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**Journal of Chemical and Pharmaceutical Research, 2012, 4(12):4975-4978**

**Research Article**

**ISSN : 0975-7384**
**CODEN(USA) : JCPRC5**

# Study on the effect of mercury (II) chloride as disinfectant on mixed culture

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**ABSTRACT**

*Mercuric chloride is very commonly used as disinfectant in biological laboratory. The study was performed to evaluate the effect of mercuric chloride at different concentration on the mixed culture. Contact time was considered as influence parameter, and spread plating-plate counting was used as numeration method for bacteria concentration. The result showed that as time and concentration of disinfectant increased, the growth of culture decreased, which revealed its sterilization efficiency. The disposal of used mercuric chloride from the laboratory cause environmental hazard, thus the proper measure should be taken before its discard.*

**Keywords:** Disinfectant; Mercuric chloride; Mixed culture; Microbial growth.

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## INTRODUCTION

Microbes are common cause for various diseases. Sterilization is one of the reliable means to control the pathogenic effect of microbes. Disinfection is a sterilization process which makes an object free from viable organisms. A disinfectant is defined as a chemical that kills or destroys nearly all disease-producing microorganisms, with the exception of bacterial spores; this term refers to agents used on inanimate objects [1]. Disinfectants can act on microorganisms in two different ways: growth inhibition (bacteriostasis, fungistasis) or lethal action (bactericidal, fungicidal or virucidal effects). Only the lethal effects are of interest in disinfection and, as the objects of treatment have no inherent means of defence, lethality is the desired objective [2]. Disinfectants are acting on bacterial wall [3], cytoplasmic membrane [4], energy metabolism [5], bacterial spores [6] etc. There are different types of disinfectant are used, physical and chemical. In case of heat sensitive objects or explants, chemical disinfectants are preferred than the physical one.

Mercuric chloride ($HgCl_2$) is a wide range of disinfectant. Mercury is an extremely hazardous chemical element because of its volatility in the metal state and ability to form numerous toxic volatile organic compounds under the action of bacteria present in aquatic ecosystems [7]. Chlorine is electronegative, therefore the chloride compound oxidizes the peptide linkages, thus denatures the protein of microbes [8, 9]. As it is having strong sterilization efficiency, mercuric chloride is most commonly used in laboratory to kill the microbes on the explants. This disinfectant is toxic not only for microbes as well as other superior organisms. It may be fatal if swallowed, causes severe irritation to eyes, skin and respiratory tract, causes allergic skin reaction, affects kidney and central nervous system, induces birth hazards also. If the used mercuric chloride was discarded from the laboratory, it will cause adverse effects in the environment. Accumulation of trace metals, especially heavy metals, like mercury, in the soil has potential to restrict the soil's function, cause toxicity to plants and contaminated the food chain [10]. Thus when the disinfectants are used, they need to be used as directed in order for them to be effective [11] and eco-friendly.

The present study reported that the influence of mercury (II) chloride on mixed culture and was described its sterilization efficiency on the basis of numbers of bacterium. At the same time different alternative approaches were found which will overcome the drawbacks of the mercuric chloride usage in the laboratory.

## EXPERIMENTAL SECTION

### Isolation of microorganisms

Five soil samples were collected from different area where the population of microbes will be maximum such as rhizosphere soil, drainage soil sample, industrial polluted soil etc. and marked as S1, S2, S3, S4 and S5. 1 gm of each of the soil sample was mixed with 1 ml of sterile distilled water. Vigorous shaking was done and all the samples were incubated for 10 minutes. Before the experiment, in order to determine the initial cell concentration, for the culture, spread plating [12] was performed on dilutions $10^{-4}$ on sterile nutrient agar plates. After that, all the plates were incubated for 24h at 37$^{o}$C and counted the colonies for numeration. With each soil sample one control plate was made.

### Preparation of disinfectants and culture media

To determine the disinfectant efficiency, four different concentration of mercuric chloride (0.01-0.04%) were prepared. The culture media for the selected mixed culture was prepared using 1% of nutrient broth (Hi-Media).The media was autoclaved to sterilize before use. The selected culture (S4) was inoculated in the sterilized nutrient broth and incubated for 24h at 37$^{o}$C in shaker incubator.

### Action of disinfectants

The action of mercuric chloride was performed using four different concentration of mercuric chloride as mentioned above. Six different contact times such as 5min, 10min, 15min, 20min, 25min and 30min were tested. For each concentration of mercuric chloride, 0.1ml of culture solution was added into 0.9ml of disinfectant and incubated for consecutive duration. After those contact time, the broths were centrifuged at 8000rpm for 5min to separate the culture from the solution. Supernatant was discarded and then the tube was refilled by deionized water and spread plating was performed for individual concentration and time. The plate counting was done on each spread plate after 24h culturing at 37°C [13]. For each set of concentration, one control plate was prepared.

## RESULTS AND DISCUSSION

Among the all the five soil samples, S4 had the maximum bacterial concentration, so that this samples was used for further purpose. Table 1 shows that the initial concentration of all the collected sample.

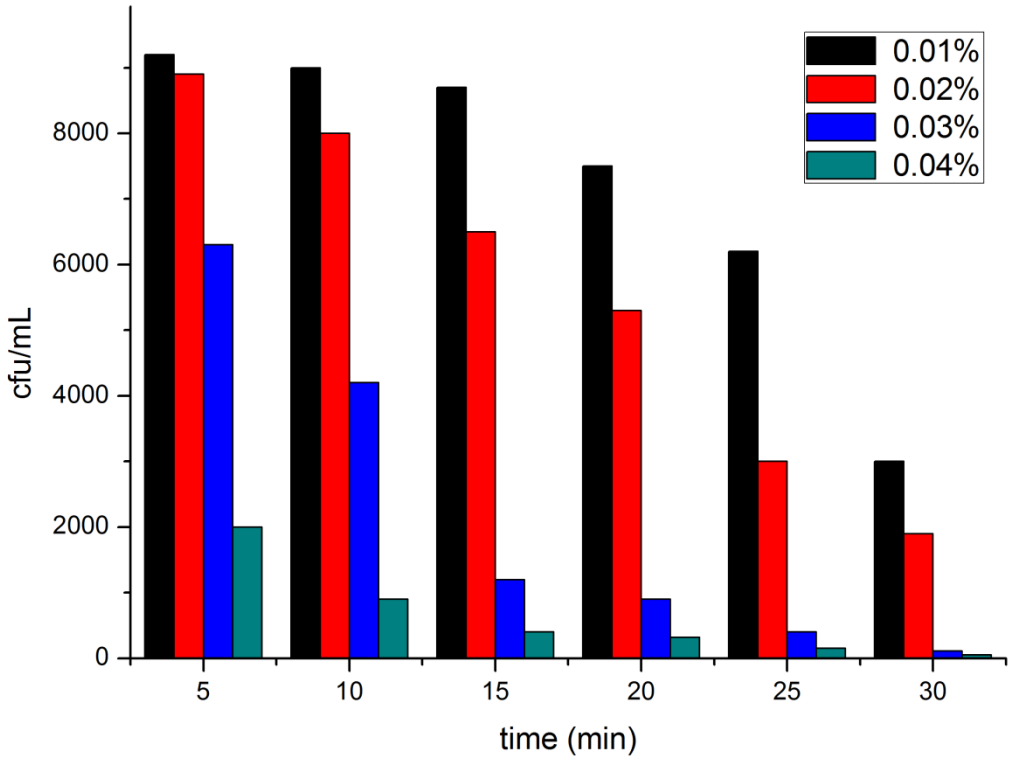


**Fig. 1 Effect of mercuric chloride of different concentration on mixed culture at various time interval**

**Table. 1 Initial concentration of mixed culture**

| Sample | Initial concentration (cfu/mL) |
|---|---|
| S1 | $2.4\times10^6$ |
| S2 | $3.5\times10^6$ |
| S3 | $3.4\times10^6$ |
| S4 | $6.3\times10^6$ |
| S5 | $4.4\times10^6$ |

Figure 1 explain that the growth of mixed culture after application of mercuric chloride of 0.01%. The CFU/ml of the culture also decreased after addition of $HgCl_2$ (0.02%, 0.03% & 0.04%) at different time interval. It indicates that as the concentration and time have been increased, the bacterial growth almost inhibited.

Heavy metals presence in the atmosphere, soil, and water even in trace concentrations can cause serious problem to all living being [14]. Heavy metals are important for environmental pollution and there is a problem of increasing significance for ecological, evolutionary, and environmental reasons [15]. The increasing influx of heavy metals into the bodies from industrial, agriculture, and domestic activities is of global concern because of their well document negative effects on human and ecosystem [16]. Heavy metal contamination affects the biosphere in many places worldwide [17, 18, 19]. Heavy metal pollutants are a major problem in aquatic environment because of their toxicity, their persistency and tendency to accumulate in organisms and undergo food chain amplification [20].Mercury is one of the common heavy metal which can exist in several form and all the forms are causing toxic effects. Mercuric chloride is used as disinfectant to remove the surface microorganisms. The result showed that the increasing concentration of mercuric chloride inhibit the growth of bacteria efficiently with increasing contact time. Thus discarding of this disinfectant in the soil or water cause soil and water pollution. This metal contamination induces the accumulation of mercury in the different parts of the plant and reduces the concentration of viable, plant-associated microbes which causes the adverse effect on soil character, plant growth and environment. Discarding in the water also affects the aquatic environment. Thus there should be the alternative approaches to dispose the mercuric chloride in the environment after it uses. Special discarding jar, paper cloth which is having the absorbance capacity can be used. The container and the material must be disposed of as hazardous waste according to Special Waste Regulations.

## CONCLUSION

The object of this study is highlighted on the efficiency of the mercuric chloride on the microorganisms. From the result it can be concluded that this disinfectant is having strong bactericidal effect which can be used for different laboratory. But the using protocol should be with proper guidelines. The used mercuric chloride can be discarded by various means like discard jar, paper towels so that it will not cause any harm to the environment. Further research is required to enumerate bacterial growth mechanism after using the mercuric chloride.

## REFERENCES

[1]M M Baddour. *Saudi Pharm. J.*, **2008**, 16(2), 165-170.
[2] P Maris. *Rev. sci. tech. Off. int. Epiz.*, **1995**,14 (1), 47-55.
[3] Centre National D'Etudes Veterinaires et Alimentaires (CNEVA), Fougères, **1989**, 235.
[4]A Dauphin; JC Darbord. Hygiène hospitalière pratique, 2nd Ed., **1988**, 715.
[5] H Guellouzh. Thesis No. 304. National Veterinary School, Tunis, **1987**, 198.
[6] AD Russell. Principles of antimicrobial activity. 3rd Ed. Lea & Febiger, Philadelphia, **1983**, 717-745.
[7] DN Ostrovskii; EI Lysak; GP Demina; VI Binyukov. *Microbiology*, **2000**, 69, 516–523.
[8] WC Barrette; DM Hannum; WD Wheeler; JK Hurst. *Biochemistry*, **1989**, 28, 9172-9178.
[9] JD Berg; PV Roberts; A Matin. *J. appl. Bacteriol.*, **1986**, 60, 213-220.
[10]K Jaya Kumar; MZC Xing; M Azzoz; CA Jaleel. *Plant Omics.*, **2009**, 2(3), 120-126.
[11]BM Layton. *Saint Martin's University Biology Journal*, **2006**, 1, 95-103.
[12] Adam Driks. *Trends Microbiol.*, **2002**, 10(6), 251-254.
[13]Comparison study on disinfectant efficiency of ethanol, bleach and anti-bacterial hand soap against E.coli and mixed culture CE 773.
[14]CH Chandra sekhar; D Sammaiah; T Shasthree; K Jagan mohan reddy. *Int. J. Pharm. Bio. Sci.*, **2011**, 2( 2), 358-364.
[15]PC Nagajyoti; N Dinakar; TNVKV Prasad; C Suresh; T Damodharam. *J.Appl.Sci. Res.*, **2008**, 4(1), 111-121.
[16]LM Metaka; EMT Henry; WRL Masamba; SM Sajidu. *Int.J. Environ.Sci. Technol.*, **2006**, 3(2), 131-139.
[17]SD Cunningham; JR Shan; DE Crowley; TA Anderson. *American chemical Society, Washington*. **1997**, 2-19.
[18]RB Meaghar. *Curr. Opin. Plant Biol.*, **2000**, 3, 153-162.

[19]I Raskin; BD Ensley. *John Wiley and Sons N. York,* **2000**, 303-306.
[20] V K. Mukke. *J. Chem. Pharm. Res.*, **2012**, 4(1), 398-401.