

RRST-Marine Biology

Influence of Chloride Ion on the Toxicity of Heavy Metal Mercury upon *Vibrio parahaemolyticus*

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Article Info	Abstract
Article History <i>Received</i> : 05-07-2011 <i>Revised</i> : 06-09-2011 <i>Accepted</i> : 11-09-2011	Heavy metal pollutants released into the coastal region certainly influence the bacterial population leading to the formation of metal resistant forms. The toxicity of mercury to different species of bacteria also vary. Among bacteria <i>Vibrio parahaemolyticus</i> play an important role in the disease of marine animals and human beings as pathogens. Hence the present study was aimed to understand the effect of mercury on the survival of two <i>V. parahaemolyticus</i> strains isolated from polluted uppanar estuary at different concentrations. (0, 1, 10, 25, 50 and 100 ppm) in the absence and presence of NaCl (1.75 and 3.5%) was carried out. Toxicity of mercury showed that <i>V. parahaemolyticus</i> gradually decreased upto 1 ppm mercury and then there was a decline at higher concentration of mercury.
*Corresponding Author <i>Cell</i> : +91-9442781999 <i>Tel</i> : +91-4427528075 <i>Email:</i> premalatha.niranjana@gmail.com	Key Words: Pollution, Heavy metal, <i>Vibrio</i> sp. Chloride ion

Introduction

Metal toxicities have received widespread attention because of increasing number of toxic metals being released into the environment, their extended persistence and toxicity to a wide variety of organisms. Haphazard rapid urbanization, industrial and waste generation along coastal region have reduced many water resources unwholesome and hazardous to living organisms, in particular to microorganisms. Heavy metals such as mercury and lead have industrial uses and have been found to be widely distributed in the marine and estuarine environments [1,2]. Microorganisms, owing to their large surface to volume ratio and high metabolic activity are important vectors in introducing heavy metal pollutants into the food web. Studies of their interaction have focused especially on conversion of mercuric ions in soil, marine and in fresh water sediments.

Considering the interaction of microorganisms and metals, particularly heavy metals, two effects can be demonstrated: that of the metal or ion on microorganisms and their activities on the metal or ion. In polluted areas receiving heavy metals, the number of heavy metal resistant forms increases or decreases according to the type of heavy metals. The toxicity to particular pollutants by the microbiota is also dependent on the physico-chemical characteristics of the environment in which the pollutants are deposited (salinity, etc.) and due to these heavy metals, microorganisms undergo physiological, morphological and genetic changes, which leads to the modification of these microorganisms to metal resistant forms. However, study about the tolerance or resistance of these bacteria to heavy metals with and without chloride ions is scanty. Hence the present study was aimed to understand the toxicity of heavy metal mercury upon *Vibrio parahaemolyticus*. The metal mercury was selected because they are used in vast

quantities, which poses a potential pollution problem. This impact on microorganisms and the effect on microbial activity on the metal or ion is much fascinating.

Materials and Methods

The method outlined by Babich and Stotzky [3] was adopted with slight modification for the present experiment. Two *Vibrio parahaemolyticus* strains (Vp no.37 and Vp no.68) isolated from water and sediment samples of uppanar estuary were selected and grown at 37° C in Brain Heart Infusion (BHI) broth for 24 h. The cells were then washed twice by centrifugation with solution containing 2.92% of NaCl and 0.45% of KCl in distilled water (pH 8) and resuspended in the same solution to yield a viable cell concentration of 10⁵ to 10⁶ cells/mL. Portions (0.1 mL) of bacterial suspensions were inoculated into screw cap tubes (13x100 mm) containing 4 mL of Sorenson Na₂HPO₄ – KH₂PO₄ buffer, at pH 8 in triplicate. The tubes were unamended and amended with different concentrations of mercury as mercuric chloride without or with NaCl (1.75% and 3.5%). All the tubes were agitated for 5 seconds by keeping on the Vortex Genie and incubated at 37° C for 4 h. Portions (0.5 mL) of the sample removed are serially diluted in Sorenson Buffer (pH 8) containing 0.56 M NaCl. One mL aliquots were taken from all the dilutions and inoculated into sterile petriplates, to which, approximately 15 mL of tryptic soy agar (TSA) was poured and mixed thoroughly. Plates in triplicate were incubated in an inverted position at 37° C and bacterial colonies were counted after 24 or 48 h.

Results and Discussions

The effect of mercury on the survival of *Vibrio parahaemolyticus* (Vp 37 and Vp 68) at different concentrations (0, 1, 10, 25, 50, 100 ppm) in the absence and

presence of NaCl (1.75 and 3.5%) was distinctly established in the present experiment (Fig 2 & 3). The survival of *Vibrio parahaemolyticus* gradually decreased upto 1 ppm mercury. Then there was a decline at higher concentration of Hg. Marked differences were noticed in the presence of NaCl, which increased the number of bacterial cells. When the toxicities of equivalent concentrations of Hg as Hg^{2+} was always found to be highly toxic to *Vibrio parahaemolyticus* than anionic complexes.

It is evident that both strains (Vp 37 and Vp 68) showed tolerance to heavy and metal mercury. Generally, the bacteria survived in mercury better in the presence of NaCl than in its absence, indicating greater of divalent Hg^{2+} than of $HgCl_3^-$ / $HgCl_4^-$. This is in accordance with the findings of Babich and Stotzky [3]. The toxicity of mercury to microorganisms in estuarine environment is influenced by many physico-chemical characteristics like chloride ion concentration and pH. Horsfall [4] investigated that pH increases the toxicity of mercury to microbes. But here the concentration of chloride ion played the key role in the toxicity of mercury to *Vibrio parahaemolyticus*. A decrease in the survival of the *Vibrio parahaemolyticus* was noticed in the 0% concentration of NaCl suspensions containing 1 ppm to 100 ppm of Hg, than in the 1.75% and 3.5% NaCl suspensions containing the same concentrations of Hg.

As suggested by Babich and Stotzky [3] the greater toxicity of cationic Hg^{2+} is due to their better binding ability to negatively charged surfaces, especially the bacterial cells. Studies of Krenkel [5] showed the better sorption capacity of Hg to negatively charged sand or clay particles. At the same time absorption of Hg to these particles decreased in the presence of higher concentration of Cl, yielding the species $HgCl_3^-$ and $HgCl_4^-$. Thus, the toxicity of Hg to bacteria is influenced by many abiotic factors, including the Cl concentration. Effluent discharge of inorganic Hg containing compounds into fresh waters or non-saline soils may evoke different responses from the indigenous microbiota than deposition of similar quantities of Hg into seawaters. This may be the reason that inspite of industrial discharge containing various heavy metals into seawater of this estuary still this type pathogens survive very well in this environment. If the level of heavy metals like mercury is low, there is no adverse effect. But when it exceeds it becomes toxic and leads to the accumulation of these metals by bacteria in general and *Vibrio parahaemolyticus* in particular and produce resistant forms with undesirable characters, which may create public health problems. So, it is essential to study about the interaction of microorganisms and heavy metals in response with physico-chemical character like chloride ion concentration.

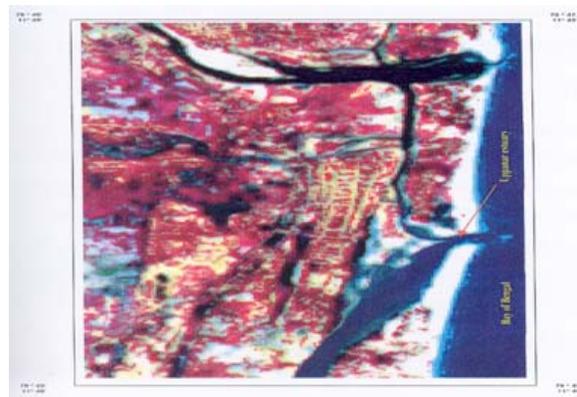


Fig1 Map showing uppanar estuary

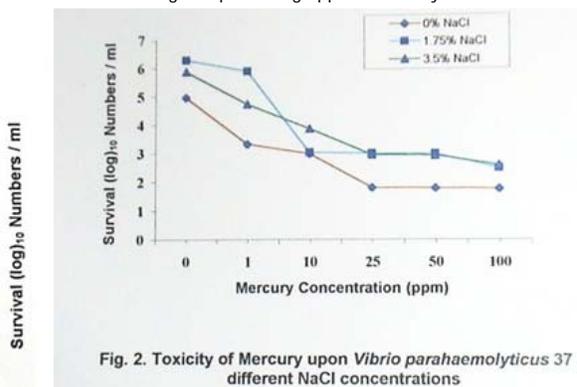
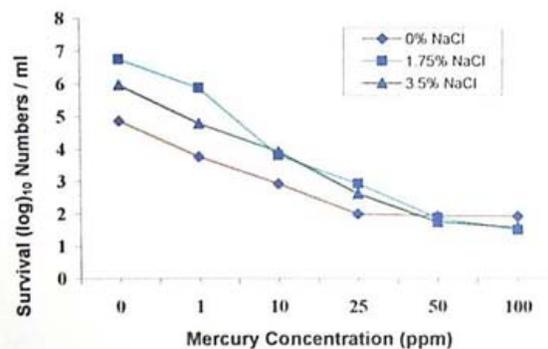


Fig. 2. Toxicity of Mercury upon *Vibrio parahaemolyticus* 37 at different NaCl concentrations



References

- [1] Sanzgiry, S., A. Mesquita and T.W. Kureishy, 1988. Total mercury in water, sediments and organisms along the Indian coast. Mar. Poll. Bull., 19:339-43.
- [2] George, M.D., 1988. Distribution of liable and non-liable forms of Cd, Pb and Cu in Lakshwadeep Lagoon Waters, Indian J. Mar. Sci., 17: 111-13.
- [3] Babich, H. and G.Stotzky, 1979. Differential toxicities of mercury to bacteria and bacteriophages in sea and in lake water. Can. J. Microbiol. 25: 1251-1257.
- [4] Horsfall, J.G., 1956. Principles of fungicidal action, Chromica, Botanica, Co. Waltham, M.A.
- [5] Krenkel, P.A., 1974. Mercury environmental considerations. Part II, CRC. Crit. Rev. Environ. Contr., 14:252-329.