iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 6 **Issue:** II **Month of publication:** February 2018

DOI:

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
Volume 6 Issue II, February 2018- Available at www.ijraset.com

Action of Commonly used Hand Sanitizers on Bacteria Isolated and Characterized from a Polluted Water Source Existing in School Premises

Ramya R Prabhu[1], Ansila B[2], Aswathy Rajendran3, Merin Johny[4], Viya Valsan[5]
[1, 2, 3, 4, 5]*Department of Biotechnology Government Arts College, Thycaud Thiruvananthapuram, Kerala, India, 695014*

Abstract: ***Fresh water is vital to sustenance of life and it is important that places of public interaction like educational institutions, hospitals, community living areas have water bodies or water sources free of contamination. Mostly water bodies are contaminated due to careless human activities or lack of well maintenance. The present study is conducted on such an ill maintained pond in a prominent high school present in the heart of Trivandrum city close to the college in which this study was conducted. The water quality was analysed for the presence of coliforms which indicated contamination. The various bacteria present in the water sample were isolated and characterised by biochemical analysis. The anti-microbial action of commonly available hand sanitizers was studied for their effect on such bacteria present in contaminated water source.***
Keywords: water quality, coliforms, sanitizers

I. INTRODUCTION

Water is an indispensable element for life and essential for metabolic activities of all living beings on earth. Human activities have contaminated our water sources; making them unfit for daily use including consumption. Safety of drinking water should be ensured for assuring a healthy population. Numerous chemical and microbiological parameters are used to determine the quality of drinking water in developing countries.

Several enteric bacteria present cause contamination of water especially pathogenic Escherichia coli which act as indicator organism for fecal contamination (Cabral, 2010) although other coliforms like Enterobacter, Salmonella, Klebsiella, Proteus etc. play an equally important role (Gogoi and Sharma, 2013) in contamination. Consumption of such faecal contaminated water causes numerous ailments and even serious outbreaks (Tallon et al., 2005) which could in turn effect a community as a whole. Therefore from health perspective, the risks associated with the consumption of drinking water contaminated with such microbes is of great concern (Tallon et.al, 2005). Hence modern drinking water testing relies on the presence of these bacteria, especially the presence of E. coli as a useful tool to analyse the extent of faecal contamination of water (Edberg et al., 2000). Hand hygiene is considered as a good health practice and has found to reduce the risk of the spread of infectious diseases (Bloomfield et al., 2007).

Disinfectants are antimicrobial agents that are applied to the surface of living or non-living objects to destroy microorganisms that are present. Sanitizers are substances that simultaneously clean and disinfect. Alcohol based hand sanitizers are common in use among health care facilities and have shown to be equal or even better than normal hand washing using soap and water alone, even in the presence of dirt or oil (Amy et al., 2010). A hand sanitizer is a liquid generally used to decrease infectious agents. As the name suggest its use on hands. Hand sanitizers are available as liquid, gel, foams etc. and are commercially available in different brands in the market. They are commonly formulations of alcohol like ethanol or isopropanol. A typical hand sanitizer contains almost 60-90 % w/w alcohol. But some non-alcoholic types of sanitisers are also available. Sanitizers containing 60-80% alcohol destroy pathogens by denaturing their proteins and hence act as disinfectant (Kampf and Kramer, 2004).

Among health facilities in developing countries, need and importance of hand sanitization had been popularized. Hand sanitizer use is slowly gaining momentum among layman around the world. Today many people have replaced the traditional hand washing process with just rubbing the palms using hand sanitisers. Many companies producing and marketing sanitizers have sprouted out which claims that it can kill about 99.9% of germs without water. Several efficacy tests for hand sanitizers that had been performed on contaminated hands has revealed that they exhibit greater efficiency than washing hands with plain soap (non- antibacterial) and water (Kampf and Kramer, 2004).

This study was conducted in an ill maintained pond situated in very prominent high school located in a strategic location in the heart of Trivandrum city close to college where this study was conducted. The water collected from this source was analysed for the presence of coliforms which indicate that it is highly polluted. Further, without prejudices and biases; the action of four different

commercial hand sanitizers on microbial contaminated water was studied. A comparative analysis of action of these sanitisers threw light to the fact that these could be of use to at least for those who would have come in contact with such a polluted water source. This work could also be extrapolated to study the effect of such sanitisers to bacteria isolated from such contaminated sources.

II. MATERIALS

Water sample, Lactose broth of single and double strength, Culture tubes, Bunsen burner, sterile pipettes, Incubator, Petri plates, Durham tubes, Loops, LAF.

Eosin methylene blue (EMB) agar, Nutrient Broth, Nutrient agar, Mac Conkey Agar, Triple sugar iron agar, Simmon Citrate agar, MRVP broth, Hydrogen peroxide (3%).

Crystal violet grams iodine, acetone, safranine for Gram Staining

4 commercial hand sanitizers-Dettol, Himalaya, Godrej and Lifebuoy; filter paper disks

III.METHODS

A. Sample collection

Water sample was collected from a school premises situated near to college where the present study was conducted. The water sample was analysed for contamination and further subjected to isolation and characterisation of bacteria present, as mentioned below.

B. Most Probable Number Test

Most Probable Number (MPN) is a method used to estimate the concentration of viable microorganisms in a sample by means of replicate liquid broth growth in ten-fold dilutions. It is commonly used in estimating microbial populations in soils, waters, agricultural products and is particularly useful with samples that contain particulate material that interferes with plate count enumeration methods.

MPN test is performed in 3 steps

1) *Presumptive test for coliform group of bacteria* : Presumptive test is a method to confirm whether water contains lactose-fermenting gas producing bacteria. However, it is used to determine the most probable number of coliforms in a sample of water besides their properties of fermenting lactose and producing gas. Coliforms if present, in water utilize the lactose present in the medium to produce acid and gas. The presence of gas is detected as gas bubbles collected in the inverted Durham tube present in the medium. The presumptive test was considered positive for tubes with gas production.
2) *Confirmed test*: Confirmed test is meant for differentiating the coliforms with that of non-coliforms as well as Gram-negative and Gram-positive bacteria. In this test, the EMB agar is inoculated with samples from previous positive tubes producing gas, for confirming the presence of coliforms. In this process, a loopful of sample from each tube showing positive test (colour change with gas) is streaked onto EMB agar. The experimental setup was incubated at 30 ± 1^{o}C for 12 hours.
3) *Completed test*: This test is required for the further confirmation. Organisms showing confirmed tests were further inoculated and grown on Mac Conkey, Nutrient agar plates and lactose broth for confirmation. Once the desired growth has been reached Gram staining was performed and the preparations were observed microscopically.

MacConkey agar is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae. Lactose fermenting strains grow as red or pink and may be surrounded by a zone of acid precipitated bile. The desired colonies showing up in confirmatory test were inoculated onto MacConkey agar plates to confirm the lactose fermenting nature of the isolated colonies.

C. Grams Staining

Grams staining was performed on the colony isolates according to the standard procedure (Adams, E., 1975)

D. Motility And Mobility Test

Motility and mobility tests were performed by hanging drop method and agar stab method.

E. Biochemical Tests

The following biochemical tests were performed to characterise the bacteria isolated and checked for utilisation of the respective medium constituents.

Simmon citrate agar, Triple Sugar Iron test, Catalase test, Methyl red, Vogus-Proskeur test.

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
Volume 6 Issue II, February 2018- Available at www.ijraset.com

F. Anti-microbial activity testing

Disk diffusion is used to test the effectiveness of antimicrobial compounds on specific microorganisms (antibiotics, disinfectants etc.). Agar plate is first inoculated with bacteria isolated form susceptible contaminated water by swabbing, and paper media disk impregnated with various antimicrobial substances are dispensed in it. It is then further incubated for about 24 hours at 35±2°C. The presence or absence of growth around the disc is measured indicating the ability of the compounds to inhibit the growth of the organism. The most commonly available hand sanitisers like Himalaya, Dettol, Godrej and Lifebuoy were used for the present study. A control plate without any hand sanitiser was also kept.

IV. RESULTS AND DISCUSSION

The presumptive tests showed the presence of gas production in the pond water sample inoculated in lactose broth with Durham tubes indicating the presence of coliforms. The sample after incubation showed turbidity indicating the presence of coliforms positively. Further confirmatory test on EMB Agar, showed the presence of three kinds of colonies varying in morphology (Table 1). Majority of the colonies formed were dark centered with green metallic sheen indicating coliforms like E.coli (Indicated as Colony B). The other types of colony morphology were slightly mucoidal with brownish colour and some were pale pink in colour with no sheen which could be non-lactose fermenting (indicated as colony A and C respectively). The growth on Mac Conkey Agar confirmed that colonies A and B were lactose fermenting whereas colony C was non-lactose fermenting (table 1). Gram staining showed that all the three kinds of colonies were gram negative, rod shaped. The motility tests using hanging drop as well as stab culture showed motility in case of all the three kinds of bacteria. The biochemical tests also helped in characterising the colonies observed which could be mostly Coliforms or Enterobacter as shown in Table 2. From the biochemical analysis done mentioned above, we could conclude that Colony A could be possibly Enterobacter, colony B could be Escherichia coli and Colony C is mostly Proteus, which could be further confirmed by molecular analysis like 16S RNA typing with the advent of techniques like Next Generation sequencing (Vierheilig et al., 2015). The effect of hand sanitisers were tested on the water sample containing the above three isolates. The commonly used sanitisers available in and around area where the study was conducted were used for the study. Four of them were found in the market viz; Himalaya, Godrej, Lifebuoy and Dettol. All of them are well known to common man and were tested for their anti-microbial activity on bacterial isolates found in the pond water. In this particular study, all of them were found to be more or less efficient with maximum zone of inhibition exhibited by Godrej. The experiments were done in triplicates and the results of antimicrobial activity is shown as the average of three experiments with standard error also plotted (Graph 1)

Table 1: Colony Morphology of the types of bacteria form water sample as seen on EMB Agar

COLONY	MORPHOLOGY ON EMB AGAR			MORPHOLOGY ON MAC CONKEY AGAR	
	Colour	Appearence	Nature	Colour	Ability To Ferment Lactose
COLONY A	Brownish black	Raised	Mucoidal	Pink	Positive
COLONY B	Dark nucleated with green metallic sheen	Flattened	Not mucoidal	Pink	Positive
COLONY C	Pink	Raised	Mucoidal	Colourless	Negative

Table 2: Characterisation of bacteria isolated form water sample using Biochemical test

COLONY	SIMMON'S CITRATE AGAR TEST	TRIPLE SUGAR IRON AGAR TEST		MRVP		CATALASE TEST
	SLANT	SLANT/BUTT	GAS PRODUCATION	MR	VP	
A	Positive	Acid/Acid	Positive	Negative	Positive	Positive
B	Negative	Acid/Acid	Positive	Positive	Negative	Positive
C	Positive	Alkaline/Acid	Positive	Positive	Negative	Positive

V. CONCLUSIONS

Water bodies present in community areas like schools, hospitals etc. could pose a threat to the life present there, if contaminated. This study was conducted in such an ill maintained pond present in school premise where about three thousand students are studying. The water sample isolated form the school pond was found to be contaminated with coliforms as revealed in the present study. Three kinds of bacteria were isolated and characterized biochemically, which could be the possible agents of contamination of pond water. Further the action of four selected hand sanitizers were studied for their anti-microbial activity on particular water sample. It was found that of the four selected, Godrej showed the most efficient anti-microbial activity on particular isolates. So we suggest the use of such hand sanitizers in cases where one comes in contact with such contaminated water sources, as it may provide protection to some extent.

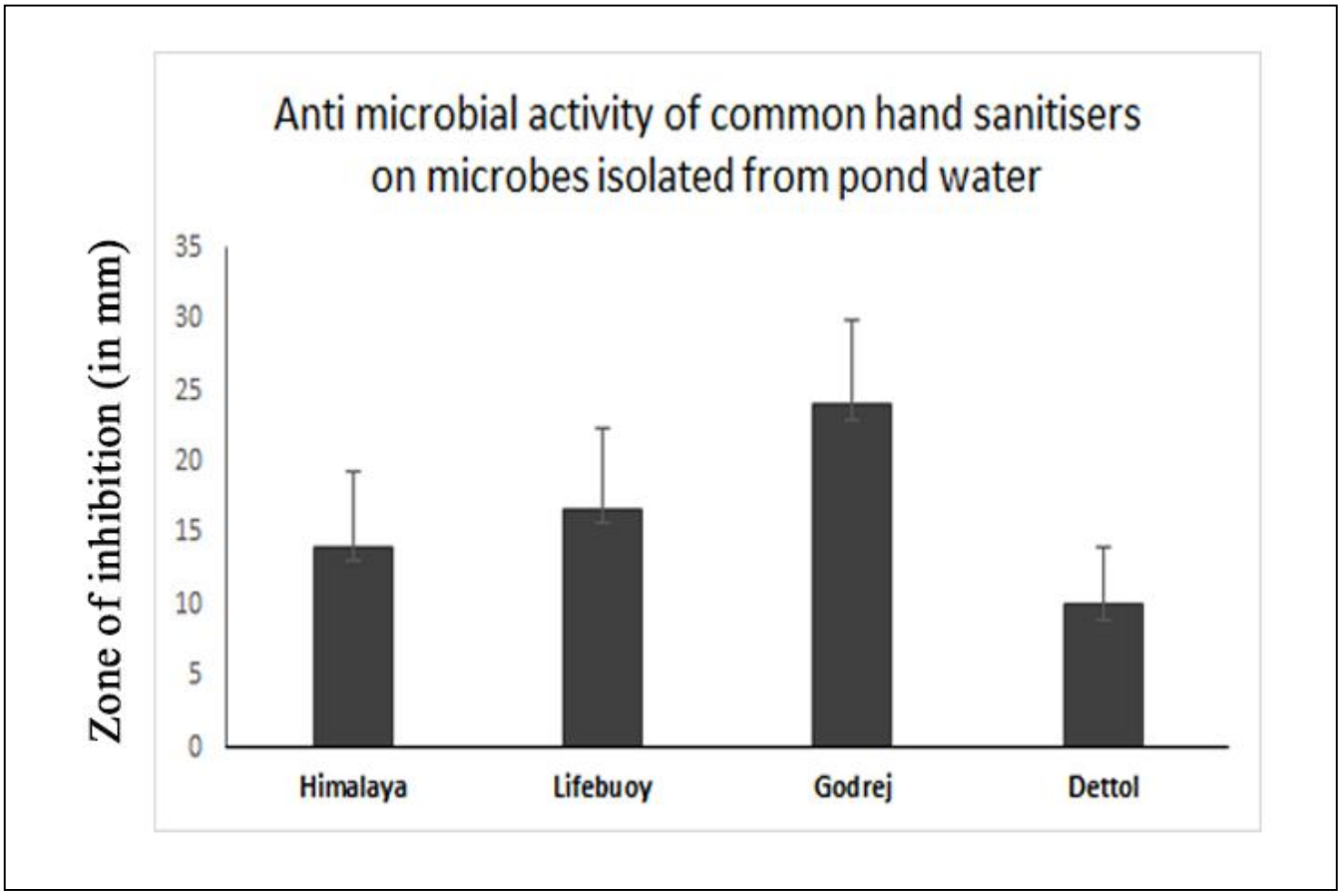
VI. ACKNOWLEDGMENT

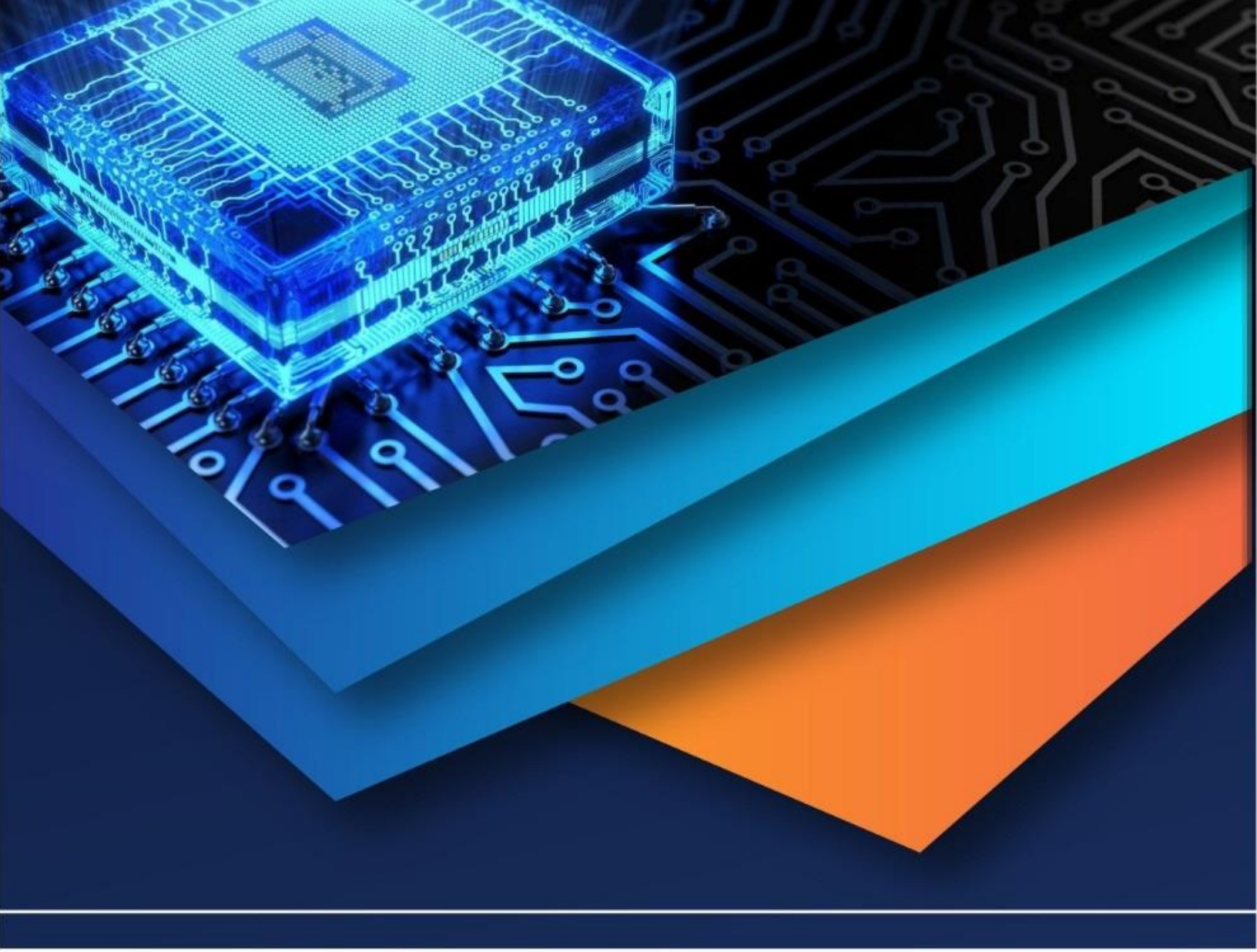
We acknowledge the Department of Botany staff, Dr. Sreelekshmi, V, Assistant professor of Biochemistry and Ayesha, Athira, Chandana, Shahinas, Subhdra, Arya, Third year BSc Biotechnology students, and the lab assistant, Mr. Sreejith who assisted us in all possible ways to conduct the study.

REFERENCES

[1] A. J. Pickering, J. Davis and A. J. Boehm, 'Efficacy of alcohol based sanitizers on hands soiled with dirt and cooking oil', J. Water and Health, 2011, 09.3, 429-433.
[2] E. Adams, Studies in gram staining, Stain Technol, 1975, 50, 227-31.
[3] G. Kampf and A. Kramer,' Epidemiologic Background of Hand Hygiene and Evaluation of the Most Important Agents for Scrubs and Rubs', Clin. Microbiol. Rev, 2004, 17, 863-893.
[4] J. P. S. Cabral, 'Water Microbiology. Bacterial Pathogens and Water', Int J Environ Res Public Health. 2010, 7(10):3657-3703.
[5] J. Vierheilig, D. Savio, R. E. Lay, R. L. Mach, A. H. Farnleitner A. H. and G. H. Reischer, 'Potential applications of next generation sequencing of 16S rRNA gene amplicons in microbial water quality monitirong', Water Sci Technol, 2015, 72, 1962-72.
[6] P. Gogoi and D. Sharma,' Microbial Contamination of Community Pond water in Dibrugarh District of Assam', Curr. World Environment, 2013, 8, 85-91.
[7] P. Tallon, B. Magajna, C. Lofranco and K. Tin,' Microbial Indicators of Faecal Contamination in Water: A Current Perspective', Water, air and Soil Pollution, 2005, 166, 139-166.
[8] S. C. Edberg, E. W. Rice, R. J. Karlin, and M. J. Allen, 'Escherichia coli: the best biological drinking water indicator for public health protection', Applied J. Microbiology, 2000, 88, 106S-116S.
[9] S. F. Bloomfield, A. E. Aiello, B. Cookson, C. O'Boyle and E. L. Larson, 'The effectiveness of hand hygiene procedures in reducing the risks of infections in home and community settings including handwashing and alcohol-based hand sanitizers', Am J Infect Control, 2007, 35:S27-64.

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
Volume 6 Issue II, February 2018- Available at www.ijraset.com





10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY