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A LITERATURE REVIEW OF THE BACTERIUM KLEBSIELLA SPP.: GRAYS HAR--ETC(U)
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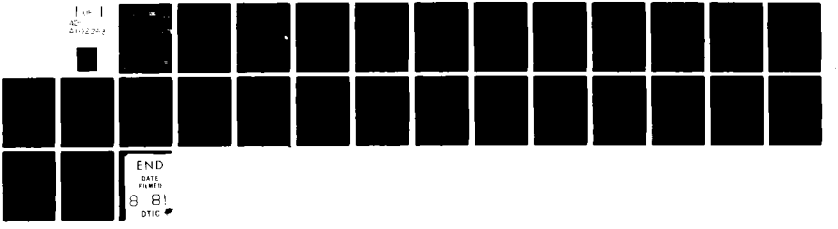

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GRAYS HARBOR AND CHEHALIS RIVER
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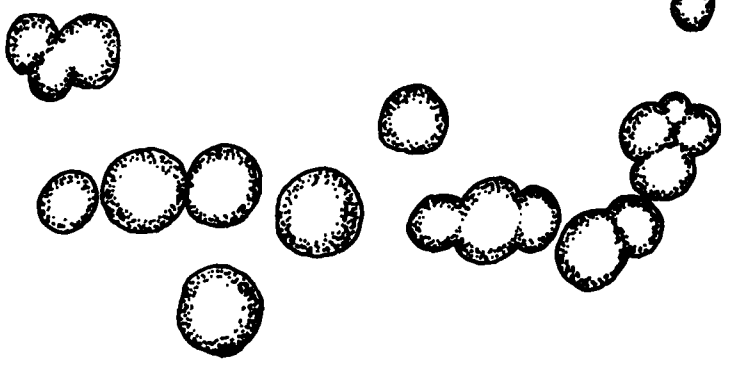
A LITERATURE REVIEW OF THE BACTERIUM KLEBSIELLA SPP.



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Cover Illustration: Macroscopic colonies of Klebsiella sp.

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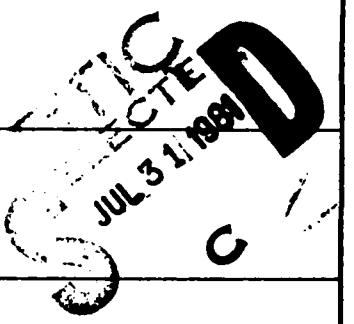

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Seattle District, U.S. Army Corps of Engineers is presently conducting studies to ascertain the environmental impacts of widening and deepening the navigation channel in Grays Harbor, Washington. This report was prompted by the concern that Klebsiella, a bacterium isolated from pulp and paper mill wastes in Grays Harbor, may be a source of contamination to Grays Harbor sediments. A critical review of its bacteriology, pathogencity, and ecology revealed that, while its taxonomy is confusing, Klebsiella isolated from the environment is not a high risk pathogen. Additionally, its reproductive capability is dependent upon the

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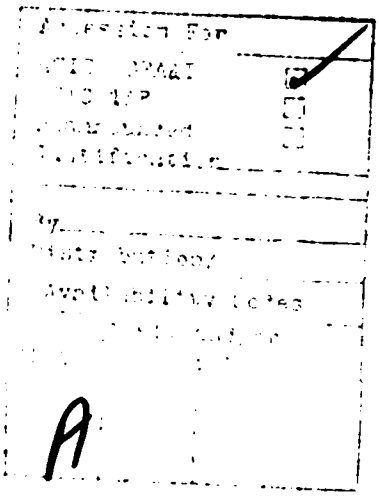

A Literature Review of the Bacterium *Klebsiella* spp.

Grays Harbor Navigation Improvement Project

by
Patricia C. Storm

April 1981

Environmental Resources Section
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ABSTRACT

Seattle District, U.S. Army Corps of Engineers is presently conducting studies to ascertain the environmental impacts of widening and deepening the navigation channel in Grays Harbor, Washington. This report was prompted by the concern that Klebsiella, a bacterium isolated from pulp and paper mill wastes in Grays Harbor, may be a source of contamination to Grays Harbor sediments. A critical review of its bacteriology, pathogencity, and ecology revealed that, while its taxonomy is confusing, Klebsiella isolated from the environment is not a high risk pathogen. Additionally, its reproductive capability is dependent upon the presence of organic wastes. It is, therefore, unlikely that the low nutrient waters at the dredging and disposal sites would provide an environment for bacterial growth. Thus, while the impact of dredging and disposal on the redistribution of Klebsiella in Grays Harbor is unknown, existing data suggest that the disturbance of sediment contaminated with Klebsiella should not represent a serious threat to human health.

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A LITERATURE REVIEW OF THE BACTERIUM *KLEBSIELLA* SPP.

1.0 Background. Seattle District, U.S. Army Corps of Engineers is presently conducting environmental studies designed to address the impacts of widening and deepening the navigation channel in Grays Harbor, Washington. The proposed project would require initial dredging of an estimated 19.3 million cubic yards (c.y.) of sand and silt and an annual maintenance dredging of approximately 2.7 million c.y. to maintain the proposed channel depths. One aspect of the environmental studies is the evaluation of the bacteriological composition of Grays Harbor sediments.

2.0 Introduction. *Klebsiella pneumonia* is a well-known opportunistic, pathogenic bacterium causing lobar pneumonia and urinary tract infection in humans and udder inflamations in cattle. *Klebsiella* sp. has been isolated from natural environments including soil, vegetation, and aquatic habitats. It is also now considered to be the dominant microorganism in treatment ponds of pulp and paper mills.

This review was prompted by concern for the bacteriological (specifically *Klebsiella*) contamination of Grays Harbor sediments from pulp and paper mill wastes. If the sediments are contaminated with *Klebsiella*, dredging by the Corps of Engineers may release *Klebsiella* into the water. These newly released bacteria may disperse throughout Grays Harbor, eventually settling into sensitive areas such as the oyster beds or in razor clamming areas along the ocean beaches.

This report will review the literature on *Klebsiella* to determine the status of knowledge regarding the bacteriology of this organism, the organism's pathogenicity, the occurrence of *Klebsiella* in natural environments including pulp and paper mill effluents, and the survivability of *Klebsiella* in sediments and the water column in fresh and marine systems, particularly in Grays Harbor. In addition, hypotheses on the impact of dredging on the redistribution of *Klebsiella* will be addressed, and any needed studies designed to test these hypotheses will be proposed.

3.0 Bacteriology. *Klebsiella pneumonia* is the first bacterium to be associated with the sanitary quality of water. In 1880, Von Fritsch[1/] described it as a microorganism characteristic of human contamination. In 1884, Escherich[1/] isolated a bacterium (*Bacillus coli* or *Escherichia coli*) from the feces of cholera patients. Based on the work of these early investigators, it is believed that the presence of such organisms in water bodies derived from warm-blooded hosts implies that the water has been contaminated with fecal matter.

1/In Geldreich, 1966.

In 1904, Eijkoff[1/] developed a test to identify intestinal (coliform) bacteria which ferment lactose at high temperature (44.5° C). These coliform bacteria (fecal coliforms) are considered to be of recent fecal origin (newly released from feces of warm-blooded animals). They are, therefore, more likely to be representative of the disease carrying enteric (intestinal) bacteria than the coliform bacteria which are not able to adapt to high temperatures. Further statistical analyses of bacterial counts have shown that as the quantity of fecal coliform bacteria increases, the probability of finding pathogenic species also increases. Based on this premise, state and Federal regulatory agencies established criteria for bacteriological quality of the waters of the United States. A synopsis of Federal and Washington State bacteriological criteria is provided in appendix A.

During the 1950's, 72 types of Klebsiella were identified in cultures isolated from hospitalized patients (Edwards and Fife, 1955). Reviews of Klebsiella bacteriology describing the range of biochemical and serological tests which were developed to differentiate Klebsiella bacilli are found in National Council of the Paper Industry for Air and Stream Improvement (NCASI) (1971) and Knittel (1975).

In the 1960's a saprophytic (soil) nonpathgenic bacterium Aerobacter aerogenes, considered to be ubiquitous in vegetation, was found to be biochemically and serologically similar to K. pneumonia. In 1963 the judicial commission of the International Committee on nomenclature adapted Enterobactor as the generic name for the motile strains of Aerobacter; the nonmotile strains are classified as K. pneumonia (see appendix B). There still remains some confusion in the identity of Enterobacter aerogenes, the saprophytic bacterium which is nonpathogenic, and K. pneumonia, the clinical pathogen.

Recent development of complex biochemical and genetic techniques has extended the subdivisions of Klebsiella (Woodward, et al., 1979; Naemura, et al., 1979) to include at least two major biotypes of Klebsiella. The subdivision of Klebsiella into fecal coliform positive and fecal coliform negative strains is the most important determination of sanitary significance. Several workers (Bagley and Seidler, 1977; Dufour and Cabelli, 1976; Duncan and Razzell, 1972) determined that of the total K. pneumonia isolated from hospitalized individuals 80 percent are fecal coliform positive. These same investigators found a smaller and more variable number (i.e., 16-24 percent) of environmental Klebsiella are fecal coliform positive.

In attempting to establish the link (etiology) between environmental and hospital isolates of Klebsiella, investigators have compared the similarities and differences between them. The only definitive difference was the increased susceptibility to antibiotics of the hospital isolates

1/In Geldreich, 1966.

(Brown and Seidler, 1973; Talbot, et al., 1980). There was no difference in the results of mouse pathogenicity tests, biochemical tests, and ability to colonize botanical environments between the environmental and hospital isolates (Matsen, et al., 1974; Bagley and Seidler, 1978; Seidler, et al., 1975; Dufour and Cabelli, 1976; Knittel, et al., 1977).

4.0 Medical Significance. Since its isolation from infected lung by Freidlander in 1882,[1/] Klebsiella pneumonia has been identified as an opportunistic pathogen, causing infections to develop in predisposed individuals. Julianelle (1926) found Klebsiella pneumonia to be responsible for 1.1 percent of 17,000 cases of lobar pneumonia, of which 60 to 70 percent of the K. pneumonia infections were fatal. Human infections associated with K. pneumonia include urinary tract infections (Edmonson and Sanford, 1967; Matsen et al, 1974), bacteremia (Steinhauer, et al., 1966), osteomyelitis (Farman, 1963), and meningitis (Spicak, et al., 1957). Klebsiella is also considered the causal bacterium in animal infections including udder infections in cattle (McDonald, et al., 1970; Braman, et al., 1973).

Klebsiella pneumonia was believed to contribute insignificantly (1.1 percent) to the overall incidence of infection. However, in the past 10 years, nonanimal K. pneumonia infections have increased and are recognized as one of the primary causes of hospital acquired infections (Knittel, 1975). Knittel (1975) outlined several reasons for the increased infections. He identified the increased longevity of the human population and an increase in antibiotic treatment as possibly predisposing humans toward bacterial infection. Aging affects a portion of the human population by lowering their resistance and increasing their susceptibility to bacterial infections. Antibiotic treatment may destroy all susceptible bacteria leaving only those which are resistant. Klebsiella pneumonia has been shown to be highly resistant to a broad range of antibiotics. Thus, it may be the chief bacterium surviving in the individual after a heavy dose of antibiotics.

K. pneumonia occurs as a normal respiratory tract bacterium in 2 to 25 percent of the human population (Finland, 1963) and as an enteric (intestinal) bacterium in 30 to 40 percent of the human and animal population (Davis and Matsen, 1974; Thom, 1970; Montgomerie, et al., 1970). A single dose of 10^5 to 10^7 Klebsiella cells/gram of food is enough to colonize the human gastrointestinal tract (Montgomerie, et al., 1970). This prior colonization may provide a reservoir of bacteria which may develop into an infectious state. In individuals whose resistance is reduced through antibiotic treatment, another disease, or age, there is an increased chance of developing a secondary Klebsiella infection.

1/In Knittel, 1975.

The complicated infectious route of Klebsiella precludes establishing causal relationships except through indirect implications. Investigators studying K. pneumonia infections in clinical cases have postulated routes of infection as: (1) autoinfections via intestinal tract, (2) contaminated food or water, and (3) contact transmission (e.g., hospital personnel hand cream, aerosals, food). Klebsiella may aggravate bacterial infections through the transfer of antibiotic resistant factors. Leary et al. (1972) hypothesized that the ingestion of antibiotic resistant Klebsiella strains may lead to the transfer of resistance to intestinal bacteria which are normally susceptible to a wide variety of antibiotics.

Bagley and Seidler (1978) found that environmental and clinical isolates of Klebsiella pneumonia are more virulent than Escherichia coli. Their investigation showed that the lethal dose for 50 percent (LD_{50}) of the test organisms (mice) exposed to E. coli is 2.5×10^5 to 2.8×10^5 cells/milliliter (ml), while the LD_{50} for K. pneumonia is 4.2×10^4 to 4.6×10^4 cells/ml. This indicates that K. pneumonia are potentially more virulent than other pathogenic bacteria, e.g., Salmonella LD_{50} 1.6×10^6 cells/ml. Bagley and Seidler hypothesized that K. pneumonia's higher degree of invasiveness (spreading into healthy tissue) and high survival rate in its host rather than its production of toxins may be responsible for its virulence.

While the environmental and clinical isolates exhibit the same pathogenicity in mice (NCASI, 1975), there is no epidemiological evidence to support pathogenicity of environmental isolates to humans (NCASI, 1972; Martin, et al., 1971). The only recorded epidemic of Klebsiella occurred in 1916-1917 in a prison camp in Germany (Zander, 1919).1/ Again, as with most Klebsiella infections, the route of infection was never clearly identified. In Craun's (1978) review of waterborne disease, Klebsiella was mentioned in association with drinking water but not as a causative factor in disease. Recreational infection (water contact) associated with Klebsiella has never been reported (NCASI, 1972; Moore, 1970). Thus, while theory supports the potential significance of infections from environmental isolates, no epidemiological evidence has been encountered. Bacteriologists (Montgomerie, et al., 1970; Selden, et al., 1971) speculate that a long time transpires between contact, colonization, and subsequent infections. Therefore, they believe it is difficult to determine the epidemiological significance of widespread distribution of Klebsiella.

5.0 Occurrence in the Natural Environment. The identification and isolation of Klebsiella pneumonia from the clinical environment has been rather extensively investigated. Its occurrence as a member of the flora of the healthy human populaton is also well documented. Klebsiella is considered to be ubiquitous as a soil and vegetation microorganism (Bagley and Seidler, 1978; Duncan and Razzel, 1972; Seidler, et al., 1977). Klebsiella has also been isolated from drinking water

1/In NCASI, 1972.

supplies (Seidler, et al., 1977). Matsen, et al. (1974), isolated Klebsiella from clean and contaminated, fresh and saline waters throughout the United States. Knittel (1975) examined waters of the Pacific Northwest and found Klebsiella to be a frequent member of the bacterial populations (see appendix C).

In 1970, Bauer (Bordner and Carroll, 1972) discovered high numbers of Klebsiella in the rivers and streams of the Pacific Northwest. These bacteria were traced to effluents of pulp and paper mills. Other investigators (Duncan, 1975; Knittel, 1975) have identified Klebsiella as a dominant member of the bacterial population of pulp and paper mill wastes.

As stated in "Standard Methods" (14th edition, 1976), the identification of fecal coliforms as opposed to total coliforms will aid in the determination of the source of pollution in water and the remoteness of pollution. Nonfecal coliforms survive longer than fecal coliforms. Therefore, fecal coliforms are indicative of recent fecal contamination. Knittel (1975) observed that of the 10^3-10^4 cells/ml total coliforms isolated from pulp and paper mills, 10^1-10^2 cells/ml were fecal coliforms; of the total coliforms, 60-80 percent were Klebsiella. While fecal coliforms comprise a portion of the bacterial population released in some industrial effluents, there is still uncertainty in their origin (Dufour and Cabelli, 1976). The route of entry into the pulp and paper mill process may be through the raw water supply or through the forest products processed in the mill. There is no conclusive evidence, however, that fecal coliform positive Klebsiella are of recent fecal origin.

The number of bacteria entering the pulp and paper mill treatment ponds is much lower (less than 100/100 ml) than the number being discharged at the outfall (2.1×10^5/100 ml) (Knittel, 1975). Investigators believe that the bacteria grow during the treatment process because of the carbon and nitrogen source as well as the continuous oxygen supply in the aeration ponds. Klebsiella dominate the bacterial populations because of their ability to metabolize the complicated nutrient source. The complex sugars produced by the sulfite mill process elicit a higher growth and reproductive rate of Klebsiella than the simpler sugars produced in the kraft mill process.

The preponderance of environments in which Klebsiella has been isolated are high in organic matter concentration; e.g., polysaccharides in wood byproducts. Grabow (1970) surmised that Klebsiella survive and multiply in these environments because they have extracellular enzymes necessary to utilize complex carbohydrates.

Several workers (Huntley, et al., 1976; Duncan and Razell, 1972) have observed that Klebsiella accumulate and reproduce in the receiving waters of pulp and paper mill effluents. The carbohydrates released in the effluent provide an excellent nutrient source for the coliform bacteria. Depending on the physical, chemical, and biological conditions of the receiving water at the outfall, the bacteria may remain viable for an extended period of time or die-off quickly.

6.0 Survival and Reproduction. When 10^3 (kraft mills) to 10^6 (sulfite mills) Klebsiella per milliliter are released in the overflow waters of wood products mills, their survival rate and destination are dependent on the environmental conditions of the receiving waters. As bacteria are discharged from outfalls, a buildup occurs in receiving water. The populations diminish as they become farther removed from their food source (in both time and distance) (Hynes, 1971). If nutrients are continuously supplied, the organisms will continue to persist. According to Brock (1966), water is a route of dispersal rather than habitat for colonization for most microorganisms. Bacteria which are denser than water sink. Therefore, as they are released in the overflow from a mill, they will enter the receiving water and either disperse with the current or settle out into the sediments. Once settled in the sediments, they begin to accumulate for some unpredictable period of time and reproduce or die.

There are several factors controlling bacterial survival in a particular area. The most important factors are nutrients, temperature, salinity, competition with indigenous microflora, predation, water movement and sedimentation/adsorbtion.

6.1 Nutrients. Bacteria below an outfall discharging organically enriched wastes are assured of a continuous supply of nutrients. Organic pollution affords opportunities for massive developments of bacterial populations. Growth of Klebsilla in muds with high organic content was observed by Dufour and Cabelli (1976) below the outfall of a textile finishing plant.

The utilization of the food source by bacteria is altered by conditions of current movement, temperature, and salinity. Fast or high flowing streams can remove the nutrient source as rapidly as it is added. Deaner and Kerri (1969) surmised that the short travel time and low nutrient concentration, due to the shallowness and swiftness of the water, prevented bacterial population settlement and reproduction in the American River near Sacramento, California.

6.2 Temperature. The effect of temperature on bacterial survivability is a complex issue. Orlob (1956) stated in his review of bacteria that it is generally accepted that bacterial survival decreases as the temperature increases. He postulated that as the temperature increased bactericidal effects increased, resulting in bacterial die-off.

Seasonal studies of Klebsiella growth in pulp and paper mill waste treatment lagoons indicate that temperature affects the diversity of bacteria that will survive (Menon and Bedford, 1973). The total coliform population will grow equally well during all seasons. Fecal coliform positive Klebsiella and other fecal coliforms require higher temperatures (28° C or greater than 15° C) and, therefore, grow more rigorously during warmer seasons.

Laboratory studies (Naemura and Seidler, 1978) have identified three Klebsiella types based on low temperature growth: group I grows at 10° C and is fecal coliform negative, group II also grows at 10° C but differs from group I in it's response to biochemical tests, and group III is fecal coliform positive and will not grow at 10° C.

These recent data as well as earlier investigations indicate that coliform bacteria are able to grow and adapt to a wide range of environmental conditions. Fecal coliform bacteria, however, are more restricted; in particular, some species favor higher temperatures and a complex mixture of nutrients.

6.3 Salinity. General reviews of the viability of bacteria in seawater (Orlob, 1956) point out a wide disparity of views. Salinity (dissolved salts) has been thought of as a toxic barrier to nonhalophilic species. Ingram (1957) observed that E. coli adapt physiologically to salinity changes. He deduced that these salinity changes did not elicit any genetic changes. Recent evidence indicates that salinity (dissolved salts) is not detrimental to bacteria (Gerba and McLeod, 1976). In some cases, increases in dissolved salts may improve bacterial growth (Zobell, 1936). However, studies show that the abundance of bacteria decreases from inland fresh waters to coastal marine waters (Jones, 1971).

6.3.1 Bactericidal Agents. Laboratory studies on the effect of salinity on bacterial growth indicate that bacterial survival increases in heat sterilized water (Mitchell and Morris, 1969; Gerba and McLeod, 1976). Investigators deduced from these studies that a bactericidal agent which is heat sensitive is responsible for the die-off of bacteria in seawater. This heat labile agent may be a predator or microbial competitor (Jones, 1971).

6.3.2 Water Movement, Sedimentation, and Adsorption. Bacterial accumulation in the sediments has been implicated as an indirect interference in estimating impacts of salinity (Carlucci and Pramer, 1959). Where the current regime permits, the organisms settle out of the water column due to their density and persist in the sediment because of the accumulation of nutrients in the sediments. Vasconcelos et al. (1976) in their investigation in the Columbia River and Kruger (1978) in his study of Grays Harbor hypothesized this mechanism for the variability in bacterial counts. Kruger observed large quantities of coliform bacteria in the sediments of Grays Harbor (appendix D). Dudley, et al. (1977) found large quantities of fecal coliforms in the sediments of Long Island Sound. Ayres (1977) observed that fecal bacteria accumulate in areas around sewage disposal sites in Liverpool Bay, United Kingdom. Orlob (1956) observed that coliforms increased below the surface waste effluents in Port Gardner Bay at Everett, Washington.

The accumulation of bacteria in sediments is also affected by the sediment composition. Bacteria will adsorb to clays and mud more firmly than to sand or silt (Carlucci and Pramer, 1959; Ayres, 1977).

Another factor affecting bacterial survival is redistribution if bacteria are released back into the water column. If the sediments are primarily sand or silt, the bacteria are loosely bound and easily released into the water column. Grimes (1975) investigated the release of sediment bound fecal coliforms by dredging operations in the Mississippi River. He found a significant short-term increase in water column fecal coliforms caused by disturbance of sediments by the dredge. Redeposition of the bacteria depended upon bottom type, direction of channel, backwater currents, and dilution.

In conclusion, it is apparent that bacterial populations are reduced in seawater. The reasons for the decreases are probably a combination of several of the factors presented above.

7.0 Prediction of Klebsiella Impacts During the Grays Harbor Widening and Deepening Project. The information collected for this review indicates that Klebsiella is a diverse group of microorganisms. Only one of the numerous types of Klebsiella (fecal coliform positive, K. pneumonia sensu stricto) has been positively identified as a pathogen. If encountered in the environment, it may induce infections in predisposed individuals. The fact that Klebsiella is ubiquitous in the natural environment and yet no epidemic of Klebsiella infection has been reported implies that (1) only a few pathogenic Klebsiella are able to survive in the natural environment and/or (2) only a small portion of the human population is susceptible to Klebsiella induced infection, and/or (3) few members of the human population are exposed directly to the bacterium. Thus, Klebsiella is a low risk pathogen. However, it is a member of the fecal coliform group of bacteria which are considered to be indicators of sanitary water quality. The presence of large numbers of Klebsiella, because of its affinity for complex carbohydrates, should be considered as presumptive evidence of organic pollution.

The presence in Grays Harbor of fecal coliform bacteria such as Klebsiella has been previously investigated. Data collected by the Department of Social and Health Services (DSHS) and the Washington Department of Ecology (WDE) (Kruger, 1978) indicate that bacterial populations in the water of Grays Harbor have on occasion exceeded state water quality criteria (see appendix E). However, only limited information about bacterial content of Gray's Harbor sediments exists (appendix D). Additional information would be necessary to establish a simple temporal and spatial model similar to Hynes' (1971) diagrammatic representation of the physical, chemical, and biological changes downstream of a sewage outfall. These results, used in conjunction with existing water quality information, would allow an empirical determination to be made of the extent of bacterial contamination of sediments in Grays Harbor.

8.0 Conclusions. Based on this literature review it is clear that some industrial and municipal effluents in Grays Harbor provide a reservoir of bacteria, as well as an excellent nutrient source for bacterial survival and reproduction. The ability of bacteria to survive and reproduce will diminish with distance from the outfalls unless the bacteria

disperse into areas with the necessary environmental conditions for survival. The relatively low nutrient, cold, saline waters of Grays Harbor and the Pacific Ocean do not offer a favorable environment for the development of a large bacterial population. Thus, while the impacts of dredging and disposal on the redistribution of Klebsiella spp. in Grays Harbor are unknown: existing data suggest that the disturbance of sediment contaminated by Klebsiella should not represent a serious threat to human health due to several factors. These factors are: (1) the low chance of direct human contact with highly contaminated sediments; (2) the high probability of reduced growth rates and reduced survival of Klebsiella in low nutrient, cold, saline water; and (3) low probability of encountering one of the few Klebsiella which are pathogenic.

Due to the low probability for bacterial contamination of the sediments, further studies are not deemed necessary at this time. However, any future bacteriological surveys of Grays Harbor should include sediment analysis as well as water column analysis.

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APPENDIX A

FECAL COLIFORM BACTERIA

1. Federal Criteria.[1/]

a. Bathing Waters. Based on a minimum of five samples taken over a 30-day period, the fecal coliform bacterial level should not exceed a log mean of 200 per 100 ml, nor should more than 10 percent of the total samples taken during any 30-day period exceed 400 per 100 ml.

b. Shellfish Harvesting Waters. The median fecal coliform bacterial concentration should not exceed 14 most probable number (MPN) per 100 ml with not more than 10 percent of samples exceeding 43 MPN per 100 ml for the taking of shellfish.

2. State Criteria.[2/]

a. Class A (Excellent).

(1) Water Quality Criteria.

(a) Fecal Coliform Organisms.

o Freshwater - Fecal coliform organisms shall not exceed a median value of 100 organisms/100 ml, with not more than 10 percent of samples exceeding 200 organisms/100 ml.

o Marine water - Fecal coliform organisms shall not exceed a median value of 14 organisms/100 ml, with not more than 10 percent of samples exceeding 43 organisms/100 ml.

b. Class B (Good).

(1) Water Quality Criteria.

(a) Fecal Coliform Organisms.

o Freshwater - Fecal coliform organisms shall not exceed a median value of 200 organisms/100 ml, with not more than 10 percent of samples exceeding 400 organisms/100 ml.

o Marine water - Fecal coliform organisms shall not exceed a median value of 100 organisms/100 ml, with not more than 10 percent of samples exceeding 200 organisms/100 ml.

1/Excerpted from EPA "Redbook," 1976.
2/Excerpted from State of Washington, Department of Ecology, Washington Administration Code, Chapter 173-201.

APPENDIX B

THE TRIBES AND GENERA OF THE FAMILY ENTEROBACTERIACEAE[1/]

Tribe	Genera
Escherichieae	*Escherichia* (includes *Alkalescens-Dispar*)
	Shigella
Edwardsielleae	*Edwardsiella*
Salmonelleae	*Salmonella*
	Arizona
	Citrobacter (includes *Bethesda-Ballerup* and *Escherichia Freundii*)
Klebsielleae	*Klebsiella*
	Enterobacter (formerly *Aerobacter*)
	Serratia
	Pectobacterium
Proteeae	*Proteus*
	Providencia

1/Excerpted from National Council of the Paper Industry for Air and Stream Improvement, Stream Improvement Bulletin No. 254, 1972.

APPENDIX C
OCCURRENCE OF KLEBSIELLA PNEUMONIAE IN LOTIC AND LENTIC WATER SAMPLES[1/]

Source	Coliforms/100 ml Total	Coliforms/100 ml Fecal	Fecal Streptococci/ 100 ml	FC/FS[2/] ratio	Enterobacteriacea Isolated[3/]
Alsea River[4/]	108	34	29	1.2	E. coli
Mary's River	14	4	6	0.7	E. coli, E. aerogenes
Unknown Creek	192	117	65	1.8	E. coli
Rock Creek	128	16	30	0.5	E. coli, K. pneumoniae
Alder Creek	26	1	3	ND[5/]	Unknown coliforms
Wiley Creek	14	1	1	ND	Citrobacter, E. cloacae
Wiley Creek[6/]	TNTC[7/]	130	21	6.1	E. coli, K. pneumoniae
Mary's River	200	30	1	ND	E. coli, Citrobacter E. cloacae
Ritner Creek	315	18	1	ND	E. coli, Citrobacter
Unknown Creek	22	6	1	ND	E. coli, Proteus, Arizona
Unknown Creek	52	16	1	ND	E. coli, Citrobacter
Luckiamute River	38	11	5	2.2	E. coli, Citrobacter
Ditch, Agricultural[8/]	228	24	15	2.3	E. coli, K. pneumoniae, E. aerogenes, Proteus
Cowlitz River	30	2	1	ND	E. coli, K. pneumoniae, Enterobacter
Snoqualmie River	120	17	1	ND	K. pneumoniae
Naches River	18	2	1	ND	E. coli
Yakima River	32	1	1	ND	E. coli,
Palouse River	390	230	ND	ND	E. coli, K. pneumoniae, Citrobacter, Providenciae, E. cloacae
Calapoola River	4	2	ND	ND	E. coli, Citrobacter
China Lake	32	2	4	0.5	E. coli, Unknown coliforms
Cape Lake	28	8	1	ND	E. coli

1/Excerpted from Knittel, 1975.

2/FC = Fecal coliform; FS = Fecal Streptococci.

3/Species of coliforms were isolated and identified from colonies appearing on M-ENDO LES agar.

4/Streams and rivers flowing through populated or agricultural areas. All other sources from areas of limited access or minimal human activity.

5/ND = Not determined.

6/Sampling point below a housing development, foam on water surface.

7/TNTC = Too numerous to count.

8/Water draining off a field.

APPENDIX D

GEOMETRIC MEAN OF BACTERIAL DENSITIES PER 100 GRAMS OF SEDIMENT TAKEN FROM GRAYS HARBOR

	Number of Samples	Fecal Coliform	Total Coliform
Chehalis River and North Channel	5	4,481	800,000
Wishkah River	3	6,782	800,000
Hoquiam River	3	2,630	439,061

Excerpted from Kruger, 1978.

APPENDIX E

TABLE 1

BACTERIOLOGICAL SURVEY OF GRAYS HARBOR
BY WASHINGTON DEPARTMENT OF ECOLOGY DURING 1976-1978[1]/
MOST PROBABLE NUMBER PER 100 MILLILITERS (MPN/100 ml)

	Jul 76		Sep 76		Oct 76		Jan 77		Feb 77		Mar 77	
	TC[2]/	FC[3]/	TC[2]/	FC[3]/	TC[2]/	FC[3]/	TC[2]/	FC[3]/	TC[2]/	FC[3]/	TC[2]/	FC[3]/
Cosmopolis STP					120	17	600	10				
Aberdeen STP		104		20	28	20	100	10		239		140
Hoquiam STP		20		20	1,248	21	100,000	20		20		20
Weyerhaeuser Pond D	4	4	195,000	60,000	40	1	4,750,000	1,600	500,000	45,000	20	3
ITT Rayonier 004	81,000	4,000	17,000	4,000	7,000	7	15	2	4	1,000	4	1,000
007							50	1,000	1,000	1,000	1,000	1,000
003	10,000	1,000	7,000,000	10	1,700,000	10	31,000,000	160	50,000	1,000	760,000	1,000
Chehalis River							7,000	30				
Cosmopolis					260	20						
Aberdeen					160	8	4,400	5				
Light Buoy No. 3	1,600	80[4]/			130	5	6,300	40				
Wishkah River					420	36[4]/	7,600	100[4]/				
Chehalis River Mouth	1,600	50[4]/			400	24	3,000	60[4]/				
CH[5]/ at East End of Pennie Island					1,400	80[4]/	13,000	64[4]/				
West End of Port of GH[5]/	4,000	6			2,000	40[4]/	1,400	5				
Hoquiam					5,500	36[4]/	190,000	15,000[4]/	8,000[4]/	250		
Moon Island Beach							1,700	10				
Mid-Channel South							3,000	5				

1/Data provided by Kruger, 1978.
2/TC = Total coliform.
3/FC = Fecal coliform.
4/Exceeds FC standard.
5/GH = Grays Harbor.

TABLE 2

BACTERIOLOGICAL SURVEY OF GRAYS HARBOR[1]
20-28 April 1976
Bacteria (MPN/100 ml) - Confirmed

Station	Coliforms		E. coli	Klebsiella
	Total	Fecal		
Water in oyster bed sites	350-16,000	2-1,300[2]	2-1,300	2-40
Oysters	APC organisms/gm	100-20,000		
Weyerhaeuser Pond D	1,100,000-9,200,000	11,000-170,000	11,000-110,000	2-7,400
Weyerhaeuser	3,300-350,000	17-24,000	12-24,000	5-2,200
Weyerhaeuser Slough	130-70,000	170-54,000	170-790	2-54,000
Cosmopolis STP	7-17,000	2-17	2-17	5-7
Aberdeen STP	2-700	2-23	2-13	5
Hoquiam STP	13-1,700	2-490[2]	2-490	2-11
Mid-Channel Stations 12, 22, 18	5-5,400	2-70[2]	2-70	2-5
East Channel Stations H5, S1	170- 1,600	2-130[2]	2-79	2-13
South Channel 31, 32, 33	2- 1,600	2-13	2-13	2-5

1/Data provided by Department of Social and Health Services, 1976.
2/Exceeds State of Washington Fecal Coliform Standard.


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