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Original Research Paper

Growth Response of *Salmonella* Species and *E. coli* to Different Metal Ions

N. S. Kulkarni, D. D. Walke, R. R. Pachori and Shiva Aithal*
Department of Microbiology, R. A. College, Washim-444 505, Maharashtra, India
*Department of Microbiology, D. S. M. College, Parbhani-431 401, Maharashtra, India

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ABSTRACT

Effect of different metal ions with their graded concentration on growth response of indicator organisms for faecal contamination and human pathogens of enteric fever was studied with individual and combined culture cultivation techniques. Both the test organisms were inhibited by almost all the metal ions analysed. However, it was surprisingly observed that the inhibitory effect by most of the metal ions was more significant against *E. coli* as compared to *Salmonella* species which indicated the possibility of confusion with respect to indicator organisms in monitoring the microbiological characteristics of water.

INTRODUCTION

The biological characteristics of wastewater are of fundamental importance in control of diseases caused by pathogenic organisms of human origin. Hence, it is necessary to keep continuous monitoring of human pathogens. However, the number of pathogens present in polluted water is usually few and difficult to isolate and identify. Therefore, use of an indicator organism viz., *E. coli*, faecal Streptococci, Enteroccoci or *P. aeurogenosa* etc. has been considered for the presence of human pathogens (Metcalf & Eddy 2006). Maier et al. (2000) suggested that the indicator organism must be present when the target pathogen or if faecal contamination is present. It has been reported that the absence of indicator organisms is taken as an indication that the water is free from disease producing organisms. However, it has been generally observed that most of the human pathogens have considerable resistance to chemical constituents in water or polluted water as compared to indicator organisms (Madigam et al. 2000) which may lead to confusion in biological water analysis. Hence, in the present investigation, comparative growth response of *Salmonella* species and *E. coli* as pathogen and indicator organism respectively, against metallic constituents in water has been studied.

MATERIALS AND METHODS

The autochthonous bacterial cultures of sewage viz., *Salmonella* species and *E. coli* were isolated and identified adopting standard biochemical and cultural methods (Bergey's Manual of Determinative Bacteriology 1994). The isolates were finally sub-cultured and maintained on nutrient agar slants. The inoculums were separately prepared by inoculating loop-full of both the cultures in 5mL sterile nutrient broth and kept for enrichment at 37°C for 6 hrs till a moderate turbidity was developed. The turbidity was matched with 0.5 Macfarland standard (Macfarland, 1993) with normal saline which corresponds to cell density of approximately 15000 CFU/mL. The inoculum was further used to inoculate in metal fortified nutrient broth.

Table 1: Effect of different metal ions on individual growth response of *Salmonella* and *E. coli.*

Metal salts conc. in the medium, mg/L	*Salmonella* CFU × 10^3/mL	*E. coli* CFU × 10^3/mL
Control	285	298
$CaCl_2$		
0.1	272	280
0.5	268	259
1.0	218	167
$NaNO_3$		
0.1	258	251
0.45	228	194
0.8	186	105
$FeSO_4$		
0.1	256	245
0.4	214	163
0.8	148	35
$MgCl_2$		
0.01	172	77
0.15	156	46
0.5	132	30
$(CH_3COO)_2Pb$		
0.015	286	268
0.3	224	183
0.6	182	101
$MnSO_4$		
0.005	188	111
0.05	136	129
0.1	132	32
$ZnSO_4$		
0.005	188	114
0.01	244	223
0.002	132	42
$CuSO_4$		
0.05	268	270
0.01	246	226
0.002	218	173

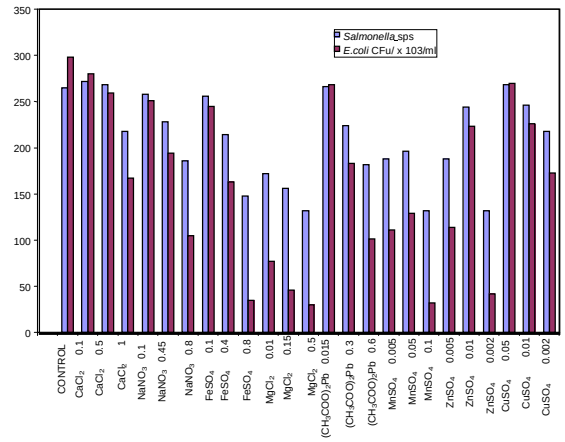

Fig. 1: Effect of different metal ions on individual growth response of *E. coli* and *Salmonella.*

Stock solution of metal salt at the rate of 100mg/mL was prepared in sterile nutrient broth and further diluted to desired concentration (APHA 1989). Among the metal salts solution total three concentration levels were used viz., lower, middle and highest, which were prepared with respect to the standard permissible limit (APHA 1985). Both the cultures were inoculated at the rate of 1 mL (15×10^3 CFU/mL) separately as well as in combinations to metal salts fortified nutrient broth and incubated at 37°C for 24 hours. The controls without metal solution for both the organisms were run using plain nutrient broth. The growth response was studied after exposure time of 24 hours. The experiment was carried out with three replicates of each concentration.

RESULTS AND DISCUSSION

The observations and results given in Table 1 and graphically represented in Fig. 1 indicate that, there was an inhibitory action of metal ions on both the test organisms. It may be due to inhibition of assimilatory process followed by cell division. The results are in agreement with experimental finding of Kandasamy et al. (1975) who reported adverse effect by high concentration of certain inorganic compounds on microbial load. However, their work was associated with

Table 2: Effect of different metal ions on individual growth response of *Salmonella* and *E. coli.*

Metal salts conc. in the medium, mg/L	*Salmonella* CFU × 10^3/mL	*E. coli* CFU × 10^3/mL
Control	276	263
$CaCl_2$		
0.1	282	275
0.5	276	270
1.0	225	260
$NaNO_3$		
0.1	278	270
0.45	248	189
0.8	286	98
$FeSO_4$		
0.1	255	248
0.4	218	155
0.8	198	31
$MgCl_2$		
0.01	182	67
0.15	166	32
0.5	152	30
$(CH_3COO)_2Pb$		
0.015	250	254
0.3	214	178
0.6	144	109
$MnSO_4$		
0.005	178	98
0.05	195	32
0.1	130	32
$ZnSO_4$		
0.005	189	109
0.01	201	34
0.002	130	30
$CuSO_4$		
0.05	278	260
0.01	266	216
0.002	238	170

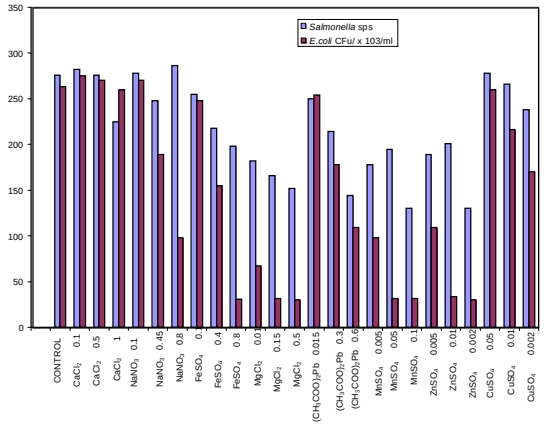

Fig. 2: Effect of different metal ion on combinational growth response of *E. coli* and *Salmonella.*

soil ecosystem. The maximum inhibitory effect on both the microorganisms cultivated separately was observed in presence of $ZnSO_4$ followed by $HgCl_2$, $NaNO_3$ and $MnSO_4$ over control which indicates that inhibitory effects of these metals was more on *E. coli* as compared to *Salmonella*. Similarly, the results found when both the organisms cultivated in combination (Table 2 and Fig. 2) indicate the inhibitory action of almost all the metal ions analysed excluding $CaCl_2$, $NaNO_3$ and $CuSO_4$ at lower concentration limits. It was surprisingly observed that the inhibitory effect of $NaNO_3$, $FeSO_4$, $MgCl_2$, $MnSO_4$ and $ZnSO_4$ was more significant against *E. coli* as compared to *Salmonella* which indicate the high possibility for disappearance of indicator organisms due to presence of metals as compared to human pathogens in aquatic ecosystems. The inhibitory action of metals under studies was less against *Salmonella.* It may be due to the plasmid confined resistance in S*almonella* whereas *E. coli* may not have such expression activity. However, such experimental findings can not be ignored since, it has been considered by almost all microbiologists that the absence of indicator organisms taken as an indication that the water is free from disease producing organisms (Metcalf & Eddy 2006). However, the inhibitory effect of metals present in aquatic ecosystems may

confuse the studies on biological characteristics of water in controlling waterborne diseases due to the variation in the existing status of indicator microorganisms. The metal induced variations in morphological changes of some microorganisms have also been reported by Paolo et al. (1992) and Nasreen (1993). However, their studies were on *Tetrahymena pyriformis*.

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