées sont associées aux effluents d'eaux usées industrielles et (ou) municipales.

RÉSUMÉ

Des levures et des champignons filamenteux ont été recueillis dans 67 stations du lac Sainte-Claire. Des organismes provenant d'échantillons d'eau ont été isolés par filtration sur membrane et culture sur un milieu donné. Des colonies de levures prédominantes représentatives, de champignons filamenteux et de quelques bactéries ont été identifiées provisoirement. Les levures et les champignons levuriformes sont des organismes largement répandus, et les chercheurs ont mesuré des densités de l'ordre de 5 à 1000 unités formatrices de colonie (UFC) par 100 ml. Le rôle des levures dans les écosystèmes aquatiques, au niveau des études de toxicité et de biodégradation est analysé.

AUTRES MOTS D'INDEX:

Grands Lacs, chenaux de communication, pollution, qualité de l'eau.

ABSTRACT

Yeasts and filamentous fungi were collected from 67 stations throughout Lake St. Clair. Organisms from water samples were isolated by filtration through membrane and cultured on defined medium. Colonies of representative predominant yeasts, filamentous fungi and some bacteria were tentatively identified. Yeasts and yeast-like fungi are widely distributed organisms, and densities of 5 to 1000 colony forming units (CFU)/100 mL were found. The role of yeasts in aquatic ecosystems and for toxicity and biodegradation studies is discussed.

ADDITIONAL INDEX WORDS:

Great Lakes, connecting channels, pollution, water quality.

INTRODUCTION

The occurrence and distribution of yeasts in aquatic habitats has been studied in the past mostly in connection with polluted waters. In many cases yeasts have been used as possible indicators of water pollution (Cook 1970, Simard 1971, Simard and Blackwood 1971, Meyer 1974, Crow et al. 1975, Buck 1977, Sherry et al. 1979a). The need for detection and enumeration of yeast-like fungi which are able to biodegrade and/or accumulate organic or inorganic toxicants, has become greater as water pollution has increased.

The Great Lakes are a very important freshwater source, and information on water quality problems has become progressively more imperative. Parts of the Great Lakes have been classified by the International Joint Commission as Class "A", "Areas of Concern". Recent research studies have focused on the connecting channels of the Upper Great Lakes - the Detroit River/Lake St. Clair/St. Clair River system (Chau et al. 1985).

Lake St. Clair is comparatively small and very shallow with an area of 1200 km² (Fisheries and Environment Canada 1979) and maximum depths of approximately 5 to 6 m. The St. Clair River/Lake St. Clair/Detroit River connecting channel is one of the world's busiest waterways, which passes through a highly industrialized area of the United States and Canada. This resource serves a wide variety of purposes, including raw water source, effluent recipient, shipping,

fishing, boating, swimming, and also supports a large variety of waterfowl.

Along the connecting channel's shores are numerous industries which require large amounts of cooling and process water. The effluents produced by these industries, in combination with wastes received from large communities, have contributed to the pollution of water and sediment in this system (Kaiser and Comba 1986). As a result, the connecting waterbody of Lake St. Clair has become a reservoir of organic and inorganic pollutants and can serve as an ideal model for ecotoxicity, biodegradation and accumulation studies. Therefore, it is very important to know the chemical composition of the environment and microorganisms in which they grow, or to which they are exposed.

The group of microorganisms known as "yeasts" is traditionally limited to the fungi in which the unicellular form is predominant. They are eukaryotic heterotrophs (Hawker and Linton 1971) that exist in nature predominantly in association with organic matter (Cooke 1958). The habitats in which they occur are normally characterised by a high content of sugars, such as various parts of plants, grain, root crops, as well as dairy products (Lund 1954, Phaff and Starmer 1980). The majority of yeasts are saprophytes and may be found wherever organic matter occurs, whereas many others are potential pathogens of animals, humans or plants (Gentles and La Touche 1969).

Soil, especially in orchards and vine yards, forms an important reservoir for sugar-tolerant yeasts, where they can survive unfavourable periods (Miller and Webb 1954). Various yeasts, salt- and acid-tolerant, have been isolated from meats, slimy sausages, bacon, ham, pickled meats and brines, where yeasts can grow in salt concentrations ranging from 0 to 24%. Also, large numbers of asporogenous yeasts have been found in paper mills and in wood pulp (Dennis 1972). Thus, in Sweden and Finland, wood chemistry research is directed towards the study of enzymatic degradation of lignocellulosic materials by yeasts (Enari 1983).

Various authors (Harris and Ricketts 1962, Westlake and Spencer 1966, Hashimoto 1970, 1973, Ahearn et al. 1971, Mills et al. 1971, Spencer et al. 1971, Neujahr 1978, Pinto et al. 1979) and others, have also shown that certain yeasts are capable of assimilating phenols, benzoic acid compounds, flavonoids and lignin breakdown products.

Yeasts are conveyed to the aquatic ecosystems from their various natural substrates. Because these substrates are widely distributed, it is not unusual to find yeasts in almost all types of soil and in oceans far from the shore, often in surprisingly large numbers (Zo Bell 1946).

In addition, yeasts have been shown to possess a wide tolerance to various environmental conditions such as temperature, moisture, extremes of pH and high osmotic pressure (Kreger-van Rij

1984). Since they form a considerable amount of biomass and have a high reproductive rate, they are an important food source for other aquatic life forms. In reservoirs, especially at considerable depths where light does not penetrate and algae do not grow, yeasts are the only food source for lower trophic level aquatic organisms, such as larger zooplankton as well as for the bottom fauna (Rodina 1972).

This study was undertaken with the aim of isolating yeasts and filamentous fungi of potential significance in the biodegradation of toxicants in the Great Lakes' basin.

The present authors are particularly interested in the role of yeasts in bioaccumulation and biodegradation of toxicants in aquatic systems for several reasons:

- a) Yeasts are eukaryotic cells with all basic structures and organelles. They can serve as model organisms for research on the environmental degradation of contaminants.
- b) There are practical advantages of using yeasts in toxicity bioassays as one can often extrapolate from the results obtained to higher organisms, including humans.
- c) Yeasts are known to be of potential significance in the degradation of organic toxicants in the aquatic environment (Kwasniewska 1986, Ph.D. Thesis).
- d) Common occurrence in aquatic habitats, under both aerobic and anaerobic conditions (oxidative and fermentative), high salt and sugar tolerance make them

important indicators for both environmental enrichment (eutrophication) and contamination by toxic chemicals.

MATERIALS AND METHODS

Sample Collection and Treatment

Subsurface water samples (at 1 m depth) were collected between 18 and 21 June 1984 in the Lake St. Clair and in several channels of the St. Clair River mouth. The sampling locations are shown in Figure 1. To prevent the proliferation of organisms, all samples were processed at a mobile processing unit on shore within four hours from collection. The water was collected in 250 mL size sterile plastic flasks. To recover the yeast-like fungi from water, the samples were filtered through membrane filters (MF) with a range of pore sizes (0.8 and 1.2 μm). For the filtration of volumes of less than 100 mL of turbid and more than 100 mL of non-turbid water, a membrane of 0.45 μm pore size was usually found to be suitable. However, a filtration with a larger pore size membrane (e.g., 0.8 μm) was found useful to reduce retention of the larger bacteria. Antibiotics were also used to inhibit the growth of bacteria on isolation media. Yeasts and fungi colonies, after three to five days incubation at room temperature, were isolated and held for further processing. The density of growing yeasts and fungi was usually estimated by colony counting colony-forming units (CFU) per 100 mL.

The number of viable propagules in the original sample was calculated using the following formula for CFU per 100 mL:

$$\frac{\text{Number of Colonies} \times 100}{\text{Sample Volume Plated} \times \text{Dilution Factor}}$$

After enumeration, yeasts and fungi were transferred to the slants of Sabouraud agar medium (Difco Ltd.) for identification and classification purposes.

Growth Media

Five types of media were used for isolation and enumeration, maintenance and classification of yeast and yeast-like fungi from the water samples.

- 1) Littman's Ox-gall Agar (Oxoid) supplemented with

Streptomycin	70 mgL ⁻¹
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Chloramphenicol	70 mgL ⁻¹
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- 2) Rose Bengal Agar (Aureomycin, Streptomycin)

	<u>gL⁻¹</u>
Glucose	10.0
Peptone	5.0

KH_2PO_4	1.0
$\text{MgSO}_4 \cdot 7 \cdot \text{H}_2\text{O}$	0.5
Rose Bengal	0.035
Agar	15.0
pH	5.4 (approximately)

The above were sterilized as 121°C for 15 minutes.

Aureomycin H	50 mgL^{-1}
Streptomycin	50 mgL^{-1}

Antibiotics were first dissolved in distilled water and sterilized by membrane filtration, using a $0.2 \mu\text{m}$ membrane, and were then added to the rest of the autoclaved medium (after approximately 50°C) and mixed thoroughly.

- 3) Sabouraud Agar (Difco Ltd.) for maintenance of isolates.
- 4) Yeast Carbon Base Agar (YCB - Difco).
- 5) Yeast Nitrogen Base Agar (YNB - Difco) at the rate of 6.7 g/L . Numbers 1 and 2 media are selective media used for isolation from natural sources. Number 3 was used for maintenance of isolates. Numbers 4 and 5 were used for the classification of yeasts. These media have been prepared according to the formulae of Wickerham, L.J. and Burton, K.A. (1948), Wickerham L.J. (1946).

RESULTS AND DISCUSSION

Yeast Distribution

In this survey of Lake St. Clair for the horizontal distribution and densities of yeasts, fungi and some bacteria, a total of sixty-seven 100 mL water samples were collected. Yeast populations in the water examined ranged from 5 to 1000 CFU/100 mL, filamentous fungi from 10 to 300 CFU/100 mL, and bacteria from 10^2 to 10^4 viable cells as determined by plate counts on Littman's medium. A detailed breakdown of these results is tabulated in Table 1. During the course of the examination, prime attention was paid to the distribution and density of yeasts. Filamentous fungi growing on Littman agar were also counted. Bacteria were considered only insofar as they were able to grow on the Littman's agar, a very selective medium, containing antibiotics inhibitory to most bacteria. Counts of bacteria, therefore, do not reflect the real abundance of these organisms in the oligotrophic water of Lake St. Clair. Another commonly used selective medium, Rose Bengal Agar, was also tested. Due to the accumulation of the rose bengal dye from the medium by unpigmented yeasts leading to confusion with red pigmented yeasts of the genus Rhodotorula, the isolates were disregarded. Simultaneously with the sampling for yeasts and other microorganisms, sampling was also conducted to determine the distribution and concentration of seven volatile halocarbon contaminants of which tetrachloroethylene and carbon tetrachloride showed the highest concentrations (Kaiser and Comba (1986).

Additional tests have been conducted by Ribo (1984, unpublished data) who examined the acute toxicity of these water samples to the marine bacterium Photobacterium phosphoreum.

From Figure 1 it can be seen that yeasts are distributed along all shorelines and throughout the water of Lake St. Clair. However, it is also apparent that the density and frequency of their occurrence varies between stations and within the same horizon. Overall, the yeast populations examined varied from 5 to 1000 CFU/mL of water.

There is an indication that the highest numbers of yeasts occur mostly in nearshore zones and adjacent to tributaries. This is particularly apparent for the Cutoff Canal and Clinton River as well as North, Middle and South Channels. Yeast populations ranged from 300 to 1000 CFU/100 mL in these areas. The important part of the lake for chemical contaminants and organisms is the mouth of the St. Clair River, downstream from Baby Point near Port Lambton, which spreads into the seven major channels forming the Delta. Yeast populations in this area ranged from 50 to 300 CFU/100 mL. It is well known (Dennis et al. 1984) that the Delta contains important marshland habitat areas for waterfowl and serves as a large wildlife refuge. There is, therefore, a potential influx of nutrients associated with bird droppings from several heavily colonized breeding sites located on islands in the Delta of St. Clair River. Bird droppings contain creatinine

($C_4H_7N_3O$), a compound that certain yeast-like fungi can use as a nitrogen source.

In general, higher yeast populations are consistently observed in the western part of Lake St. Clair. Yeast populations in the waters of this part of the lake ranged from 200 to 1000 CFU/100 mL. It is also evident from Figure 1 that similar yeast populations are found in the navigation channel between the St. Clair and Detroit Rivers. This is particularly visible at stations #206, 208, 220, and 221. Our findings suggest that large quantities of some urban or industrial effluents, particularly sewage, may enter the lake near these sites. Also, frequent dredging of the navigation channel in Lake St. Clair may spread these readily utilizable sources of nutrition and increase their availability for yeasts and filamentous fungi. The horizontal and vertical mixing of effluents, combined with increased aeration, would give an additional explanation for the predominance of these organisms in the channel.

The role of sewage in the development of high microbial counts in polluted water is discussed in the studies of Cooke (1963, 1976). Some of our previous studies (Kwasniewska 1972, unpublished results) support Cooke's findings that yeasts and filamentous fungi are present in large numbers at every stage of the sewage treatment process and occur in elevated densities in natural waters that have received an input of sewage effluent. Meyer (1974) also noted that in areas directly affected by industrial or urban effluents yeast

populations generally exceeded 300 CFU/100 mL, as opposed to baseline levels of approximately 20 CFU/100 mL.

Thus, it is not surprising that very high numbers exceeding 1000 CFU/100 mL of several species of yeasts have been found in these areas of the lake. The urbanization of the Detroit and Windsor areas, the development of marinas and the increased usage of the west side of the lakeshore for summer recreational activities all serve to augment nutrient levels available to microorganisms.

In the eastern part of the lake, smaller but still substantial numbers of yeasts were found. Yeast densities in the eastern near-shore water were approximately 200 CFU/100 mL. An explanation for the presence of substantial yeast populations in this area can be found in water quality surveys that indicate a continuous zone in which phenolic contaminants, petrochemicals, styrene alkali chemicals, chemicals from rubber fabrication and dye manufacturing, as well as many other contaminants, are discharged into the upper St. Clair River along the eastern shore and enter Lake St. Clair (IJC 1968). Some of these chemicals, including azo dyes, can be readily utilized by red yeasts of the genus Rhodotorula (Kwasniewska 1985). The ability of many yeast species to degrade phenolic compounds is well known (Neujahr 1978, Summerbell 1983, and many other studies).

Yeast Species

Classification of yeasts is based primarily on their ability to ferment or assimilate different kinds of sugars (Kreger van Rij 1984). However, another useful parameter for rapid characterization of yeasts is pigment production. This breakdown, which separates important taxonomic and ecological groups, was found to be a convenient tool for the purposes of this study. Pigments in yeasts are synthesized in various shades of black, brown, red, pink, orange, yellow, cream or white. These pigments have been identified as melanin in Aureobasidium pullulans, carotenoids in the genus Rhodotorula and pulcherrimin in Metschnikowia pulcherrima and in some Kluyveromyces species. The predominant red yeasts of Lake St. Clair were identified as Rhodotorula rubra, Rh. minuta, Rh. glutinis, and Sporobolomyces spp. White yeasts included members of the genera Candida, Cryptococcus, and Debaryomyces. Black yeasts included Aureobasidium pullulans and other species yet to be identified.

From Figure 2, it can be seen that red, white and black yeasts are present along all shorelines and throughout Lake St. Clair water. A sample plate from Station #220 shown in Figure 3, depicts their density in 1 mL of sub-surface (1 m depth) water. The major portion of the yeast mycota here consisted of members of the genus Rhodotorula. The wide distribution of Rhodotorula yeasts in aquatic ecosystems has also been observed by other investigators of both freshwater reservoirs and seawater (Ahearn et al. 1969, Novozhilova et

al. 1981). Red yeasts are frequently abundant in surface layers of water with elevated oil concentrations (Crow et al. 1975, Novozhilova et al. 1981). This suggests the utilization of oil by these organisms (Ahearn et al. 1971).

Biochemical analysis of red yeasts and related species (Eijk and Roeymans 1982) showed that they produce orange to red pigment called carotenoids. The carotenoids of the red yeasts have been intensively studied by Nakayama et al. (1954), who showed that β -carotene, γ -carotene, torulene and torularhodin are the major carotenoids. The carotenoids are always detected in red yeasts, but are present in various proportions as has been shown by Nakayama et al. (1954), Kvasnikov et al. (1978) and others, these quantitative differences are greatly influenced by environmental conditions, particularly nutrient availability and temperature.

The presence of these carotenoids may explain the predominance of Rhodotorula spp. and other carotene-producing organisms in the upper layers of the lake water. During periods of high sunlight, carotenoids function to protect the photosynthetic apparatus of plants and algae against photodestruction (Goodwin 1981). Similarly, they may also protect the vital structures and processes of Rhodotorula spp. Several other very important cellular compounds, such as volatiles, sterols and fatty acids, have also been identified in red yeasts. Many important components, such as the cytoplasmic and

cell-wall carbohydrates of red yeasts, have been extensively documented, but will not be discussed here.

Although the number of red yeasts in our survey exceeded the numbers of black and white yeasts in both density and frequency, the presence of black yeasts, in particular within the aquatic environment should not be overlooked. The number of black yeasts isolated per 100 mL varied from 5 to 200. These small numbers could be due to an inadequate selective medium. Unfortunately, lack of information on nutrient requirements of black yeasts limited the extent of our investigations at the time of the lake survey. These organisms undoubtedly play an important role in certain ecosystems and they therefore deserve more attention in future studies.

Bacteria and Filamentous Fungi Distribution

The bacteria which grew on the Littman agar appeared to fall into two distinct groups. A selected isolate from one group was identified as Pseudomonas aeruginosa, while an isolate from the second group was determined as Pseudomonas putida. These species are commonly found in association with habitats which have a high content of phenolic compounds (Dagley and Patel 1957, Ornston and Stainer 1966, Nakazawa and Yokota 1973) and others. High densities of 10^2 to 10^4 CFU/mL and broad distribution as shown in Figure 4, suggest that these organisms do not require a specific environment. Their

occurrence along the shorelines and main channel across the lake may indicate sewage waste effluent since these bacteria are known as sewage, water and soil inhabitants (Havelaar et al. 1985). Both species produce diffusible fluorescent pigment and a characteristic odour.

A comparison of the densities and distribution of Pseudo-
monas spp. with the densities and distributions of red yeasts shows that these two groups of organisms tend to proliferate at the same sites. This implies that they may be responding to the same pollutants, and may share major responsibility for the breakdown of toxic organic chemicals in Lake St. Clair. However, the information available today on the role of yeasts in ecosystems is fragmentary and does not permit drawing firm conclusions as to their overall importance in the breakdown of pollutants.

The predominant filamentous fungi occurring in the sampled water included members of the genera Trichoderma, Fusarium, Mucor, Rhizopus, Penicillium and Paecilomyces. Some of these fungal records may represent only dormant propagules originating from adjacent land habitats. However, as Cooke (1958) has shown, active mycelia of filamentous fungi occur frequently in heavily polluted waters. These organisms therefore may also play a role in the breakdown of contaminants in aquatic ecosystems. However, much more research is necessary before a comprehensive evaluation of the roles of individual filamentous fungal species in aquatic systems can be carried out. Like the

yeasts, these organisms are richly deserving of greater attention from toxicologists of fresh water systems.

CONCLUSION

The data obtained here show the distribution and densities of yeasts and filamentous fungi and antibiotic-tolerant bacteria in Lake St. Clair water. The results of 67 samples of subsurface water indicate that members of three important subgroups, the red, white and black yeasts, were present along all shorelines and throughout Lake St. Clair water. Particularly high densities of red yeasts were found; the majority of these were members of the genus Rhodotorula.

In general, higher yeast populations were consistently observed in the westerly part of Lake St. Clair as compared to the easterly part. Yeast populations in the waters examined ranged from 5 to 1000 CFU/100 mL, filamentous fungi from 10 to 300 CFU/100 mL and antibiotic-tolerant bacteria from 10^2 to 10^4 /mL.

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FIG. 1. Yeast densities in water samples from Lake St. Clair with station locations and numbers.

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PREDOMINANT PLANTS, FUNGI AND BACTERIA FOUND IN LAKE ST. CLAIR WATER

[illegible]

6A. How did you feel about the experience?

* See Figure 1

TABLE I

Yeast densities in water samples from Lake St. Clair
with station locations and numbers

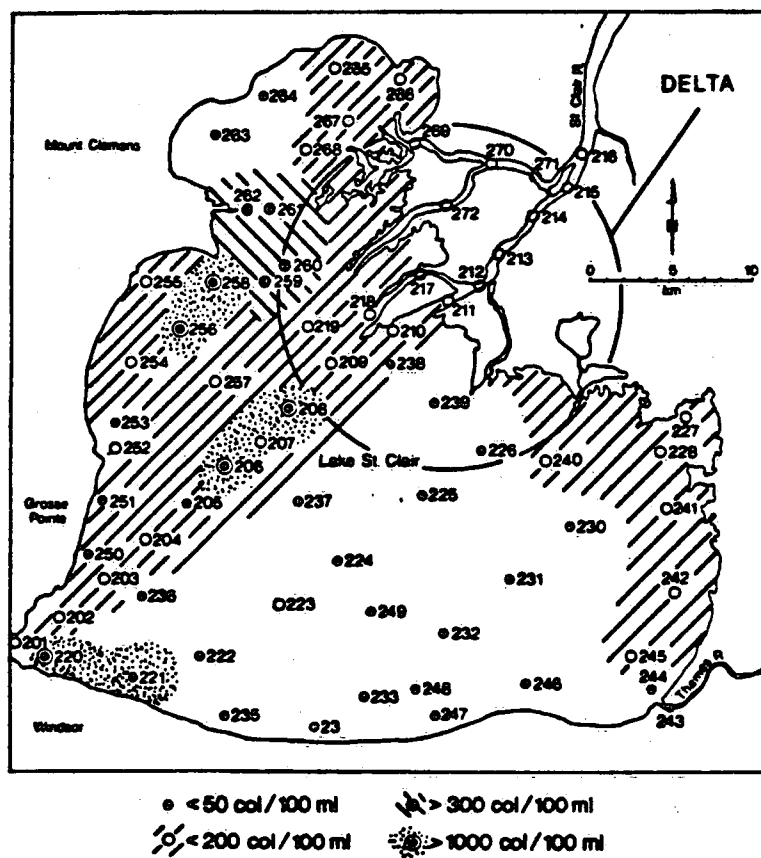


Figure 1

Frequency of isolation of red, white and black yeasts in Lake St. Clair water

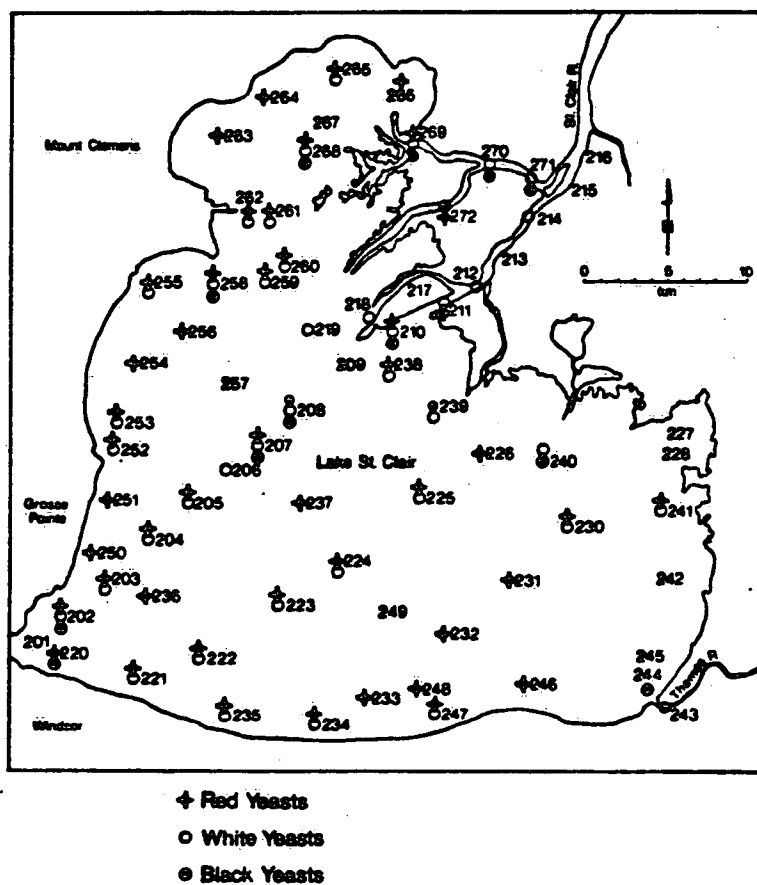
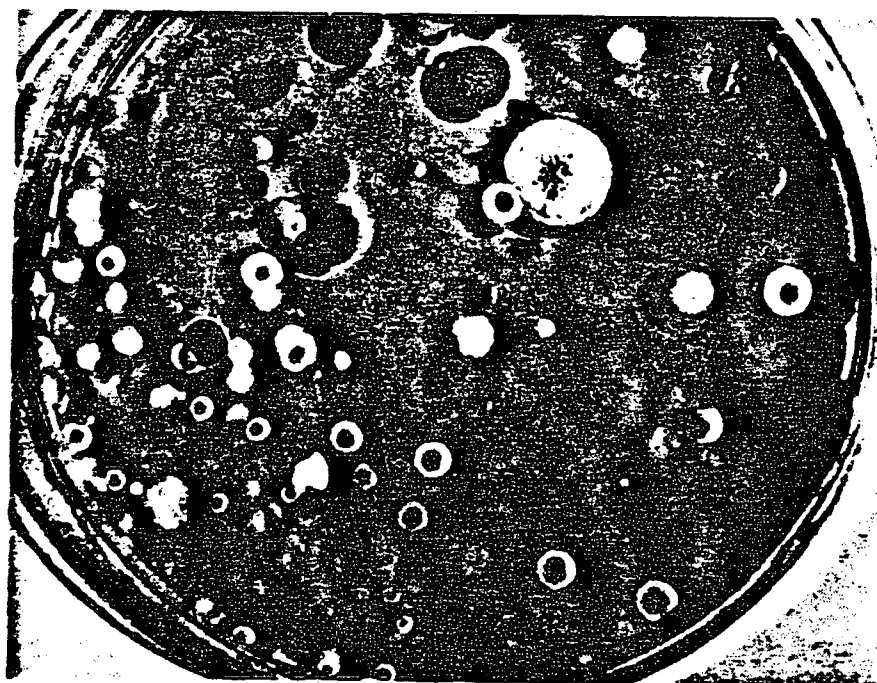
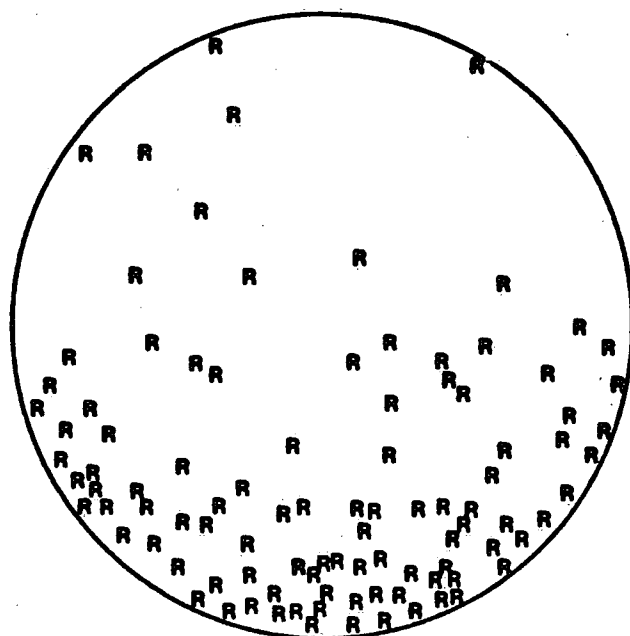


Figure 2



LAKE ST. CLAIR 1984

Inoculum of 1 mL Lake St. Clair water from station 220, showing the predominance of red yeasts over black and white yeasts.

Abundance of bacteria in Lake St. Clair water

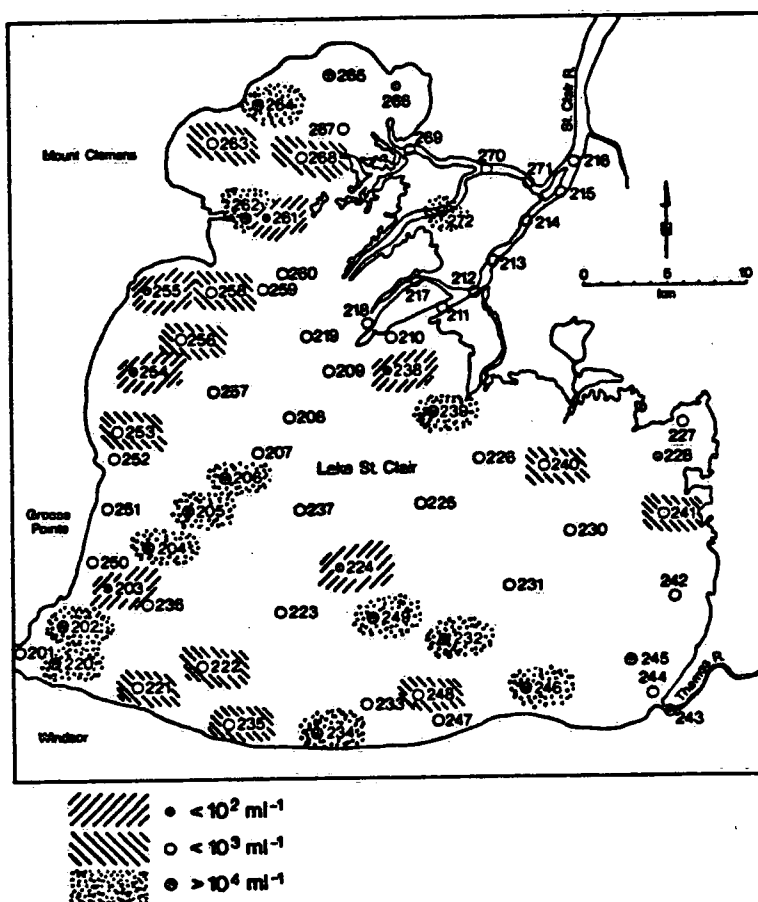


Figure 4