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Telephone: 0491 2541149 Fax: 0491 - 2542681 Website: http://:www.mercycollegepkd.com E-mail:mercycollege@satyam.net.in



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Mercy College, Palakkad

Phone : 0491 - 2541149

fax : 0491 - 2542681

Website : http://:www.mercycollegepkd.com

E-mail : mercycollege@satyam.net.in

# Editorial

Greetings to all our contributors and readers.

We are happy to release fifth volume of **Scientia** with three review articles and ten full length papers. The topics are varied and span from Microbiology to Computational Biology.

Over the last four decades the molecular revolution in biology has transformed the life Sciences, washing away the bridges between many allied disciplines. Many years ago, Arthur Kornberg lamented that turbulent advance of "Molecular Biology" had washed away the bridges to biochemistry and the traditional approach to the molecules of life. Fortunately, the dramatic rise of structural biology, fuelled by X-ray diffraction, NMR Spectroscopy and computational methods has ensured that chemistry and physics are essential for the solution of important problems in biology. Year of science, a review in this volume pay homage to the extraordinary scientists.

We at the editorial office are happy to see the continued growth of numbers as well as the diverse branches of science manuscripts being received. While thinking all of you, we look forward to your continued interaction.

With warm personal regards.

Sd/-**Dr. S. Jayasree**(Chief Editor)

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#### Photovoltaic cells – present and future

Lakshmi M.

Department of Physics, Mercy College, Palakkad 678008, kerala, India

#### Introduction

It is high time that mankind come up with a solution for the energy crisis. As we all know, the natural reserves of fossil fuel are not going to sustain long. Tidal energy and wind energy cannot cater to our needs beyond a minimal level. The next potential is nuclear energy which has got many problems and controversies about safety lapses and nuclear wastes. The most viable, eco friendly and inexhaustible source of energy that can meet the requirements of the next generation is definitely solar energy. Although silicon solar cells are the dominant cell type used in photovoltaics today, other cell types have been developed that compete either in terms of reduced cost of production or in terms of improved efficiencies. This article is a review on various types of solar cells that have been developed till date with special emphasis on their performance. It also shines light on the novel ideas of the next generation solar cells.

Cost is the major factor that restricts the usage of solar cells for day today needs. Neglecting this factor, today photovoltaic cells find its supreme position in supplying power to remote locations (where other sources of power cannot reach), for consumer products (like electronic calculators and street lights) and applications in space (for satellites and space vehicles).

Before evaluating merits and demerits of various types of solar cells here is a brief attempt to explain the operation of a ordinary solar cell: Consider a pn junction with a very narrow and heavily doped n region. The illumination is through the thin n side. Then depletion region or the space charge layer extends primarily into the p side. There will be a built in field  $E_0$  in this depletion layer. The electrodes attached to the n side must allow illumination to enter the device. A thin antireflection coating on the surface reduces reflections and allows more light to enter the device.

As the n side is very narrow, most of the light is absorbed within the depletion region and within the neutral p side. This leads to the generation of electron hole pairs (EHPs) in the region. EHPs generated in the depletion region are immediately separated by the built-in field  $E_0$  which drifts them apart. The electron drifts and reaches the neutral  $n^+$  side where upon it makes this region negative. Similarly, the hole drifts and reaches the neutral p side and thereby makes this side positive. Consequently an open circuit voltage develops between the terminals of the device with the p side positive with respect to the n side. If an external load is connected and the circuit closed then work can be extracted due to the flow of current.

#### The present senario

#### **Crystalline Silicon Solar cells**

For crystalline silicon (Si) devices, Boron doped p-type silicon is grown using the Czochralski method and wafers are sawn from it. Crystalline Si have an indirect energy band gap resulting in a low optical absorption coefficient, with the consequence that the wafers need to be greater than 200 nm thick to absorb most of the incident light. The wafer surfaces are 'textured' to minimize reflection losses and to refract the light entering the Si to high angles of refraction and this enhance the optical path length in the Si. Screen printed silver contact fingers are used on the n-type surface to make electrical contact while also allowing light to be transmitted to the junction region. Aluminium paste is used to make contacts at the back p-type surface. This is annealed to introduce a p<sup>+</sup> doped region at the back of the cell to lower the contact resistance and supply a back surface field that reflects minority carriers back towards the junction, fig.(1). An antireflecting coating is deposited over the top surface to complete the device. As screen printed contacts are formed to reduce the device efficiency considerably, advanced methods like photolithography or laser scribing is used in high technology cells.

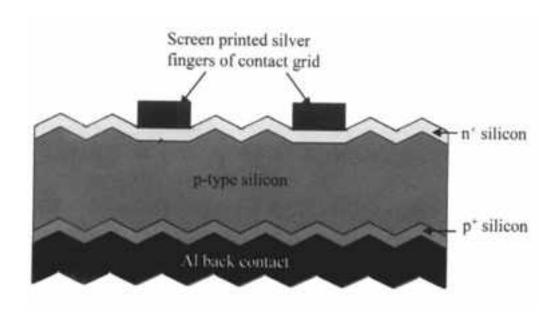


Fig. (1) Cross-sectional view of a silicon solar cell

The highest efficiency Si solar cell produced in the laboratory is the "passivated emitter rear locally diffused" solar cell, which has an efficiency of 24.7%. Higher efficiency is achieved by improving the surface texturing and by the inclusion of a  $SiO_2$  layer at the back of the device to passivate the back

surface. A silicon solar cell is 'concentrator systems' reduces the required Si layer thickness and also incorporate 'light trapping' features, fig. (2). However, with such systems, there is a need to track the sun and concentrator systems can only be used in parts of the world where there is unimpeded sunlight.

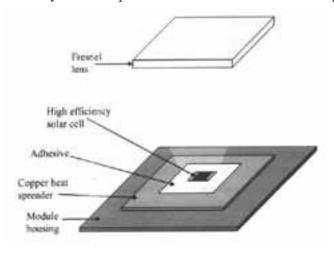


Fig. (2) Schematic view of a concentrator

#### Multicrystalline Silicon solar cells

Molten Silicon is poured into a container and then allowed to cool, resulting in Si ingots with large columnar grains (typically 0.3 mm diameter) growing from the bottom of the container. The grains are so large that they expand through the wafers cut from the solidified block. The incorporation of hydrogen during device processing plays an important role in passivating the grain boundaries in the devices formed.

Advantages of using multicrystalline growth over Czochralski method include lower capital cost, higher throughput, less sensitivity to quality of silicon feed stock used and higher packing density of cells to make a module because of the square or rectangular shape of the cells. The best module made with multicrystalline silicon generally have efficiencies 2-3% less than those of crystalline silicon and but the production cost is only approximately 80% of crystalline silicon cells.

#### Amorphous silicon solar cells

Here the material used is hydrogenated amorphous silicon (5-20 at.%H), where hydrogen plays the important role of passivating the dangling bonds that result from the random arrangement of Si atoms. This has a direct optical band gap of 1.7 eV and an optical absorption coefficient greater than  $10^5 \text{ cm}^{-1}$  for photons with energies greater than the energy band gap. This means that only a few microns of material are need to absorb most of the incident light, reducing material usage and hence cost.

It is possible to absorb the solar spectrum more efficiently and to improve cell stability by using multiple p-i-n structures (i stands for intrinsic) in the double junction or triple junction structures, fig. (3). The highest reported stabilized efficiency of a double junction is greater than 9.5% and for a triple junction module it is greater than 10%.

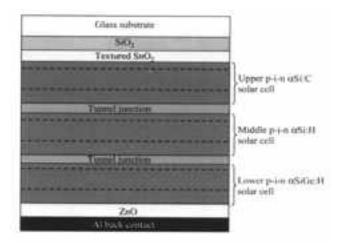


Fig. (3) A cross sectional view of a triple junction amorphous silicon solar cell

#### III -v solar cells

GaAs, InP and GaSb have direct energy band gaps, high optical absorption coefficients, and good values of minority carrier life time and mobility. These qualities make III-V compounds excellent materials for high energy solar cells. GaAs and InP has near optimum energy band gaps of 1.4 eV. The highest conversion efficiencies confirmed under standard conditions are 25.8 % for GaAs and 21.9% for InP single junction cell. The disadvantage of using III-V compounds in photovoltaic devises is in the very high cost of producing device-quality substrates or epitaxial layers of these compounds. Crystal imperfections, including unwanted impurities, severely reduce device efficiencies and alternative lower cost deposition methods cannot be used. These materials are also mechanically weaker than Si. The high density of material is also a disadvantage, in terms of weight, unless very thin cells can be produced to take advantage of their high absorption coefficients. These drawbacks have led to III-V compounds not being considered as promising materials, for single junction terrestrial solar cells. The development of III-V devices have been under taken primarily because of their potential for space applications. Here the high conversion efficiency together with good radiation resistance in the demanding environment of space power generation mitigates against the high material cost.

In a single junction Si solar cell, 56% of the available energy is lost because photons with energies

less than the band gap are not absorbed and photons with energies greater than the band gap 'thermalise', such that the excess energy over the band gap is lost as heat. A range of studies have shown that using multijunction solar cells such losses can be minimized leading to much higher efficiency devices. Stacked cells grown on to GaAs, InP and Ge substrates has shown appreciable results in the resent past.

#### Thin film solar cells based on compound semiconductors

In the case of CdTe only a few micron thickness is required to absorb most of the incident light. This is due to the fact that CdTe has a direct band gap of 1.5 eV and high optical absorption coefficient for photons with energies greater than 1.5 eV. The material cost can be reduced considerable here as only thin layer is required. In a CdS/CdTe solar cell the front contact is provided by depositing a transparent conductive oxide on to the glass substrate. This is followed by the deposition of a CdS window layer, the CdTe absorber layer and finally the back contact. Efficiencies up to 16.5% have been reported in this structure.

Solar cells based on chalcopyrite compounds are also popular. CIGS i.e Cu(In,Ga)Se<sub>2</sub> has proved to be better than CuInSe<sub>2</sub>. CIGS has a direct band gap of 1.3 eV and high absorption coefficient. So this leads to reduction in required thickness of the layer and hence the reduction in the production cost. These cells have reported an efficiency of 19.5% (13.4% in the module level). A junction is made in this CIGS by the deposition of a thin (50-80nm) window layer. CdS has been found to be the best material but alternatives such as ZnS, ZnSe, Zn(O,S) can also be used. A 50 nm intrinsic ZnO buffer layer is then deposited and it prevents any shunts. The transparent conducting layer is usually ZnO:Al 0.5-1.5 micrometer. The cell is finally completed by depositing a metal grid contact Ni/Al for current collection.

#### The future generation

#### Quantum wires and quantum dots

Silicon nano structures consisting of quantum wire or quantum dot super lattices can result in control of the effective band gap of silicon and this is a promising route towards more efficient solar cells. Si quantum wires have recently shown energy confinement for a well thickness of 1 nm – 2.7 nm. But such Si layer of thickness less than 3 nm is found to have low crystallographic quality. Silicon in quantum dot form gives greater control over band gap with a less stringent size requirement. For example the confinement energy in a 2 nm diameter quantum dot will be the same as in a one nm wide quantum well. By this process 2 nm quantum dot is found to have a band gap of 1.7 eV. This is the ideal band gap for a 2 cell tandem photovoltaic cell with bulk silicon forming the bottom cell. A 1.4 nm diameter quantum dot supper lattice would on the same basis give a 2.3 eV band gap, high enough for the top cell in a five cell tandem on bulk silicon.

Silicon quantum dot supperlattices are fabricated by alternate deposition of silicon rich oxide (SRO) and  $SiO_2$  layers by co-sputtering of Si and  $SiO_2$  targets and reactive plasma  $SiO_2$  deposition, respectively. SRO which is actually  $SiO_x$  (where x is less than 2), is thermodynamically unstable and hence leads to precipitation of silicon on annealing at  $1173^{\circ}$  C. If the film thickness of SRO film is on the scale of a few nanometers and enclosed by an insulator, the phase separation in the SRO film creates self organized nano-scale Si quantum dots. If the packing density is high enough, overlapping of the wavefunction of the dots should allow a quantum dot supper lattice to form.

Surface energy minimization would favour the formation of near-spherical dots of as large as a size as possible (i.e. the thickness of the SRO layers). Their maximum size is therefore determined by the thickness of the SRO layer as is the depth co-ordination of position. This feature offers uniform size controllability of Si quantum dots.

#### Up/Down converters

This new theoretical idea is now being experimentally tried as a promising approach to reach high conversion efficiencies. In down conversion cells a luminescent converter is located on the front surface of a solar cell, which has a band-gap energy Eg. High energy photons with energy greater than 2 Eg are absorbed by the converter and efficiently down converted into two lower energy photons with energy greater than Eg, which can be absorbed by the solar cell.

In up converters, the converter is placed on the rear side of the solar cell. It absorbs low energy photons transmitted by the cell and re-emits photons above the band gap of the cell. In both cases the solar cell and the converters are electronically isolated from each other. Theoretical analysis has reveled that a solar cell with band gap energy of 2 eV and with an optimum up converter attached to its rear side can reach an efficiency of up to 50.7%. One of the appealing aspects of this approach is that they can be applied to existing solar cells and therefore experimental work can be carried out with relatively uncomplicated structures. In initial studies, the focus was on the up-conversion systems, mainly due to the availability of efficient luminescent phosphors like NaYF4: Er<sup>3+</sup>.

#### Hot carrier cells

The concept underlying the hot carrier solar cells is to slow down the rate of cooling of photoexcited carriers, caused by phonon interaction in the lattice. This allows time for the carriers to be collected whilst they are still "hot" thus enhancing the voltage of the cell. This tackles the major photovoltaic loss mechanism of thermalisation of carriers. Here carriers must be collected over a very small energy range with selective energy contacts.

The property of a narrow selected energy transmission range is a key requirement in choosing a suitable contact structure. The transmission energy range should be kept small to approach the entropic ideal, but remain large enough to support the appropriate rate of carrier extraction. Other properties of critical interest are the ease of manufacture of the structures and thermal insulation. Quantum mechanical tunneling structures satisfy most of the above requirements with resonant tunneling as the appropriate process. Resonance, in this context refers to a high level of electron transmission within the range of resonant energies with low transmission outside this range. The quality of resonance is evident by the narrowness of the transmission range. This offers the possibility of very good energy selection..

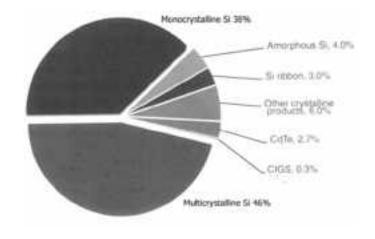


Fig. (4) Market share of various types of solar cells

#### **Conclusion**

In the first part of this article, though we have discussed a variety of solar cells, it is interesting to note that more than 90% of the market is still based on silicon material, fig.(4). The ideas discussed in the second half can contribute to the next generation of advanced solar cells. If we want to witness a day where photovoltaic is the prime supplier of energy, then we have to go a long way in development. Today the total world power production is estimated to be around 14 TW. Providing 10 TW of power will require building a 1GW coal, nuclear or wind power station every day for the next 27.5 years. (Note that today's largest wind farm in USA is only 0.73 GW capacity!). If instead, we want to rely only on photovoltaic, and if we are going for only the highest photovoltaic power instillation of today, i.e. 14 MW (at US air force base at Nevada), then it would mean building one such plant every hour for the next 81 years!! So no doubt that if photovoltaic is to lead our planet earth, then a miraculous leap has to happen and we are waiting for that.

Vol. 5, No. 1, Jan - Dec. 2009, pp 7 - 14

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#### **Artificial Neural Networks**

Reena T. J.

Department of Computer Science, Mercy College, Palakkad-678006

#### Introduction

Neural network simulations appear to be a recent development. However, this field was established before the advent of computers, and has survived at least one major setback and several eras. Many important advances have been boosted by the use of inexpensive computer emulations. Following an initial period of enthusiasm, the field survived a period of frustration and disrepute. During this period when funding and professional support was minimal, important advances were made by relatively few researchers. Currently, the neural network field enjoys a resurgence of interest and a corresponding increase in funding. The first artificial neuron was produced in 1943 by the neurophysiologist Warren McCulloch and the logician Walter Pits. But the technology available at that time did not allow them to do much. Presently, neural networks are being successfully applied across an extraordinary range of problem domains in areas as diverse as finance, medicine, engineering, geology and physics.

#### What is an artificial neural network?

An artificial neural network is a system based on the operation of biological neural networks, in other words, is an emulation of biological neural system. The implementation of artificial neural networks performs certain tasks that a program made for a common microprocessor is unable to perform.

#### Advantages:

A neural network can perform tasks that a linear program can not.

When an element of the neural network fails, it can continue without any problem by their parallel nature.

A neural network learns and does not need to be reprogrammed.

It can be implemented in any application and without any problem.

#### Disadvantages:

The neural network needs training to operate.

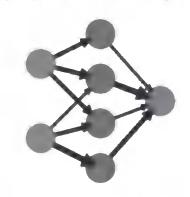
The architecture of a neural network is different from the architecture of microprocessors therefore needs to be emulated.

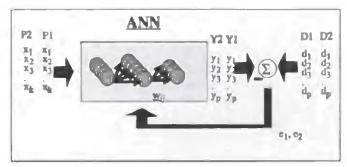
Requires high processing time for large neural networks.



#### A simple neural network

Input layer Hidden Layer Output Layer





The style of neural computation.

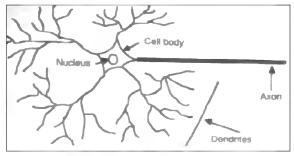
The simple and neural network style in neural computation is as shown in figure 1:

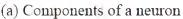
An input is presented to the neural network and a corresponding desired or target response is set at the output. Error can occur from the difference between the desired response and the system output. This error information is fed back to the system which adjusts the system parameters in a systematic fashion. The process is repeated until the performance is acceptable. It is clear from this description that the performance hinges heavily on the data. At present, artificial neural networks are emerging as the most sought after technology which has various applications such as pattern recognition, prediction, system identification, and control.

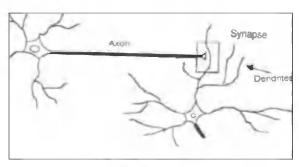
#### The Biological Model

Simplified neurons of McCulloch and Pitts were presented as models of biological neurons and as conceptual components for circuits that could perform computational tasks. The basic model of the neuron

is founded upon the functionality of a biological neuron. "Neurons are the basic signaling units of the nervous system" and "each neuron is a discrete cell whose several processes arise from its cell body".





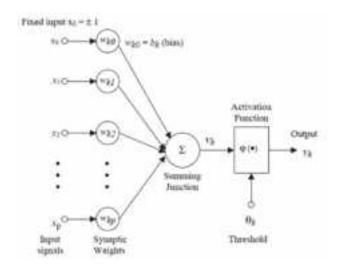


(b) The synapse

Figure 2:-The neuron has four main regions to its structure. The cell body or soma has two offshoots the dendrites and the axon, which end in presynaptic terminals. The cell body is the heart of the cell, containing the nucleus and maintaining protein synthesis. A neuron may have many dendrites which branch out in a treelike structure and receive signals from other neurons. A neuron usually has only one axon which grows out from a part of the cell body called the axon hillock. The axon conducts electric signals generated at the axon hillock down its length. These electric signals are called action potentials. The other end of the axon may split into several branches, which end in a presynaptic terminal. Action potentials are the electric signals that neurons use to convey information to the brain. All these signals are identical. Therefore, the brain determines what type of information is being received based on the path that the signal took. The brain analyzes the patterns of signals being sent and from that information it can interpret the type of information being received.

#### The Mathematical Model

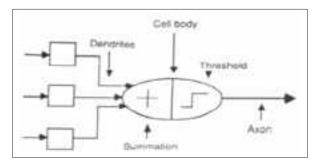
When creating a functional model of the biological neuron, there are three basic components of importance. First, the synapses of the neuron are modeled as weights. The strength of the connection between an input and a neuron is noted by the value of the weight. Negative weight values reflect inhibitory connections, while positive values designate excitatory connections [Haykin]. The next two components model the actual activity within the neuron cell. An adder sums up all the inputs modified by their respective weights. This activity is referred to as linear combination. Finally, an activation function controls the amplitude of the output of the neuron. An acceptable range of output is usually between 0 and 1, or -1 and 1. Mathematically, this process is described in the figure 3:



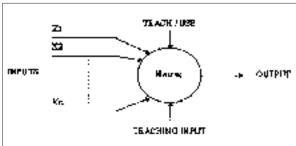
From this model the interval activity of the neuron can be shown to be:

$$v_k = \sum_{j=1}^p w_{kj} x_j$$

The output of the neuron, yk, would therefore be the outcome of some activation function on the value of vk. The architecture shown in figure 4:-



(a)The neuron model



(b) A simple neuron

#### A more practical example

The same neural networks in brain process familiar and newly learnt words:-A series of experiments conducted as part of the Academy of Finland's Neuroscience Research Programme (NEURO) have shown that the brain uses the same neural networks to process both familiar and newly learnt words.

In one experiment, participants learnt the name and/or purpose of 150 ancient tools. They had never heard those words before. Their brain function was measured by means of magnetoencelography during the naming of the tools, both before and after the learning period. It was observed that their brains used the same neural networks to process both familiar and newly learned words. Academy Professor Riitta Salmelin, HUT Low Temperature Laboratory, who is in charge of the research, revealed that the names of objects were processed in the left temporal and frontal lobe within half a second of showing the image of the tool to the subject. If the subject had only recently learned the name of the tool, the naming process induced an activation that was just as strong as or stronger than the activation induced by the image of a familiar object. The learning of the meaning of ancient tools did not cause corresponding clear differences in the function of the brain. The processing of meanings in the brain differs essentially from the processing of names. On the other hand, the performance results indicated that new definitions were learnt even faster than new names.

#### Artificial Neural Networks (ANNs) and their Role in Our Lives

Scientists mathematically define artificial neural networks by models similar to figure 3.

The mathematical model and equation defines biological neurons' processes through a mathematical function (Rios, 2007-2008). In a biological network, the "summing junction" represents the spinal cord, while the "activation function" serves as the brain. Output Yk represents the reaction the brain forces a person to perform.

While scientists know what artificial neural networks consist of, researchers disagree on whether or not there exists a "central planner" that collects information from any location in the system. Andy Clark states that in the human body, the brain represents a "central planner," and experiments prove the organ's ability to comprehend multiple sources of data. Clark notes that in certain ANNs, independent devices employ themselves in separate locations, each with an individual pathway to convert sensory inputs into actions.

Discoveries in neuroscience lead to intriguing inventions in artificial intelligence and provide humans with computational power unrivaled in the past. As an example, computers prove theories proposed by mathematicians hundreds of years ago. Solutions to the approximate sum of an infinite series of numbers could only be deciphered by a writing utensil and paper. Only individuals blessed with minds like Newton or Einstein contemplated how to solve these problems, but today, ANNs aid all people with a scientific calculator in resolving an infinite series by hitting a few buttons.

Andy Clark, Director of the Cognitive Science Program at Indiana University, describes how

digital technology enables humans to ignore distance as a limiting factor of production. In the experiment linking the monkey brain to the robotic arm, electronic impulses were transferred from the monkey's brain to the robot. This connection did not require human intervention. With this type of technology, organizations like NASA possess the ability to control probes in other areas of the solar system and human knowledge applications extend farther than a restricted physical area.

Andy Clark views cell phone as another link for a person to theoretically be in two places at a time (2003). Hundreds of years ago, the actions of a human in one area would not effect a situation far away. Today, an individual can eat lunch, run their business in America, and deal with foreign import companies at the same time. While these artificial networks enhance the ability to focus on multiple projects, they divide the person's attention span into separate places. This leads us to the detriments of artificial neural networks, specifically the fact that it separates humans from actual experience.

Students of the modern age students rely on intricate visual and audio triggers to stimulate brain attention whereas students of the past generations relied on basic verbal and written communication. Perhaps prolonged exposure to digital age technology creates a new attention deficit disorder towards teachers' words and blackboards. An Australian survey found that people who watch television for only an hour daily do better on memory tests than those who watch more. 'Couch potatoes,' increase their risk of developing Alzheimer's disease as the brain literally zones out (Tesh, 2008). While these facts present themselves repeatedly, artificial neural networks and digital media continue to impede the development of biological neural systems.

As Professor Wesch implies, the same theories proposed prior 500 AD apply to researching the quality of college institutions (2007). Some believe that universities with a low faculty to student ratio have below average educational programs. The personal attention given by philosophers like Socrates, Aristotle and Plato remain an important aspect of education. Justinian disowned the methods taught by the Academy because it was more important to industrialize education than actually create systems that enhanced enlightenment. Military societies did not need smart soldiers, so they rushed students through school and onto the battlefield. Obviously, modern college education represents commercial investment when hundreds of students cram into lecture halls where they have limited opportunities to learn. Ironically, our sophisticated digital systems make education more difficult for masses.

Artificial neural networks transmit data in ways not previously experienced by humans, and while modern machines process information faster than ever before, the new technologies limit the abilities of biological neural networks. Even with these facts, artificial neural networks play a crucial role in the development of humans, creating biological neural pathways that cannot comprehend education through

classroom reading, listening, and writing. Only the future will tell whether ANNs lead to human success or failure.

#### **Applications for Neural Networks**

Detection of medical phenomena. A variety of health-related indices (e.g., a combination of heart rate, levels of various substances in the blood, respiration rate) can be monitored. Neural networks have been used to recognize the predictive pattern (symptoms) so that the appropriate treatment can be prescribed.

Stock market prediction. Neural networks are being used by many technical analysts to make predictions about stock prices based upon a large number of factors such as past performance of other stocks and various economic indicators.

Credit assignment. After training a neural network on historical data, and personal information (age, education, occupation) neural network analysis can identify the most relevant characteristics and use those to classify applicants as good or bad credit risks.

Monitoring the condition of machinery. A neural network can be trained to distinguish between the sounds a machine makes when it is running normally ("false alarms") and those made on the verge of a problem. After this training period, the expertise of the network can be used to warn a technician of an upcoming breakdown, before it occurs and causes costly unforeseen "downtime."

Engine management. Neural networks have been used to analyze the input of sensors from an engine. The neural network controls the various parameters within which the engine functions, in order to achieve a particular goal, such as minimizing fuel consumption.

Speech and Vision recognition systems. Neural Networks are increasingly becoming part of such systems. They are used as a system component in conjunction with traditional computers.

White goods and toys. As Neural Network chips become available, the possibility of simple cheap systems which have learned to recognize simple entities (e.g. walls looming, or simple commands like Go, or Stop), may lead to their incorporation in toys, washing machines, etc. Already the Japanese are using a related technology-fuzzy logic- in this way.

#### Conclusion

Human brain is the most wonderful creation of God. The development of science and technology has enabled its further improvement and applications. Supplements like Artificial Neural networks will definitely improve the quality of life and living as evident from the various experiments stated above. A

proper assessment of its functioning and clear awareness of its drawbacks will enhance its benefits and reduce its bares.

The computing world has a lot to gain from neural networks. Their ability to learn by example makes them very flexible and powerful. Furthermore there is no need to devise an algorithm in order to perform a specific task; i.e. there is no need to understand the internal mechanisms of that task. They are also very well suited for real time systems because of their fast response and computational times which are due to their parallel architecture.

Neural networks also contribute to other areas of research such as neurology and psychology. They are regularly used to model parts of living organisms and to investigate the internal mechanisms of the brain. Perhaps the most exciting aspect of neural networks is the possibility that some day 'conscious' networks might be produced. There are a number of scientists arguing that consciousness is a 'mechanical' property and that 'conscious' neural networks are a realistic possibility.

It is thus evident that neural networks have immense potential, but we can make the best out of them only when they are integrated with computing, AI, fuzzy logic and related subjects.

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#### Year of science- 2009

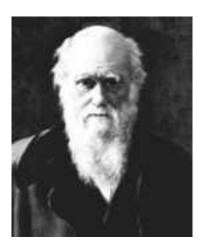
Namitha Issac and Srilakshmi Balakrishnan Department of Physics, Mercy College, Palakkad 678008,kerala,india

#### Introduction

Human beings are curious by character. The curiosity of man unveils the mysteries of nature. With a highly developed mind, man can observe precisely, correlate the results of observations meaningfully and also predict future happenings logically. It has freed man from the clutches of ignorance and superstition. This overall development is what is known as Science. It is worth dedicating a year for the celebration of Science and its contributions that has helped us advance in all fields of technology.

The year 2009 is celebrated as the Year of Science. It is with reference to some seminal events and significant anniversaries that are on the horizon in 2009. The celebrations are carried out round the year. The theme of the celebration is "How We Know What We Know". Some of the significant events include the following:

The 200<sup>th</sup> anniversary of the birth of Charles Darwin and 150<sup>th</sup> anniversary of the publication of his "The Origin of Species".



Charles Darwin was born in 1809 in England in a small town. He joined the 'H.M.S. Beagle' expedition around the world, and during this voyage, he made innumerable observations, critical notes on organisms and returned in 1836. On the 24<sup>th</sup> of November, 1859, he elaborated his views in his work, "On The Origin Of Species". He died on the 19<sup>th</sup> of April, 1882.

The 400th anniversary of the publication of Johannes Kepler's Laws of Planetary Motion.



Johannes Kepler was born in the year 1571 in Weldester in Germany. He studied about the planets on the basis of Copernicus theory. He was the first to propose that six planets revolve around the sun. He published the three Laws of Planetary Motion in his book, "New Astronomy" and it is considered to be the foundation of modern astronomy. He died on 15<sup>th</sup> November, 1630.



#### The 400<sup>th</sup> anniversary of Galileo's first use of a telescope.

Galileo is one among the earliest scientists who paved way for the scientific revolution. He was the one who observed the planets through a telescope for the first time. Made of wood and leather, Galileo's telescope had eight times magnification with a convex main lens and a concave eyepiece that presented the image the right way up.

The 200th anniversary of the birth of Abraham Lincoln.



Abraham Lincoln, the 16<sup>th</sup> President of the United States of America, was born on 12<sup>th</sup> of February, 1809. He is remembered during the Year of Science as he was the founder of the "National Academy of Science". He was assassinated on the 15<sup>th</sup> of April, 1865.

#### The 200<sup>th</sup> anniversary of Dalton's theory.



Dalton is known as the father of modern atomic theory (1807). His theory states that atom is the fundamental particle of matter.

#### Round the year celebrations

The 12 month event consists of 12 scientific themes, each theme dedicated to each month. The 12 scientific themes are the following:

#### January-Process And Nature Of Science

As we launch into the Year of Science, we begin with the theme highlighting the process and nature of Science. The process of Science circles back on itself so that the ideas are tested and retested- and the most useful of those are built upon and used to learn more about the natural world.

#### **February-Evolution**

To many, evolution is a thing of the past- an idea that Darwin developed, or an explanation of fossils and things that lived long ago. But evolution is not only alive and well in 2009, it is a global phenomenon and critical to our understanding of many aspects that influence our lives. Understanding evolution helps to solve biological problems that influence our lives.

#### **March-Physics And Technology**

In a broad sense, Physics is the Science of nature. From the tiniest quarks to the biggest galaxies, everything around us has something to teach us about how the universe works. The laboratory of the physicists extends from the edge of the universe to the inside of the nucleus of an atom.

#### **April-Energy Resources**

Energy is indispensable for human existence. It is an inevitable component in economic as well as technological development. We must continue to improve energy efficiency- getting as much work as

possible out of a unit of energy and reducing our waste. We should thrive forward for renewable and ecofriendly sources of energy.

#### May-Sustainability And Environment

Sustainability in a broad sense is the capacity to endure. It can be defined as the ability of an ecosystem to maintain ecological processes, functions, biodiversity and productivity into the future. Sustainability represents the way of thinking, living and acting to ensure that our choices do not impact the future generations' ability to enjoy a high quality of life.

#### June-Ocean And Water

Earth truly is a 'water planet' with the oceans covering 97% of the earth's water. They are the store house of minerals. By exploring the ocean frontier, we will advance our understanding of ocean resources. Understanding that we share and enjoy the same watershed should give us the reason to appreciate and protect the water that we have and use everyday.

#### **July-Astronomy**

Astronomy, the oldest Science in history, played an important role in most cultures over the ages. Astronomy has included disciplines as diverse as astrometry, celestial navigation, observational astronomy, the making of calendars and even astrology, but professional astronomy is nowadays often considered to be synonymous with Astrophysics.

#### **August-Weather And Climate**

Weather is a set of all phenomena occurring in a given environment at a given time. Climate encompasses the statistics of meteorological element in a given region over long periods of time. What we are celebrating is how much we continue to learn about the dynamic forces within our atmosphere.

#### **September-Biodiversity And Conservation**

Biodiversity is the measure of the richness of life on earth. Life comes in all shapes and sizes and manages to persist in even the most extreme environments. Biodiversity, also known as the array of life, is a global issue of great importance and provides an indicator of the overall health of our planet.

#### October- Geosciences And Planet Earth

Geoscience also known as earth science is the Science related to planet earth. It is a combination of both Geophysics and Geochemistry. The more we know about earth's geologic resources and processes, the better we can appreciate its grandeur and beauty and respect its occasional disruptions.

#### **November-Chemistry**

Of all the branches of Science, Chemistry in particular has played a vital role in improving the quality of human life. It provides us health care, pharmaceuticals, cosmetics, detergents, preservatives and many advanced materials like fibres, propellants, etc. In short, life without Chemistry would have been impossible.

#### **December-Health And Science**

Health Science builds upon an integration of Biology, Chemistry and Physics and has resulted in major advancements in medicine, new surgical instruments and increased longevity of human beings. Let us celebrate Health and Science this December as it is worth toasting as we prepare to ring in the next New Year.

#### Conclusion

After reading all about the Year of Science, one may ask why actually we are celebrating the Year of Science. As we know the theme of the project is "How We Know What We Know", we celebrate the Year of Science just to understand how the nature and the universe work though we know that they are working. It makes us keen to learn of all the basic scientific concepts behind every little object in the whole universe.

The Year of Science is celebrated to bring about awareness among the people, most of whom remain ignorant to the changes on the earth and also in the universe, and the role that Science plays in the change. It also provokes us to do something for our planet against all the environmental issues.

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# Phenol biodegradation by a microbial consortium developed from activated sludge

Aswathy N<sup>1</sup>, Karthikeyan P<sup>1</sup>, Dhanya K<sup>1</sup>, .Chandrasekaran M<sup>2</sup> and Elyas K.K<sup>3</sup>

<sup>1</sup>Department of Biotechnology, Cochin University of Science and Technology, Cochin

<sup>3</sup>Department of Biotechnology, Calicut University, Calicut

<sup>2</sup>Department of Botany & Microbiology, College of Science, King Saud University

Riyad-11451, Kingdom of Saudi Arabia

#### **Abstract**

Activated sludge is known to contribute significantly to the spontaneous bioremediation of waste water in nature. The microbial consortia present in the activated sludge are in fact the real workhorses to accomplish this task. In the present study, bacteria associated with the activated sludge of the industrial effluent of FACT,Kochi, Kerala were isolated, evaluated for the potential for phenol degradation under *in vitro* conditions. The species of *Vibrio*, *Pseudomonas* and *Aeromonas* were found to play a dominant role in phenol degradation. Hence; a microbial consortium was formulated and developed using these strains. Growth curve and phenol degradation pattern by the microbial consortia were estimated. More than 60% of phenol in the media was degraded by the cultures before they entered the stationary phase. The results indicate the scope for application of microbial consortia for effective management of waste water rich in phenol. *Vibrio sp.* is found to show enormous potential for phenol degradation in combination with *Pseudomonas sp.* It is concluded that activated sludge bacteria are ideal for development for microbial consortia for the treatment of industrial effluents.

**Key words**: Activated sludge, bioremediation, microbial consortia, phenol degradation

#### Introduction

Rapid growth of population, industrial development and globalization has led to an alarming increase in the rate of waste generation in industrial sector. As this involves health and environmental concerns, the scientific community is focused on improving the ways to treat the wastes and detoxify the harmful chemicals. Phenol is considered an extremely hazardous substance and is listed as a hazardous substance under the Occupational Safety and Health Act (OSHA). The major sources of phenolic wastes are oil refineries, coal gasification and liquefaction plants, chemical plants, resin and paint industries. The microbial ecosystem is highly complex in an activated sludge plant Butterfield (1935) had studied the same and his studies laid the foundation for the later research. The existing removal methods have the

inherent drawbacks due to the tendency of the formation of secondary toxic materials (Bandhyopadhyay et al, 2001), complexity, and high cost of treatment (Gonzalez et al, 2000). Thus, in some cases, biological treatment has turned out to be a favourable alternative for phenol degradation.

A number of microorganisms have been reported to degrade toxic chemicals like phenol. These organisms include *Pseudomonas spp.* (Bayly et al,1973), *Alcaligenes spp* (Hughes et al, 1983)., *Streptomyces setonii* (Antai et al, 1983), and yeasts like *Trichosporon cutaneum* (Neujahr et al, 1973) and *Candida tropicalis* (Neujahr et al, 1974). The biodegradative potentials of the activated sludge microbial flora is yet to be explored.

#### **Materials and Methods**

#### Sample Collection

The samples were collected from the activated sludge plant of Fertilizers and Chemicals Travancore Ltd. (FACT), Kochi in sterile bottles.

#### Isolation of Microorganisms

The sample was serially diluted and pour plating was done to obtain the single colonies. The colonies were differentiated based on the morphology, shape, elevation size, colour, and texture. Pure cultures were obtained by sub-culturing and streak plate method in nutrient agar. Paraffin stocks of the cultures were prepared and stored at room temperature.

#### Characterization of the Microorganisms

The pure cultures of the isolates were subjected to gram staining and several biochemical tests.

#### **Biodegradation studies**

The pure cultures were used as inoculum in nutrient media supplemented with 8mM phenol. Phenol biodegradation and growth curve of each strain were simultaneously studied.

#### Microbial consortium

The best degraders were selected from the 7 strains and used to formulate a consortium and the phenol biodegradation was studied. Strains 4, 5 and 7 were mixed in the ratio1:1:3 and was used to inoculate 100 ml of media with 8 mM phenol. Strains 5, 7 and 3 were mixed in the ratio 2:2:1 and was used to inoculate 100 ml of media with 8 mM phenol.

#### Analytical methods

Phenol degradation studies were carried out using Folin's Ciocalteau Phenol Method.

#### Gallic Acid Stock Solution

In a 100-mL volumetric flask, dissolve  $0.500\,\mathrm{g}$  of dry gallic acid in  $10\,\mathrm{mL}$  of ethanol and dilute to volume with water.

#### Sodium Carbonate Solution.

Dissolve 200 g of anhydrous sodium carbonate in 800 mL of water and bring to a boil. After cooling, add a few crystals of sodium carbonate, and after 24 hr, filter and add water to 1 L.

To prepare a calibration curve, 0, 1, 2, 3, 5, and 10 ml of the above phenol stock solution was added into 100 ml volumetric flasks, and then diluted to volume with water. These solutions have phenol concentrations of 0, 50, 100, 150, 250, and 500 mg/L gallic acid, t 0.5ml of the culture was pipetted out, centrifuged at 8000 rpm for 15 minutes at 4C. 20 microlitre of the sample was used for the estimation.

From each calibration solution, sample, or blank,  $20~\mu L$  was pipetted out into separate cuvettes, and to each 1.58 ml water was added, and then  $100~\mu L$  of the Folin-Ciocalteau reagent was added, and mixed well. After an incubation period of 5min,  $300~\mu L$  of the sodium carbonate solution was added, and shaken to mix. The solutions were incubated at 40C for 30 min before reading the absorbance at 765 nm against the blank. he effective range of the assay.

#### **Results and Discussion**

Out of the 20 bacteria isolated,7 strains which showed promising potential for phenol degradation were identified to various genera tentatively based onm their morphological, biochemical and physiological characteristics. They were tentatively placed under the following genera as given below:

Vibrio sp. BTASP 1, Vibrio sp. BTASP 2, Vibrio sp. BTASP 3, Pseudomonas sp. BTASP 4, Vibrio sp. BTASP 5, Aeromonas sp. BTASP 6 and Vibrio sp. BTASP 7. The property of phenol degradation was studied along with the growth characteristics. The growth curves obtained are of the typical sigmoid curves while the phenol degradation curves show decline till around 40 hours and then plateaus out (Fig.1). Strains 1 and 2 showed a sharp decline in phenol concentration in these 40 hours while strain 7 traces a curve. Strains 3, 4, 5 and 6 show a reduction in the rate of degradation in the period between the tenth and twentieth hours. Strains 5 and 7 showed continuous degradation and are potential candidates for the treatment of phenolic wastes.

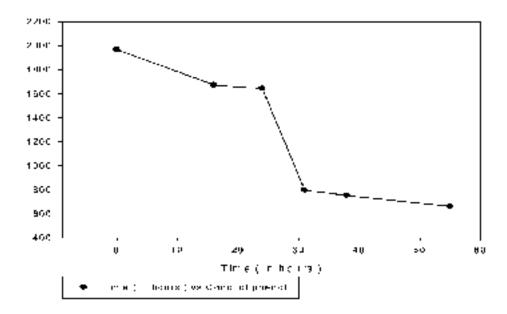


Fig.1.Phenol Degradation Curve

Out of the two microbial consortia tried, the combination of strain 3, 5, and 7 showed consistent biodegradation compared to the combination of strains 4, 5 and 7. Strain 3 was selected as it grew even in 10 mM phenol indicating high tolerance. Pseudomonas is reported to have high potentials for biodegradation of xenobiotics and was selected to be used in the second combination.

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# Comparative study of Mitotic activity and Chromosomal behavior in root meristems of Allium cepa L. in different soil samples for sustainable agriculture\*

Jayasree. S.
Department of Zoology, Mercy College
Palakkad- 678006, Kerala

#### **Abstract**

Pollution of the agricultural area as a result of non degradable solid waste dumping turns to be a global problem for contemporary mankind. Sustainable agriculture provides protection of the environment and requires a conversion period. Response of the Allium cepa root meristem to the presence of potential cytotoxic and genotoxic substances in the environment was used to evaluate the toxicity of soil collected from five different sites of Mercy College campus. Soil samples were collected from solid waste dumping site, open compost site, vermicompost, vermicompost amended with solid waste leachate (10; 1) and normal ground soil. For insitu monitoring of the cytotoxicity level, the inhibition of mitotic division in meristematic cells was assayed. For testing the genotoxicity of the collected samples chromosome aberration (CA) assay in mitotic cells and micronucleus assay in (MN) interphase cells were carried out. Mitotic index was normal in ground soil (15.50%), higher in vermicompost (45.90%) minimum in solid waste dumping site (6.32%) and intermediate in vermicompost -solid waste amended, soil (34.10%). Chromosome aberration (CA) and micronucleus formation was significantly high in solid waste soil, less in open compost and significantly nil in normal and vermicompost soil; intermediate in solid waste-amended vermicompost. The result indicates that soil of the solid waste dumping site and open compost site accumulates different toxic chemicals which are manifested through decreased mitotic index and significant increase of chromosome aberrations. Solid waste amended with vermicompost helped to decrease the toxicity indicated by increased mitotic index from 6.32% to 34.10% which is higher than control (15.50%). The present study indicates the feasibility of vermicomposting for the clean up of toxic soil to mitigate the genotoxicity. Use of vermicompost help to replenish the toxic material and rejuvenate the soil, a boost for sustainable agriculture.

**Key words:** Mitotic index, chromosome aberration, genotoxicity, vermicompost, solid waste, *Allium cepa*.

#### Introduction

The increasing discharge of hazardous chemicals into the environment has affected the balance of natural ecosystems and has consequently called the attention of several researchers and government agencies to the health of living organisms (Andrade, 2008). It is well established that pollution lowers the quality of life in various aspects. Besides the direct health effects, the subtle danger of pollutants lies in the fact that they may be mutagenic or toxic and lead to several human afflictions like cancer, cardiovascular diseases and premature ageing (Grover and Kaur, 1999). Agricultural soil are often contaminated with genotoxic chemicals (Krishnamurthy et al, 2006: saxena et al, 2004). The soil has been traditionally the site for disposal of genotoxic chemicals which need to be treated .Unlike organic compounds ,metals cannot be degraded (Salt et al., 1995) and their cleanup requires requires conventional remediation techniques (Jadia and Fulekar, 2008). Soil conditions can be defined by physical, chemical and biological methods depending on the functional aspects of the investigation. Bioassays provide a means of assessing the toxicity of complex mixtures like soil, without prior knowledge about their chemical composition. (Watanable, 2001). Accumulation of solid waste in the environment causes environmental pollution, toxic for all living organisms (Yuzbasioglue et al, 2008). When some toxic chemicals accumulated within the food chain to a toxic level, these chemicals affect directly the public health (kaymack and Goc Rasgele, 2009).

Cytogenetic tests in plants are relatively inexpensive and can easily be handled. Due to their size of their chromosomes, higher plants are suitable to cytological analysis and they have shown good correlation with other bio-testing systems (Fiskesjo, 2005) plant roots are extremely useful in biological testing. The root tips are often the first to be exposed to chemicals dispersed naturally in soil or in water .Allium cepa also enables the evaluation of different endpoints (Leme et al, 2009).

The objective of this study was to perform a comparative evaluation of the genotoxicity of five different soil types from Mercy College Campus using Allium cepa chromosome aberration (CA), micronucleus assays (MNC) and calculation of mitotic index to prove best soil type for sustainable agriculture.

#### **Materials and Methods**

Onion (Allium cepa (2n=16) were used as test organism. Ten clean and healthy bulbs of Allium cepa were chosen for each treatment groups. Soil samples were collected from 5 different sites of Mercy College campus- solid waste dumping site (SWL), open compost site (OC), windrow turned vermicompost (VC), vermicompost amended solid waste (SWL+VC) (10:1) and ground soil (GS) as

control. Before starting the experiment dry scales of bulbs were removed and allowed to germinate in ground soil for first 24 hrs at 25 ° C with regular light cycle. The healthy bulbs were selected and transferred to experimental plots. Ten bulbs with well developed roots were chosen from each experimental plot. Seed germination percentage, shoot length and root length were observed in different treatment groups. For mitotic studies, root tips of Allium cepa were fixed in farmers fluid, and hydrolysed in Acetic acid: HCl solution (45% Acetic acid: 1 M HCl) for 10 minutes and heated for 5 min. at 50°C. Root tip squashes were prepared in 2% acetocarmine solution. Atleast 1000 cells of each meristem were analysed. Different phases of mitosis were counted and chromosomal abnormalities were observed to calculate mitotic index, phase indices and total abnormality percentage at different phases. The mitotic index was calculated as ratio between the cells in mitosis and the total number of analysed cells in percents. The microscopic analysis includes mitotic index, micronuclei presence in interphase cells and chromosomal aberrations in different stages. The index of each phase of mitotic division was calculated as a ratio between the cell number in the respective period and the number of dividing cells in percents. The frequency of aberrant cells was calculated as percentage of the total number of analysed cells. The chromosome aberrations were characterized and classified in the following categories: bridges, fragments, laggards, micronuclei and disturbed metaphase etc.

The results were expressed as the Mean  $\pm$  SE and statistical comparisons were done by using student's t-test with P<0.05 indicating significance.

#### **Result and Discussion**

Seed germination was hundred percentages in vermicompost site where as less in open compost and solid waste leachate in comparison with the control. Germination potential enhanced in vermicompost amended solid waste sample. Morphological studies of Allium cepa indicated coiled and wavy roots in the plots of Solid waste leachate and open compost soil but no root abnormality was reported in VS. Mitotic index was normal in ground soil (15.50%), significantly higher in vermicompost (45.90%), minimum in solid waste dumping site (6.32%) and intermediate in vermicompost -solid waste amended soil (34.10%) (Table-1). Microscopic observations of squashes of Allium cepa . root meristem cells showed solid waste leachate induced a number of mitotic abnormalities when compare with control. The most common abnormalities were metaphase bridges, clumbed chromosome, laggards, disturbed anaphase, polypliod and micronucleus. The types and percentage of these abnormalities are given in Table-2 and Fig.1. Chromosome aberration (CA) and micronucleus formation was significantly high in solid waste soil (90.6±0.18%), less in open compost (29.28±2.08%) and significantly nil in contol and vermicompost soil; intermediate in solid waste-amended vermicompost (1.73±0.05%) (Table-2). The

result indicates that soil of the solid waste dumping site and open compost site accumulates different toxic chemicals which are manifested through decreased mitotic index and significant increase of chromosome aberrations. Solid waste amended with vermicompost helped to decrease the toxicity indicated by increased mitotic index from 6.32% to 34.10% which is higher than control (15.50%). It could be concluded that solid waste dumping at the campus is toxic to the plants and animals in the near vicinity. So the better solution for sustainable agriculture is to reduce the toxic potential by vermicomposting or use the vermicompost amended solid waste in the ratio 10:1.

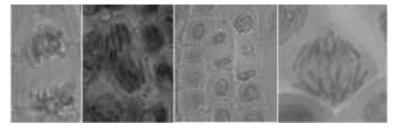


Fig.1- Some mitotic abnormalities observed in the root tips of *Allium cepa* L. (a) Disturbed anaphase (b) Mitotic bridge (c) Micronucleus (d) Diagonal anaphase

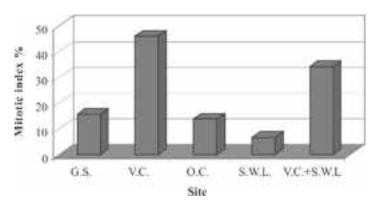


Fig. 2. Comparison of mitotic index between sites

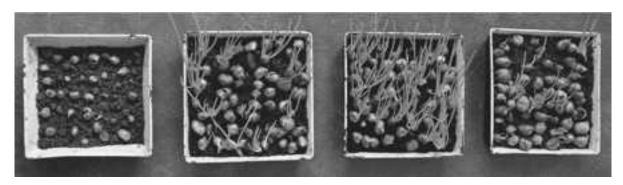


Fig. 3. Germination of A. cepa in different soil types. (a) SWL (b) SWL+VC (c) VC (d) GS

Allium cepa test has often been used for the determination of cytotoxic or genotoxic effects of various substances (Leme and Martin-Morales, 2009). It is considered to be a standard procedure for quick testing and detection of toxicity and pollution levels in the environment (Rank and Nielson, 1998). The cytotoxicity levels can be determined by the decreased rate of mitotic index. Mitotic index decrease below 22% of the control causes lethal effects on test organisms (Antonsie-Wiez, 1990) significantly high mitotic index in vermicompost site compared control site was due to the fact that earth worm activity enhances soil fertility. While decrease of mitotic index below 50% usually have sublethal effects (Panda and sahu, 1985) and is called cytotoxic limit value (sharma, 1983). In the present study, solidwaste leachate sample and open compost sample showed a low mitotic index 6.52% and 13.73% respectively. Significantly low mitotic index of solid waste leachate indicate sublethal effect of soil (Table- 1). The vermicompost is a rich source of beneficial microorganisms and nutrients and is used as a soil conditioner (Hattenschwile and Gaser (2005). Increased rate of mitotic index in vermicompost amended solid waste was in agreement with the findings of Jadia and Fulekar (2008). Earthworm activity enhances soil organic matter, improves nutrition and reduces toxicity (Welke and Parkinson, 2003). It is obvious from the result of the present investigation that solid waste leachate is cytotoxic on meristematic cells of plant tests (Fig.1 &Table-2). The cytotoxic effect has been evaluated at micro and macroscopic levels. Macroscopically we have observed reduction of root growth in SWL and OC. The cytogenic analysis showed that inhibition of root growth was due to the toxicity of SWL through disturbances of mitotic process and induction of chromosome aberrations and cell death. (Andrade et al, 2008). The inhibition of mitotic index can also be attributed to be the effect of environment chemicals on DNA/protein synthesis of the biological system (Chauthan et al., 1988). It is evident from the results that the mitotic index varied considerably in different treatment groups. The drop in mitotic index is very steep in the solid waste leachate site. It showed the mitodipressive activity of solid waste. Similar results were obtained by Keymak and Goc Rasgele (2009) in A. cepa. The reduction in mitotic activity may result from a blocking of G1 stage suppressing DNA synthesis (Mohandas and Grant (1972). SWL was toxic to a remarkable extent but vermicomposting of sludge might be beneficial for bioremediation and recommended before land filling (Srivastava et al. (2005). A. cepa has been used to evaluate DNA damages, such as chromosome aberrations and disturbances in the mitotic cycle (Leme et al. (2009).

Genotoxic activities of the solid waste samples induced micronuclei in the roots of *Allium cepa* indicates indicates the efficiency of Allium MN system in detecting clastogenic potential of soil pollution, these observations are in agreement with Ma *et al.* (1995); Minissi and Lombi (1997). The induction of micronuclei in root meristems of *A. cepa* is the manifestation of chromosome breakage and disturbance of the mitotic process due to spindle abnormalities (Dash *et al.*, 1988; Grover and Kaus, 1999). Micronuclei

were considered as in indication of a true mutation effect (Aeurbach, 1962), thus, the high percentage of the micronuclei induced in solid waste leachate soil sample indicates the mutagenic effect of them. On the other hand, the percentage of aberrant metaphase as well as anaphase cells for solid waste leachate and open compost soil sample indicates genotoxicity of the soil samples. High genotoxicity of the solid waste samples may be attributed to the accumulation of heavy metals and other mutagenic substances (Minissi and Lombi (1997). It was shown that metals could induce clastogenic and aneugenic effect including mitosis and cytokinesis disturbances (Dovgaliux *et al.*, 2001).

Table-1. Mitotic index test in *Allium cepa* root cells grown in five different soil samples at Mercy College campus.

Sint	Total cells	Dividing odby	Miratio Mass	%		r ı	hase (=de×
				Propheso	Motopheo	Апарінен	ToAophuse
c.s.	0.8 1000-	1554018	15.5 <b>-4.26*</b>	03.2240.12	passau, ba	7,7 <del>44</del> 0(())8	J4.19 0. J3
V.C.	1002-1.4	400+12	45.90±0.18*	48,3540,18	15.1840.08	11,690,13	24,21±0.06
n.c.	1440_1.12	1994/0.95	19.73±0.21■	41.9_1.35	21.910.12	12. <del>60</del> 1Jk.12	23.46_0.11
S.W.L.	LUKSE_UKO	7LLD.5	6.52_0.12*	35.0.11.26	10:010:04	10-Quo-un	45.0L:0.2X
V.C.:S.W.L	1000 -1.21	34140.62	]4,[_0,R]*	42.5 <u>2.0</u> 0.[9	28.73LJL17	13,48±0,07	17,59=0.06

\*P< 0.05. GS-Ground soil; V.C.-Vermicompost; O.C.-Open compost; SWL-Solid waste leachate

Table-2. Chromosome aberration and micro nuclear assay in Allium cepa

Sin	Това	Divis	Chromo	Chromosoma sherratiente (%)					
	47dls	n∎ co#n	C Bridge	Climbed chromosom c	<b>।</b> स्टुज्ञार्यः	mathrase q (problem	prejopilus	BENC	Total Alamonrating %
G.5.	1000+ 0.8	1.55±4 .R	ш	เฉ	a.o	DO .	B.O	0.0	1.20 =0.13*
V.C	1002±1. 4	46041 .2	na	6.15	NO.	nл	no On	no	0.25 <b>±0.0</b> %*
ë	1449±1 22	19 <b>05</b> 91	<b>K</b> 31	624	2 <b>.6</b>	920	9 <b>,6</b> 1	<u>4</u> 7	2 <b>4 28±2,198*</b>
¥.₩.⊥	100 <del>31-0</del> 0. 9	71 <del>14</del> 0. 5	6.7	25.11	7.1	45.5	3.0	4.0	90.6-0.18*
V/C+6. W L	]00 <b>6</b> ±1.71	341±+ 182	00	125	0.15	ų (ix	00	025	L/15 <b>-0.0</b> 54

<sup>\*</sup>P< 0.05GS-Ground soil; V.C.-Vermicompost; O.C.-Open compost; SWL-Solid waste leachate

The present study indicated a decrease of the soil genotoxicity after amending the solid waste leachate with vermicompost. Allium test might be used for cytogenetic monitoring of soils without preliminary extraction of the chemicals they contain (Dragova *et al.* 2009). The present study indicates the feasibility of vermicomposting for the clean up of toxic soil to mitigate the genotoxicity. Use of vermicompost help to replenish the toxic material and rejuvenate the soil, a boost for sustainable agriculture. Biremediation of solid waste disposal site with vermicompost in the ratio 10:1 was ideal before starting agriculture.

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Structural and electrical properties of Antimony (iii) Selenide thin films

Jessy Mathew N.

Department of Physics, Kongunadu Arts and Science College, Coimbatore, TamilNadu.

Abstract

Thin films of Sb<sub>2</sub>Se<sub>3</sub> with different thicknesses deposited at room temperature by vacuum evaporation technique. The films are characterized by structural and surface morphological analyses by means of X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM) respectively. Films were found to have amorphous structure. The current - voltage characteristics in the temperature range 300-453K at thickness range 50-300nm are ohmic in the lower field regime followed by non-ohmic behaviour in the higher voltage regime. It is satisfactorily explained by Poole-Frenkel effect. The frequency dependence of the dielectric loss of each sample demonstrates the ohmic nature of the loss. Properties such as dielectric constant, loss function and electrical conductivities as a function of both frequency and temperature are reported.

**Keywords:** Thin films, X-ray diffraction, Electrical properties, Antimony Selenide.

Introduction

Antimony Selenide (Sb<sub>2</sub>Se<sub>3</sub>) has wide applications to thermo electric cooling devices and optical devices owing to its high thermoelectric power and photoconductig properties. Antimony Selenide is a semi conducting chalcogenide of the V<sub>B</sub> and VI<sub>B</sub> groups of elements. Generally the electrical property of semiconductors depends on the chemical composition.

The aim of the present investigation is to obtain a high dielectric semiconducting material and to monitor the temperature and frequency dependence of its dielectric constant and dielectric loss.

**Materials and Methods** 

The powder of Antimony selenide [99.99% purity], was kept in the molybdenum boat of 200 amps and heated with high current controlled by a transformer. Deposition of Sb<sub>2</sub>Se<sub>3</sub> on preplanned glass substrates under the pressure of 10<sup>-6</sup> Torr. The thickness of the films was measured by Quartz crystal monitor. The samples prepared in a similar environment were used for studying their various properties. The structural analysis can be performed by using various techniques such as X-Ray Diffraction (XRD), Scanning Electron Microscopy.

-40-

(SEM). Scanning Electron Microscopy technique was employed to the present study to analyze the surface morphology of as deposited and annealed  $Sb_2Se_3$  films of thickness 3000 Å. In SEM the secondary electrons are primarily used.

The dielectric studies on Sb<sub>2</sub>Se<sub>3</sub> films were carried by forming Metal Seciconductor-Metal (MSM) structures. The capacitors were formed on a substrate with the dielectric layer in between the two metal electrodes so as to form an MSM structure. Aluminium has been used for electrode deposition in the present work. The capacitance of (C) and the dissipation factor (D) for MSM structure in the frequency range 100 Hz to 100 KHz at different temperatures (300 to 483 K) were measured using a Digital LCR meter. All the measurements were carried out under a rotary vacuum condition. A copper-constantan thermocouple is employed to sense the temperature. Area of the capacitor was measured using traveling microscope.

Current-Voltage characteristics of antimony selenide films of different thicknesses were studied for dc electrical measurements by measuring the values of current for different voltages at different temperatures.

#### Results and discussions

X-ray analysis for the composition of Sb<sub>2</sub>Se<sub>3</sub>

X-ray diffraction patterns of Sb<sub>2</sub>Se<sub>3</sub> films are shown in Fig. 1. It is clear that all the investigated films are amorphous. The annealed film with 423K shows single peak. This shows microcrystalline structure (Fig2).

# **SEM** studies

The SEM photograph is presented in Fig. 3 for  $Sb_2Se_3$  film with thickness 3000 Å annealed for  $150^{\circ}C$ . The film shows microcrystalline nature only. As seen in the photograph, the surface of the film is homogeneous.

#### **AC Conduction studies**

The defects on the as deposited films can be removed by heat treatment. Vacuum deposited MSM structures were annealed at about 373K in a rotary vacuum. Annealing in vacuum improves the dielectric properties. This is because annealing is a process related with stress relief and local structural rearrangement. Fig. 4 indicates the variation of dielectric constant () with frequency. The dielectric constant decreases with increasing frequency. When the frequency is increased, the orientational

polarization decreases since it takes more time than electronic and ionic polarization.

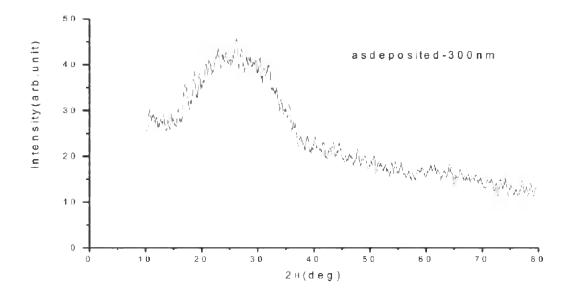


Fig 1.XRD Pattern for Sb<sub>2</sub>Se<sub>3</sub> Film (as deposited) of 3000Å thicknesses

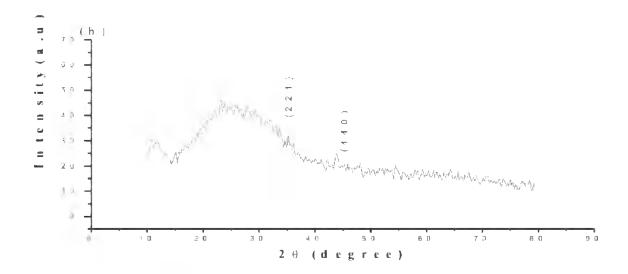


Fig. 2. XRD diffractogram of annealed  $Sb_2Se_3$  thin films (423K) of 3000 Å



Fig. 3. SEM images of anneald for Sb<sub>2</sub>Sb<sub>3</sub> thin film of 3000 Å thickness.

The variation of tan ä with frequency is shown in Fig. 5. The loss factor increases with increase of temperature at high frequencies and it is almost a constant at low frequencies. At high frequencies the loss factor increases which may be due to the effect of lead resistance.

The temperature dependence of the permittivity for various frequencies has been shown in Figs. 6. Increase of with temperature can be attributed to the fact that the orientation polarization is connected with the thermal motion of molecules, so dipoles can not orient themselves at low temperatures.

Fig. 7 shows the temperature dependence of ac conductivity. In all the three cases, it is observed that the conductivity increases with temperature. The activation energies have been determined from the slopes of these curves at different frequencies. (Table 1)

Frequency	<u>Activati</u>	on Energy(e	<u>V)</u>
(KHz)	Th	ickness Å	
	500	1000	3000
1	0.2859	0.5952	0.6448
10	0.2422	0.4364	0.4761
100	0.1489	0.3472	0.3968

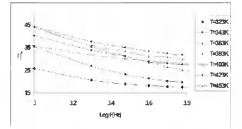


Fig.4 Frequency dependence of dielectric constant (t=3000Å)

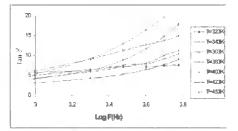
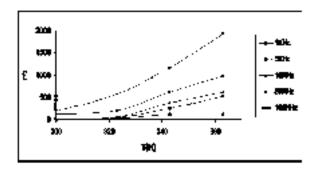


Fig.5 Variation of Tan  $\theta$  with Frequency



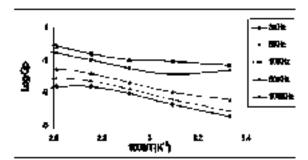


Fig.6 Variation of permittivity with temperature

Fig. 7 Dependence of temperature on conductance at different temperatures

# **DC** Conduction studies

For different thickness the current voltage characteristics of  $Sb_2Se_3$  films are shown in Fig.8 at different temperatures. From these figures it is clear that the current (I)- voltage (V) dependence is of the form  $IV^n$ . The theoretical values of  $\hat{a}_{PF}$  and  $\hat{a}_{RS}$  are  $2.48 \times 10^{-5} \, (\text{mV})^{1/2}$  and  $1.24 \times 10^{-5} \, (\text{mV})^{1/2}$  respectively. The experimental values of  $\hat{a}_{PF}$  are given below.

Thickness(û)	Temp.(K)	$\hat{a}_{exp}*10^{-5}(mv)^{1/2}$
	343	2.46
500	383	2.24
	423	2.58
	343	2.47
1000	383	2.32
	453	2.42
	343	2.43
3000	363	2.55
	403	2.49

The conduction mechanism for thermally evaporated  $Sb_2Se_3$  film of various thicknesses may be of the Poole-Frenkel type. The experimental values of  $\hat{a}_{PF}$  for film of different thicknesses are almost equal to the theoretical value by Poole-Frenkel equation.

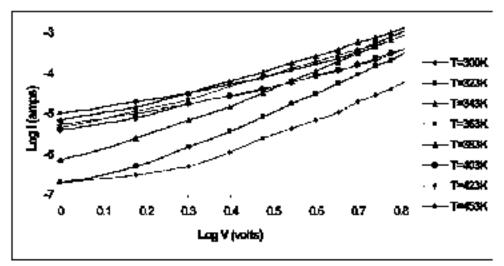


Fig. 8. I - V Characteristics of Sb<sub>2</sub>Se<sub>3</sub> film at different temperatures

The structural analyses have been performed by X–ray diffraction technique showed that the as deposited Antimony Selenide thin films are amorphous in nature. The surface morphological studies have been done by Scanning Electron Microscopy (SEM). The micrographs of SEM studies showed that the surface of the film is homogeneous. The studies on AC conduction of these thin films predict the semiconducting features based on a hopping mechanism. The type of transport phenomenon observed in the films under DC field, ascribed to a modified Poole-Frenkel mechanism.

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# Study of water quality parameters for iron removal

Manju priya. P.

Department of Chemistry, Mercy College,
Palakkad, 678008, Kerala, India.

#### **Abstract**

The quality of water is of vital concern for man kind since it is directly linked with human welfare. The water required for public water supplies need not be pure but it should be portable. Treatment of drinking water supplies is a matter of great public health importance and to assist in food assimilation it should contain some minerals. The amount of mineral and other elements in water should be in appropriate proportion. The study is mainly concerned with the analysis of iron content in water, which is a serious problem encountered by common people. The greatest problem faced by the modern man is the water crisis. So it is not possible for us to waste even a drop of water due to iron contamination. By using the aeration technique we can purify and use this type of water without wasting it. Moreover this technique is inexpensive and can be carried out easily without the use of any chemicals by common man. Water is an essential or basic need for the existence of all living organisms. In the borewell samples the concentration of iron is greater. This occurs due to the occurrence of rocks in palakkad district. Total hardness of the last two samples exceeds the limit to a greater amount. Its consequences are fatal because hardness may cause a series of biological and physiological impact. To ensure safety to public health, economy and utility in industries, it is the duty of the authority to thoroughly check, analyze and treat the raw available water to safe and permissible limits before supplying to public. This must be strictly followed when water is supplied for domestic uses as drinking, bathing, washing etc. The authorities should take necessary actions to remove iron content of water to ensure public health.

Key words: Water treatment, water crisis, iron content.

# Introduction

Water is the most precious gift of the nature to mankind.water is contaminated by different factors and iron is one of such contaminant. Iron is the second most abundant metal after aluminium and fourth most abundant element in the earth crust. Because of its high abundance, iron is often found as an impurity in other metals. Under proper conditions, iron will leach into the water supply from the rock and soil formations. Water having a low pH tends to be corrosive and may dissolve iron in objectionable quantities from pipes, pumps and other equipment. Iron is often difficult to treat. This is due primarily to the fact that iron can be present in several forms, and each form can potentially require a different method of removal.

A common method was adapted and iron removal was effectively conducted. To check the physical and chemical parameters of drinking water sample collected from different area of Mundur panchayath and Chittur block. To find out the samples having iron above the desired limit. (0.3ppm to 1ppm). To remove the excess of iron by using the "aeration" method. To check the water quality parameters after the removal of iron. To find out whether the treated water is portable or not, thus making a useful contribution to society.

#### Materials and methods

#### Study area

For the physico- chemical analysis of water and removal of iron content in water samples are collected from the following sources:

Sample No: 1(open well) this sample is taken from open well situated near IRTC in Mundur panchayath.

Sample No: 2 (pond) this sample is taken from pond situated near IRTC in Mundur panchayath.

Sample No: 3 (bore well This sample is taken from a bore well situated 3Km away from IRTC in Mundur panchayath.

Sample No:4(bore well) This sample is taken from a bore well situated in Chenthoni in Chittor block.

Sample No:5 (bore well) This sample is taken from a bore well situated 1/2Km away from the above bore well situated in Chenthoni in Chittor block.

# Analysis of water quality parameters

#### physical parametres

#### Electrical conductivity and Total dissolved solids

EC and TDS of the water sample is determined with the instrument ELICO EC-TDS ANALYSER CM 183.

# **Chemical parameters**

# Determination of pH

The pH of the sample is tested using the instrument ELICO pH METERS, L1120/L1160.

# **Determination of chloride: (Argentometric method)**

Chloride is determined by the titration with standard AgNO<sub>3</sub> solution in which white precipitate of AgCl is first formed. After the conversion of all chlorine to AgCl, the excess AgNO<sub>3</sub> reacts with potassium chromate to form reddish brown precipitate of silver chromate. The amount of AgNO<sub>3</sub> utilized for acquiring the end point is equivalent to the chloride content of the sample.

$$AgNO_3 + Cl \qquad \qquad AgCl + NO_3$$

$$(White)$$

$$2AgNO_3 + K_2Cr_2O_4 \qquad \qquad Ag_2CrO_4 + 2KNO_3$$

$$(Reddish brown)$$

#### **Determination of hardness**

The concentration of calcium and magnesium expressed as equivalent CaCO<sub>3</sub> is considered as a measure of total hardness. The calcium and magnesium ions of the sample are titrated with EDTA to form stable Ca-EDTA and Mg-EDTA complex. A small quantity of eriochrome black T (EBT) is added to water sample, buffered at pH10 to form a solid wine red complex. During titration the EDTA will first complex all the free calcium and magnesium ions. These ions would then dissociate from their complexes with EBT, to form more stable complexes with EDTA, and EBT is set free. The solution assumes the blue colour of the free EBT. This marks the endpoint.

When EDTA is added to water containing both calcium and magnesium, it combines first with calcium. Calcium can be determined directly using EDTA, when the pH is made sufficiently high that the magnesium is largely precipitated as the hydroxides and an indicator is used that combines with calcium only. Murexide is used as the indicator. On titration with EDTA, the solution turns from pink to purple.

#### **Determination of alkalinity**

The alkalinity of water is a measure of its capacity to neutralize acids. NaOH is standardized against KHP using phenolphthalein as indicator. Using this NaOH 0.1N HCl is standardized .Exact 0.02N HCl is prepared from 0.1N HCl. 50ml of the given water sample was pipetted out into a clean conical flask. Added a drop of methyl orange indicator. Titrated with HCl till yellow colour changes to orange red. Titrations are repeated for concordance.

#### Determination of fluoride (SPADN'S Method)

The SPADN'S colorimetric method is based on the reaction between fluoride and a zirconium dye lake. Use a 10ml sample or a portion diluted to 10ml with distilled water and 2ml SPADN'S reagent. Mix well and read the absorbance at wavelength 570nm using fluoride pocket colorimeter.

#### Determination of iron - Phenanthroline method

The ferric form of iron is reduced to ferrous form by boiling with HCl and hydroxyl amine hydrochloric acid. Phenanthroline is added at pH between 3.2 and 3.3 to form chelated complex of orange red colour with iron. Three molecules of 1, 10-phenanthroline is required to form a chelated complex of iron with each ferrous ion. The colour obeys Beer's law and the intensity of colour is independent of pH from 3-9. 25ml of well mixed sample is taken in a conical flask. 2ml concHCl and ml hydroxylaminehydrochloride solution are added. It is then boiled for 25 minutes to ensure dissolution of ferric iron. This is then cooled and transferred to standardflask.10ml ammonium acetate buffer and 2ml 1, 10 phenanthroline solution are added and diluted to 100ml. Mix thoroughly and allow at least 10-15 minutes for maximum colour development and absorbance is measured at 510nm. The values of iron are directly determined from graph. A graph is drawn by taking absorbance along Y-axis and standards along X-axis. A straight line is obtained from which unknown concentration of iron is obtained according to Beer's law.

#### Removal of iron

#### Aeration

Aeration is the technique used for the removal of iron. 1L of iron contaminated water is allowed to expose in air .Then ferric hydroxide is precipitated and settled at the bottom of the container. This is the carefully filtered using cotton. The filtrate is collected. Iron content in this is analyzed using phenanthroline method. For large quantities of water air is bubbled up through the water. The iron will precipitate, since the air has oxygen contained within it. Following the aerator a settling tank and filter should be utilized to remove the precipitated iron. This method can effectively remove a large amount of iron dissolved in water. This method involves the following reaction:

$$4\text{Fe}^{2+} + \text{O}_2 + 10\text{H}_2\text{O} \quad 4\text{Fe} (\text{OH})_3 + 8\text{H}^+$$

The process of converting ferrous iron to ferric iron is dependent upon several factors. The most important factor for effective iron removal is the pH level of the water. Higher levels are more favorable for effectively oxidizing the iron. A pH level of 6.8 or higher is desirable. Low pH water can also be effectively treated for iron removal but the process becomes a little more involving. Rate of precipitation

and filtration are accelerated in practice by contact and catalysis. Water is allowed to trickle over coke or crushed stone. The deposition of hydrated oxides of iron and bacteria on the contact media is believed to act as catalyst which accelerates the oxidation of iron. Thus this is a simple and inexpensive treatment for the removal of iron and this can be easily carried out by common people without the use of any chemicals.

# **Result and Discussion**

From the Table-1 it is clear that the iron content of the samples 4 and 5 are very much greater than the permissible limit. So these two samples are subjected to iron removal by the simple technique of aeration. The results are tabulated in Table-2.



water sample collected from chenthoni



Iron containing water after aeration



Water after iron removal

Table 1 Water quality parameters analysed before removal of iron

PARAMETERS	SAMPLE	SAMPLE	SAMPLE	SAMPLE	SAMPLE
ANALYSED	1	1	3	1	1
pii	7.5	7.2	7.8	7	7.53
EC(µs)	197	226.4	197	1883	761.3
TDS(ppm)	120.3	140.8	120	1106	485.4
Hardness	94	28.2	136.3	678.9	3196
Ca- hardness (ing/l)	33.91	7.54	47.J	143.17	67.82
Mg-hardnesa (mg/l)	2.29	2.29	4.57	126.83	36.56
Chloride (regil)	13.72	36.59	22_87	546.63	54.89
Alkaihi(y(ng/l)	118.56	80.56	190	688.2	643 80
Fluoride(mg/l)	0.24	N.D	0.24	0.84	0.93
Iros (mg/l)	0.94	1.16	0.32	10	3.2

Table-2 Comparison of water quality parameters analysed

PAHAMRTERS ANALYSED	BEPOMERRE	MOVAL OF IRON	AFTER RESIDVAL OF IRON		
	SAMPLE	SAMILE	SAMPLE	SAMPLE	
	4	ā	4	3	
DE1	•	9.53	1	1/12	
EU (ca)	1443	761.3	1551	< % T	
TDS(ppm)	11.08	455.4	1008	*44	
Li ardinesa (mw Li	975.5	219.6	775.3	300.5	
Ca- hawthrea(ing3)	140. 7	65.82	1 340	247	
Me-bardacesqug-II	.25 83	36,36	109.6	-1.7	
4. il lertdormg/ii	MSC	24,59	19.7	757	
Alkalinity(mg/r	568.2	(43.HH	230 (1	222.7	
Mույո <b>ւկա</b> (ուցի)	कारु।	Ous	385	034	
tros(mg/l)	10	3.2	925	920	

The range of pH for potable water should be 6.5-8.5. From the analysis report it can be followed that all samples are with in this limit. The permissible limit for electrical conductivity is between 50-500 ms. Here sample 1, 2 are with in the limit. But all the other bore well samples exceeds this limit. But the value of EC decreases after the removal of iron. The permissible limit of TDS is 500ppm. All samples except sample 4 are in the permissible limit. Even though the value decreases after the removal of iron it is not in the permissible limit. The permissible limit of total alkalinity is 200ppm.Sample1, 2, 3 have permissible limit of alkalinity. But 4 and 5 have high alkalinity due to the precipitation of Fe(OH)<sub>3</sub>. After the removal of iron it decreases very much. But then also it is slightly higher than permissible limit. The desirable limit of total hardness for drinking water is 300 mg/l. This type of water is used for domestic purpose. Here sample 1, 2,3 are with in the limit and are useful for domestic purpose. But the hardness of sample 4 and sample 5 are greater than the desired amount. After the removal of iron sample 5 became potable. But sample 4 is again very hard. The desirable limit for calcium hardness is 75mg/l. All the four samples are with in this limit. But sample 4 is having greater value of calcium hardness. The desirable limit of magnesium hardness is 30-150 mg/l. All the samples have magnesium hardness with in the limit. The desirable limit for chloride is 250mg/l. All the four except the sample 4 have chloride content with in this limit. Sample 4 have high chloride content due to geographical condition of that area and due to over exploitation of water. The desirable limit for fluoride level is 0.6-1.2. Low fluoride levels are linked with dental carries and above 1.5 it may cause fluorosis. In three samples the fluoride content is with in the limit. But the sample 1 and 2 has low fluoride content. The desirable limit for iron is 0.3ppm. This may be extended up to 1ppm. Samples 4 and 5 have high iron content. So a simple method was adopted for the removal of the excess iron content. In this method, involving the aeration technique, the iron content decreased from 10 ppm to 0.55ppm which is in the tolerance limit. But if we subject the same water for iron removal by using KMnO<sub>4</sub> the result will be more efficient and more iron content will be removed. But the after effect of this method is that purified water will have a purple colour. Thus this water cannot be used for drinking purpose. According to Michael C Keller in his iron removal methods oxidation due to KMnO<sub>4</sub> suffers the following disadvantages: It is not easy to work with as it stains just about any thing it contacts. It is toxic and is expensive, and, if overfed, the water will have a pink to purple colour. It consumes a lot of time.

Thus it becomes difficult for the common people to use  $KMnO_4$  for the removal of iron. The aeration technique is also very efficient for the removal of iron as it consume less time for the precipitation of iron. The efficiency of this method is clear from the above result. More over this technique can be easily followed by common people as it requires no chemicals. Thus aeration is a more acceptable method.

To ensure safety to public health, economy and utility in industries, it is the duty of the authority to thoroughly check, analyze and treat the raw available water to safe and permissible limits before supplying to public. This must be strictly followed when water is supplied for domestic uses as drinking, bathing, washing etc. The authorities should take necessary actions to remove iron content of water to ensure public health.

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# Study of water quality parameters with special emphasis on fluoride and its effective removal methods

Nishamol B.

Department of Chemistry, Mercy College, Palakkad, 678006, Kerala.

#### Abstract

Different samples of drinking water were collected from Mundur and Chittur block of Palakkad District. The physical and chemical parameters of the collected samples were checked. The parameters include the analysis of pH, Electrical Conductivity, Total Dissolved Solids, Total Hardness, Total Alkalinity and Fluoride level in drinking water. The fluoride content was determined colorimetrically using SPADN's reagent. Two of the collected samples had fluoride content above the desirable limit. The high fluoride containing samples were subjected to defluoridation techniques like 'Nalgonda' and 'Alum'. 'Nalgonda' is a technique in which aluminium sulphate and lime is used as the coagulant whereas 'Alum' is a technique in which only alum is used as the coagulant. These techniques were employed because this is the cheap, best and the most effective method which can even be used by a common man. The physico chemical parameters were analysed before and after defluoridation and found that fluoride level had decreased to considerable extent after defluoridation. The other quality parameters were also found within the desirable limit and water obtained after defluoridation was found potable, and hence the project makes a useful contribution to the society.

**Keywords:** pH, Electrical Conductivity, Total Dissolved Solids, Total Hardness, Total Alkalinity, Fluoride, SPADN's reagent, Nalgonda, Alum, defluoridation.

# Introduction

The desirable limit of fluoride in drinking water is 1ppm as set by World Health Organisation. Fluoride is actually like a two edge sword. High concentration of it causes endemic fluorosis whereas low concentration of it causes dental caries. Hence fluoride poor water should be fluoridated and fluoride rich water should be defluoridated to maintain the fluoride level within the desirable limit.

#### **Materials and Methods**

The physico chemical parameters of the collected samples are analysed. pH which expresses the acidity or alkalinity of the solution is measured by Electrometric method using a pH meter. Electrical Conductivity – It is the water's capacity to carry electric current which also determines the quantity of dissolved salts in the water sample is measured by using an EC analyzer. TDS -Total Dissolved Solids

include both dissolved solids and suspended solids are measured using a TDS analyzer. Total Hardness include the concentration of both calcium and magnesium salts in water is measured titrimetrically using EDTA as titrant and Eriochrome Black-T as indicator. Total Alkalinity which is a measure of its capacity to neutralize acids is also determined titrimetrically using standard HCl. The important parameter – 'fluoride' is determined by colourimetric method. Here the two solutions viz. SPADN's solution – Sodium-2-(parasulphonyl azo)-1,8 dihydroxyl – 3,6- naphthalene disilphonate and Zirconyl acid – ZrOCL2.8H2O – Zirconyl chloride octahedreate are mixed in equal volumes to obtain SPADN's reagent. This serves as the colouring agent.

#### Procedure

10 ml of the sample and 2 ml of SPADN's reagent are mixed thoroughly. The fluoride present in the sample reacts with the reagent to produce a red coloured complex whose intensity is measured using a colorimeter at 570 nm filter using fluoride pocket colorimeter.

# Defluoridation methods - Nalgonda and Alum

Both the methods are based on the simple principle of coagulation flocculation-sedimentation and filtration.

Nalgonda technique involves the use of two chemicals like aluminium sulphate and lime. First of all, lime is added to make the water alkaline followed by aluminium sulphate and vigorously stirred for 10 minutes when 'cotton wool' like flocs develop (aluminium hydroxides) which is then subjected to simple settling followed by filtration. The main contents of the fluoride are removed along with the flocs. The physico-chemical parameters are checked at every 2 hours of contact time.

The Alum technique also involves the same principle and procedure with the only difference that lime is not added in this technique.

# Results and discussion

Samples were collected from different sources like pond, openwell and borewell. Among the samples analyzed, two of the samples which are collected from Chittur had fluoride concentration above the desirable limit. The other quality parameters like TDS, hardness and alkalinity are also found high in these samples.

Table.1-Chemical analysis of watersample

Ac Samble	Source	ħΠ	lw. g≴vem	TDS ppin	Total Handress ppn	Dbu. Ca	Mg	Al calinity ppm	duorice ppn
-	Pund	7.2	226.4	140.8	28.29	7,559	3,292	80.56	0.35
2	OW	7.5	197	120,3	94,3	34-01	2,292	118.5	0) 34
3	BW	7.8	197	120	136.73	4724	4.584	190	0.28
-1	HW	<b>6</b> .5	732.1	4584	315.9	79.37	28.65	172.7	2 84
5	ΒW	7.9	788.3	4658	437 3	122 8	36.67	273.7	3.5

# **Defluoridation studies**

One of the samples with high fluoride concentration was defluoridated using Nalgonda technique and the other sample using Alumtechnique.

Table 2. Defluoridation studies

Lype	Chemicals used	Amauut in 94	Time of stirring	Carit <b>act</b> time
Nalgonda	Alum ling	0.5+0,25	10 minutes	8 hours
Alum	Alum	1	15 minutes	8 Eours

# Nalgonda technique

The initial fluoride level before treatment was above the desirable limit. The Nalgonda technique was employed and checked for fluoride level at the interval of every 2 hours. At the 8<sup>th</sup> hour, it was reduced upto desirable limit. When checked after 8 hours, the fluoride level was increased. Thus 8 hours is the optimum contact time where maximum fluoride removal takes place.

Table 3. Nalgonda technique

Confuet <b>Gi</b> ne	Flooride ppm	£111	Ю: µ8/ст	TDS ppm	foful Hardness ppm	hbin (,ar	Mg ppm	Alkalinity pptd
Before treatment	2.84	6.5	722.1	1587	ف.دا و	79.37	28,65	172.7
2 hours	1,25	5.8	728,2	4572	330,2	\$0.43	27,42	195.3
4 hours	1.13	8.7	722.4	1569	331.1	81.62	27.12	198.2
( hours	1.15	8.6	719.3	4564	335.4	\$2,42	27,42	200.3
8 hours	1.05	8.5	714.6	4558	336.9	83.14	27.42	302.4

# Alum technique

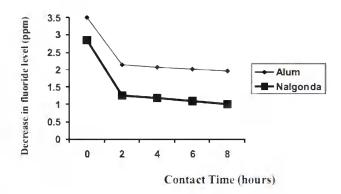
The Alum technique also follows the same trend as that of Nalgonda i.e., with increase in contact time, there is decrease in fluoride level. Here also,  $8^{th}$  hour is the optimum contact time where maximum fluoride removal takes place.

Table 4. Alum technique

# Comparison of fluoride level

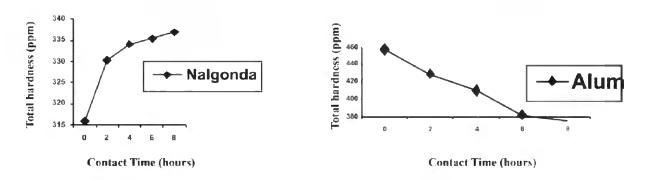
Cambet time	Fluoride ppm	µ11	PC µS/cm	TDS ppm	Total Hardness ppm	bbm Ca	Mg ppm	Alkalinity ppm
Before treatment	1 <sub></sub>	7.0	788 (	4658	457.33	0.3.8	34.67	274.7
2 hours	1,14	6.9	776.4	4975	429 56	119,1	32.09	178.2
4 hours	2.06	6.8	770.7	4971	410.20	103.9	32.09	169.4
6 haurs	2.0	67	760 1	50119	381.91	1013.1	\$2.0V	ñl a
в нашта	1.17	ħη	757, 4	5014	374 70	93.63	37.09	158.4

In both the techniques, there is decrease in the fluoride level with increase in contact time till 8 hours. Defluoridation capacity of both the techniques was calculated and found that Nalgonda was more efficient than Alum technique.



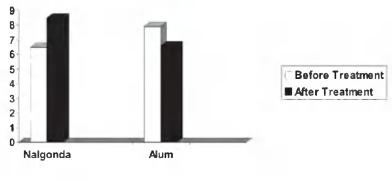
# Comparison of total hardness

The study shows that, in Nalgondatechnique, there is increase in total hardness with increase in contact time, which is due to the addition of calciumoxideduring defluoridation process. In the Alum technique, there is decrease in total hardness with increase in contact time.



# Comparison of pH

The variation of pH in both the techniques before and after treatment was found out. The pH of the treated water in Nalgonda increases becomes slightly alkaline. It is due to the addition of CaO and can be reduced by optimum amount of CaO during defluoridation process. The pH of the treated water in Alum technique decreases, which can be minimized by using optimum amount of Alum during defluoridation.



Physical and Chemical parameters of the collected samples were analysed. High fluoride containing samples were defluoridated using Nalgonda and Alum techniques. Different contact time were carried out during defluoridation and found that 8th hour is the optimum time. Nalgonda technique was found to be the better of the two because of its high defluoridation technique, low residual aluminium in the treated water. Lastly, my suggestion is that, the mass media like newspaper, television, radio should come forward to spread the knowledge of its illeffects caused by fluoride in water and also to spread the knowledge of the defluoridation techniques available to the common man so that the 'fluoride devil' can be conquered at the domestic level.

# Acknowledgement

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# Study on adsorption efficiency of Tamarind Nut Carbon and Tamarind Nut Peel for the removal of Zinc, Chromium and Iron from Polluted Water

Rashmi Ramachandran

Department of Chemistry, Mercy College,
Palakkad, 678008, Kerala, India.

#### Abstract

Tamarind (Tamarindus indica) is one of the common spices grown widely all over India. India is the major producer of tamarind in the world. In the tropic zone, tamarind is used in many dished and traditional drinks, but the commercial cultivation of the crop was intiated only recently. Every part of the tree is useful, specially the fruit. Recently, much attention is given to the various constituents in tamarind which find use in both medicinal and industrial field. Even through traditional processing is widespread; its commercial uses are unknown and underdeveloped. There is no doubt that tamarind has got a glorious future ahead, provided sufficient attention.

Adsorption using agricultural product has great significance now a days. Tamarind is a common tree grown in India especially in kerala. Their climatic conditions suit this area very well. But most of the tree parts are disposed as waste. The carbon prepared from tamarind nut and powdered tamarind nut peel can be used as adsorbents for the removal of metals like Chromium, Iron, and Zinc, Chromium, Iron from the polluted water. The study also focuses on the comparison of the removal efficiency of tamarind nut carbon with activated charcoal and verification of Langmuir and Freundlich Adsorption Isotherm for the removal of Zinc from polluted water using Tamarind Nut Carbon.

Key words: Tamarind nut peel, activated charcoal, Tamarindus indica.

# Introduction

Removal of toxic metal contaminants from polluted water is one of the most important environmental and economic issue today the ever increasing demand for water of high quality has caused considerable attention to be focused towards recovery and re-use of waste water.

Freshwater resources all over the world are threatened not only by over exploitation and poor management but also by ecological degradation. The main source of fresh water pollution can be attributed to discharge of untreated waste, dumping of industrial influent, and run-off from agricultural fields. Industrial growth, urbanization and the increasing use of synthetic organic substances have serious and adverse impact on fresh water bodies. All of these have caused a great increase in the concentration of toxic heavy metals.

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Since most of heavy metals are non-degradable, their concentration must be reduced into acceptable levels before discharging them in to the environment. Otherwise these could pose threat to the world health and affect the quality of protable water. According to the world Health organization the metals of most immediate concern are chromium, zinc, iron, mercury and lead. Adsorption, by low cost absorbents such as saw dusts, moulds, yeasts, agricultural products such as wool, rice straw ect... is the most versatile and widely used method. The aim of the study is: To find the effectiveness of tamarind nut carbon for the removal of zinc from polluted water and compares its efficiency with activated charcoal and to verify langmuir & Fruedlich adsorption Isotherm To find effectiveness of tamarind nut peel for the removal of chromium and iron. Quantitative estimation of zinc, iron and chromium is determined by spectrophotometer.

#### Materials and methods

# Preparation of tamarind nut carbon

Tamarind nut procured is washed with distilled water, dried at  $110^{\circ}$ c, cut in to small pieces and sieved. Then it is treated with concentrated sulfuric acid in 1:1 weight ratio and kept in an air oven at  $150^{\circ}$  cf or 24 hours. The carbonized material was washed with distilled water to remove free acid and dried at  $105^{\circ}$ C. Then it was repeatedly soaked in 1% sodium bicarbonate until effervescence ceased and finally soaked in sodium bicarbonate solution for 2 days to remove any residual acid. The material was then washed with distilled water, dried at  $105^{\circ}$  and again sieved. This Tamarind nut carbon is used for the analysis.

# Preparation of Tamarind Peel Powder

Tamarind nut procured is washed, dried and cut into small pieces and sieved. It is then roasted and the peel of the tamarind nut is taken separately. The Peel is then sieved and powered.

Preparation of Stock Solution

**Zinc:** 1g of zinc dust is accurately weighed dissolved in minimum amount of dilute nitric acid and is diluted to 1000ml using distilled water.

**Chromium:** 2.5g of potassium dichromate is accurately weighed, dilute with distilled water and made up to 250ml.

**Iron:** 1g of Ferric alum is weighed and dissolved in concentrated hydrochloric acid, diluted to 1000ml using distilled water.

#### **Procedure**

Zinc: The stock solution was dilute to get a series of solution in the range 10-50 ppm (taking 4, 6, 8, 10, 12ml and made up to 100ml) and transferred to shaking flask. Added accurately weighed 1g tamarind nut carbon, shaken for 1 hour and immediately filtered through Whatmann no: 1 filter paper, 20ml of filter was pipetted into a beaker, added 5ml acetate buffer, 1ml sodium thiosulphate solution, 10 ml Dithiazone reagent. Shaken well for 4 minutes and kept for 10minutes. The CCl<sub>4</sub> layer was run to dry boiling tube and the optical density was found out at 520nm.conducted a blank. Experiment was repeated using activated charcoal as adsorbent.

Chromium: The stock solution was diluted to get a series of solution in the range 35-225ppm (taking 1,2,3,4 ,5,6ml and made up to 100ml) and transferred to shaking flask. Added accurately weighed 1g tamarind nut carbon, shaken for 1 hour and immediately filtered through Whatmann no: 1 filter paper. Added 3.3 ml 6N H<sub>2</sub> SO<sub>4</sub>, 1 ml diphenyl carbazide to 1 ml of filtrate and made up to 100 ml. absorbance is found at 520 nm. Absorbance of original set before adsorption is also found out shaking time and amount of adsorbent was varied. Experiment was repeated with tamarind nut peel as adsorbent.

Iron: The stock solution was diluted to get a series of solution in the range 35-225 ppm (taking 1,2,3,4,5,6 ml and made up to 100 ml) and transferred to shaking flask. Added accurately weighted 1g tamarind nut carbon, shaken for 1 hour and immediately filtered through Whatmann no: 1 filter paper. Added 5 ml 4N HNO3, 10ml 20% potassium thiocyanate to 10 ml of filtrate and made up to 100 ml. Absorbance was found at 490 nm. Absorbance of original set of solution before adsorption was also found out. Shaking time and amount of adsorbent was varied. Experiment was repeated with tamarind nut peel as adsorbent.

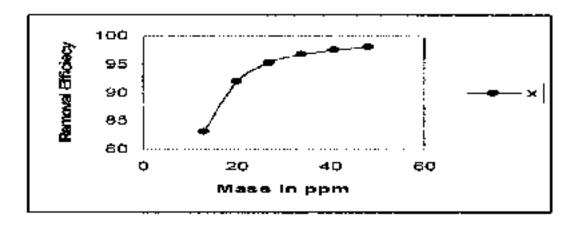
#### **Results and Discussion**

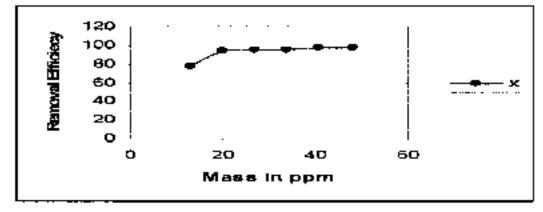
Adsorption using tamarind nut carbon as adsorbent was carried out for all the three stock solutions; zinc, chromium and iron. It was found that zinc could be removed efficiently using tamarind nut carbon. The adsorption efficiency of tamarind nut carbon as adsorbent for the removal of zinc was compared with the removal efficiency of activated charcoal. The shaking time of the solution was increased from 1 to 2 hour, and was found to be independent of shaking time. It was found that iron and chromium could not be removed using tamarind nut carbon. Increasing the shaking time from 1 to 2 hour and amount of adsorbent also has same effect. As Tamarind Nut carbon was found to be ineffective for removal of chromium and iron Tamarind Nut peel was used.

# Adsorbent: tamarind nut carbon

Table -1.zinc & charcoal adsorbent: tamarind nut carbon shaking time: 1hour

Initial Concentration (ppm)	Removal Efficiency (%)	Zinc Charcoal
13	83.12	77.56
20	91.94	93.75
27	95.3	95.8
34	96.77	95.8
41	97.6	97.6
48	98.17	97.2



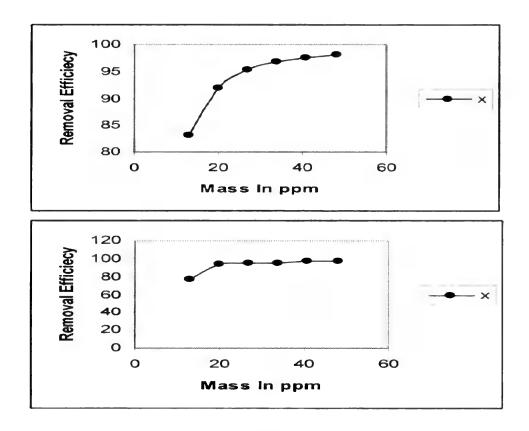


It was found that both iron and chromium could be removed efficiently using tamarind nut peel was used. It was found that both iron and chromium could be removed efficiently using Tamarind Nut peel. Adsorption studies are carried out for the adsorption of zinc by Tamarind Nut carbon.

Iron shaking time: 1 hour(concentration: 11.5 ppm)

Table-2 zinc & charcoal shaking time: 2hour

Initial Concentration (ppm)	Removal Efficiency (%) Zinc Charcoal					
13	83.12	77.56				
20	91.94	93.75				
27	95.3	95.8				
34	96.77	95.8				
41	97.6	97.6				
48	98.17	97.2				



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Table-3 chromium shaking time: 2hour

Initial Concentration (ppm)	Mass of Chromium After Adsorption
	(ppm)
35.35	35.35
70.71	70.71
106.10	106.16
141.475	141.475
176.84	176.84
212.213	212.213

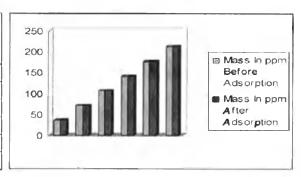
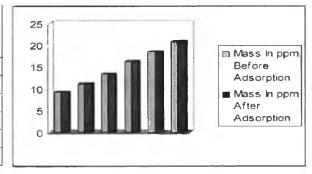


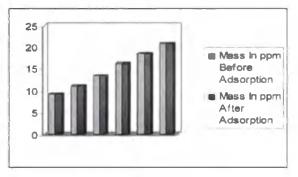
Table – 4 iron shaking time

Intial Concentration (ppm)	Mass of Chromium After Adsorption (ppm)	
9.26	9.26	
11.5	11.5	
13.38	13.39	
16.21	16.21	
18.53	18.53	
20.84	20.84	



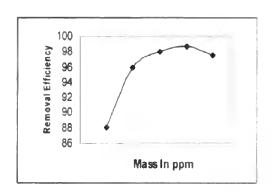
Iron shaking time: 1 hour(concentration: 11.5 ppm)

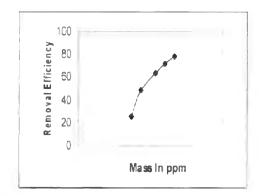
Amount of Adsorbent (g)	Mass of Chromium After Adsorption (ppm)	
1	11.5	
2	11.5	
3	11.5	
4	11.5	
5	-11.4	
6	11.4	



Chromium adsorbent: tamarind nut peel shaking time: 1hour

Initial Concentration (ppm)	Removal Efficiency	
35	88.07	
70	95.99	
106	98	
141	98.75	
176	97.60	
212	97.21	





# Adsorption studies for the removal of zinc by tamarind nut carbon freundlich adsorption studies

Freundlich proposed an empirical equation and was known as Freundlich Adsorption Isotherm. The equation is as follows: Where, X=amount of substrate, M=amount of adsorbent, C= concentration of solute in g/1, K and n are constants.

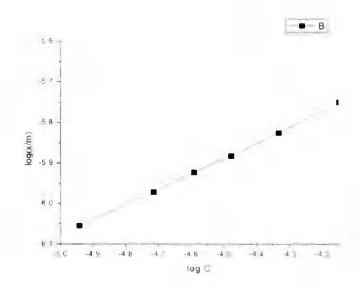
Taking logarithm of the equation we get.

# Log(x/m) = log k + (1/n) log c

 $If log \, (x/m) \, is \, plotted \, against \, log \, c \, a \, straight \, line \, should \, be \, obtained. \, The \, slope \, of \, the \, line \, will \, give \, the \, value \, of \, 1/n \, and \, the \, intercept = log \, k.$ 

Aplot of log(x/m) vs. log c is found to be linear for adsorption of Zinc by tamarind nut carbon.

Log(x/m)	Log c
-4.9411	-5.6343
-4.7218	-5.7793
-4.5816	-5.8827
-4.4778	-5.9546
-4.3948	-6.0087
-4.3254	-6.0555

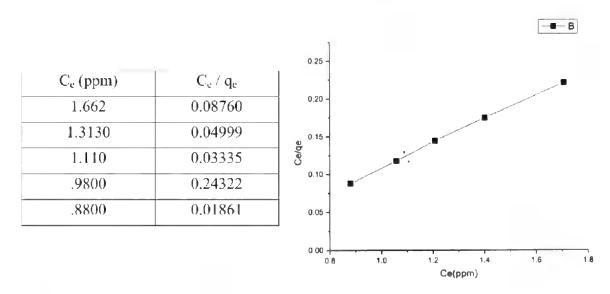


# Langmuir adsorption studies

Langmuir proposed an empirical equatum for monolayer adsorption into a surface of finite number of identical sites. The quantitative relationship is:  $(C_e/q_e) = (1/Q_0.b) + (C_e/Q_0)$  Where,  $C_e =$  equilibrium concentration in mg/1.qe = amount adsorbed at equilibrium.  $Q_0$  and b are Langmuir constants.

Langmuir constants are related to adsorption capacity and energy of adsorption respectively. If  $C_e$  /  $q_e$  is plotted against  $C_e$  a straight line should be obtained.

A plot of  $C_e/q_e$  vs.  $C_e$  is found to be linear for adsorption of Zinc by tamarind nut carbon.  $Q_0$  and b are determined from Langmuir plots and is found to be 139.3922 mg/g and 1.4348 mg/1.



In this study it is found that: Adsorption using agricultural waste, tamarind nut carbon and tamarind nut peel is an effective, simple and low cost method for the removal of inorganics like Zinc. Chromium and iron from polluted water. For the removal of Zinc from polluted water Tamarind Nut Carbon is a more effective adsorbent than Activated Charcoal. Chromium and Iron can be removed efficiently using Tamarind Nut Peel. Adsorption of Zinc using Tamarind Nut Carbon obeys Freundlich and Langmuir Adsorption Isotherms.

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# **Evaluation of Porphyrin derivative for potential use in Phytodynamic therapy of cervical cancer cell lines-He La**

Revathy. K. A.

Cancer Biology Department, Rajeev Gandhi Centre for Biotechnology,
Thiruvanathapuram, Kerala.

#### **Abstract**

The present study was done to evaluate the phototoxicity of a Porphyrin derivative (SJR103) in cervical cancer cell lines –HeLa .A general cytotoxicity assay (MTT assay) was carried out to determine the toxicity of this drug and dose dependent growth inhibition was obtained in light. Cell cycle analysis was done using FACS using Propidium Iodide and the cells treated with the drug were found to be arrested at G2-M phase. Finally PDT induced apoptosis was confirmed by flow cytometry using AnnexinV-FITC and fluorescence microscopic studies using Hoescht dye and AnnexinV-FITC

Key words: Porphyrin derivative, cervical cancer, HeLa, cytotoxicity assay.

#### Introduction

Cervical cancer or cancer of the cervix is an abnormal growth of malignant (cancer) cells in the cervix. It is the second most common cancer in females and it is successfully curable in the early stages. Cervical cancer occurs most commonly between 40 and 55 years of age. In India, despite the grim reality that eight women die every waking hour to cervical cancer, only 5% to 6% of them, who are more than 35 years, take the diagnostic test. India's millions need a miracle to rein in the largest cancer killer-74,000 women in the country die every year of it. On an average, 1.2 lakh new cases of cervical cancer are detected annually. The situation calls for a public health miracle.

Killing of tumor cells by chemotherapy, radiotherapy, immunotherapy, or suicide gene therapy is predominantly mediated by triggering injury in cancer cells which leads to cell death. The medical need for advances in cancer treatments has made only a modest overall impact on mortality. Cancer chemotherapy suffers major drawback as chemoresistant cells develop within the tumors due to their heterogeneous nature, most importantly in response to therapy under different treatment regimens, and due to inadequate drug delivery methods. Hence, the significance of discovering new targets, pathways and strategies for therapeutic intervention in cancer is obvious objective.

A recent addition to cancer treatment is the photodynamic therapy. The isolation of porphyrins and their inherent tumor localizing properties coupled with its ability to generate reactive singlet oxygen

when activated by light of particular wavelength which in turn results in cytotoxicity led to the emergence of a new modality namely, photodynamic therapy (PDT) as a therapeutic tool, which could be the answer. The higher degree of selectivity offered by this modality and fewer side effects when compared to chemotherapy and radiotherapy has prompted the researchers around the globe to generate new photosensitizers. Porphyrins and expanded porphyrins are one class of molecules under intense investigation due to their photosensitizing ability for PDT application. Here evaluation of a Porphyrin derivative (SJR103),was done for potential use in Photodynamic Therapy in Cervical Cancer cell lines, HeLa.

#### Materials and methods

#### Revival of preserved cell line

The cryovial was taken from -80°C and transferred to a 37°C water bath for thawing. To this, fresh medium supplemented with 10% serum was added. This was centrifuged at 3000 rpm for 5 minutes at 4°C. The supernatant was discarded and pellet was resuspended in 20% medium. The flask was viewed under an inverted microscope and incubated at 370C in a carbon dioxide incubator.

#### Maintenance of cell culture.

HeLa from ATCC (American Type Cell culture), were grown in DMEM containing 10% Foetal Bovine Serum and 1% antibiotic antimycotic cocktail. Cultures were examined daily using an inverted phase contrast microscope (10x to20x). The general morphology and freedom from microbial contamination was ensured. A medium change was given after 2 days, using a sterile pipette, and spent medium was replaced with fresh one. When the cells were observed to be 80 to 90% confluent, as seen by the area occupied by the growing cells at the basement surface of the flask, media was removed and the cells were washed twice with PBS-EDTA solution. (EDTA is a chelating agent which chelates calcium ions which are needed for cell attachment. The washing step will help to remove fetal bovine serum also from the flask which is an inhibitor of trypsin). PBS-EDTA solution was removed and 0.5ml of trypsin (0.25%) was added. The flask rocked and kept in the incubator for 5 minutes. The progress of the enzyme treatment was checked every few minutes with an inverted phase contrast microscope. Cells were found to be rounded up as time prolongs. Once all the cells were seen to be detached from the surface, 5 ml of fresh medium was added to the flask slowly. The cells were resuspended in 1 ml fresh media to remove the action of trypsin which may become toxic to the cells on prolonged exposure. Cell counting was performed using a hemocytometer.105cells were seeded in a new flask and kept in incubator for further growth. All the procedures were carried out aseptically in a laminar air flow unit.

# Cell viability assay

Confluent flasks were removed from incubator and checked under the microscope for contamination and cell morphology. Cells were enzymatically disaggregated and to appropriate number of cells were plated into 2 microtiter plates and kept for incubation. Next day after removal of the media drug was added in approximate concentration after serial dilution. A control was also kept using the same amount of DMSO. After hours of incubation 1 plate was exposed to light and the other was kept in dark. After 48 hours of incubation, plates were removed from the incubator and 10microlitre of MTT (5mg/ml stock) was added to each well of the plate. The plates were placed in the incubator for 4 hours. After 4 hours, the supernatant was removed carefully taking care that the formazan crystals formed are not removed and added 100 microlitre of isopropyl alcohol to each well. The plates were covered with aluminium foil and kept on a shaker until the crystals are dissolved. The absorbance was read at 570nm.

Percentage growth inhibition was calculated as

% Growth inhibition = ((control-test)/control)  $\times 100$ 

# Analysis of apoptosis

# Flow cytometry using Annexin V-FITC

3ì l AnnexinV-FITC was added to the cells treated with drug and also to the control and vortexed and incubated for 15 minutes in dark. The cell suspension was diluted using 200 µl binding buffer. This suspension was subjected to FACS analysis. From the tubes the cells were deposited on glass microscopic slides by a cytocentrifuge, and analyzed by fluorescence microscopes.

# Staining using Hoescht dye

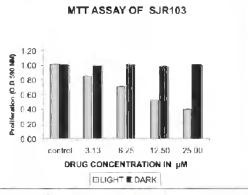
The cells treated with the drug and the control were fixed with 4% formalin, stained with the DNA binding dye Hoechst 33258 (4 mg/ml), deposited on glass microscopic slides by a cytocentrifuge, and analyzed by fluorescence microscope.

#### Results and discussion

# A. MTT Assay

Photocytotoxic effect of porphyrin derivative (SJR103) in HeLa cell lines was initially investigated using MTT assay. Cytotoxicity of the drug was analysed with and without irradiation using Sodium Vapour lamp (600 nm) and the following results were observed. In the presence of irradiation there is significant growth inhibition and the IC50 value obtained was 13  $\mu$ M whereas in the absence of light or drug there is no growth inhibition. From the graph it's clear that growth inhibition is dependent on concentration of the drug.

#### Cell Proliferation in Light and Dark



D.Annexin-FITC staining

## % Growth Inhibition in Light

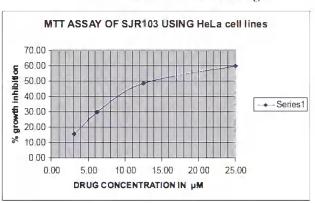




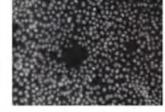




Figure: 1 Figure: 2 Figure: 3

Annexin V-FITC is a protein that has got high affinity towards the phospholipid Phosphatidyl Serine. It can be used to indicate apoptosis exploiting the fact that apoptosis causes a flip-flop of PS from the inner membrane to the outer membrane of plasma membrane thus exposing the binding site of PS to the dye. This is visualized in a fluorescence microscope. In the above diagram Figure: 1 indicates the picture of the cells treated with both light and drug, taken by phase contrast microscope. Figure: 2 represents the picture of the fluorescence emitted by the same cells when they were treated with the dye Annexin V-FITC. Figure: 3 is the superimposed picture of figure 1 & 2. fom these figures it can be inferred that apoptosis has occurred and that only the cells in which apoptosis has occurred bind the dye and fluoresce.

E. Hoescht staining



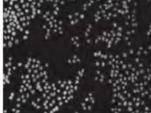




Figure: 1 -light control

Figure: 2 -dark control

Figure: 3-test

Hoechst is a dye that binds to the nucleus and not to the cytoplasm. It can be used to indicate chromatin condensation which is the preliminary process that occurs during apoptosis. In the above picture, Figure: 1 represents the cells that were treated with light alone and here the nuclei of all the cells fluoresced more or less uniformly. Figure:2 represents the cells that were treated with the drug alone. Here also the observations were similar. Figure:3 represents the cells treated with both the photosensitizer and light. Here the intensity of fluorescence was different among the different nuclei of the cells. In some nuclei intensity was more indicating more uptake of the dye due to chromatin condensation, an indication of apoptosis. In this figure there is also a shrinkage of the nuclei of the cells which again confirms apoptosis.

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# Control measures of the larva of Asura conferta

Sheeba.K.M ¹and T.N.Sarasu²

¹ Department of Zoology, Mercy College, Palakkad
²Department of Zoology, Govt. Victoria College, Palakkad

#### Introduction

The moths and butterflies included in the order Lepidoptera are soft bodied and vary greatly in size from minute to very large insect.. They are the very beautiful insects but at the same time many of the lepidopteran larva acts as serious pest in the crop field. The larva of many moths cause serious health problem also. One among them is the larva of *Asura conferta* walker coming under the family – Arctiidae and subfamily – Arctiinae. K.P. Srivastava (1996) defined pest as "an insect whose population increases to such an extent as to cause economic loses to crops or a nuisance and health hazard to man and his livestock." In that sense *Asura conferta* walker which is a public nuisance insect can also be considered as a pest.

Asura conferta are mostly seen through out Kerala. It is found during rainy season only since the larva of the insect feed on algae and moss plants found during this time. Body of the larva is long, vermiform, and fleshy with a thin black skin. Three yellow horizontal lines are seen on the dorsal surface of the body. The urticating or irritating hairs which cover the larval body cause irritation either by mechanical action or by secreting a poisonous substance. Asura conferta is a holometabolous insect. Average number of eggs laid is 250-300 in large clusters. Incubation period is 7 – 8 days. Eggs hatches in to larva having seven instars. The sixth and seventh instars larva bears a tuft of hairs on the upper region of the two thoracic segments. Crochets are biordinal and mesoseries. The seventh in star larva undergoes pupation. Pupa is pupa obtecta and the pupal shelter is made by its own hairs. The adult month emerges from the pupa.

The larval hairs cause serious health hazards such as skin irritation, inflammation, itching etc. so control of the larva is an urgent requisite. This can be control by means of physical methods like collection and burning or using chemical methods by the application of insecticides or using biological methods. Biological method using biological agents are widely accepted process. Because *Asura conferta* are seen in the vicinity of human being so the control measures should not cause any harm to humans.

Insect population increases every year. The occurrence and the Public nuisance created by the larvae have been reported from many places by many dailies. Much work has not been done in this regard.

The larval hairs cause serious health hazards such as skin irritation, inflammation, itching etc. so control of the larva is an urgent requisite. This can be control by means of physical methods like collection and burning or using chemical methods by the application of insecticides or using biological methods. Biological method using biological agents are widely accepted process. Because Asura conferta are seen in the vicinity of human being so the control measures should not cause any harm to humans.

Insect population increase every year. The occurrence and the Public nuisance created by the larvae have been reported from many places by many dailies. No study has been conducted anywhere in this world regarding this. So we have selected this problem of insect for my present project.

#### Materials and methods

Adults and larvae were collected from many places in and around Palakkad district. The larvae were reared in the laboratory. They were fed with algal groups identified as cyanophyceae and chlorophyceae and some mosses. Adult were allowed to lay eggs. The eggs were incubated. Hatched larvae were also utilized for the experiments..





Table-1 Collection sites

DATE	PLACE OF COLLECTIONS
2:06:2007	5 <sup>th</sup> mile, chittur (p.o), Palakkad (Dis)
6:08:2007	Chittur, Palakkad (Dis)
7:09:2007	Puthunagaram, chituu (p.o)
8:09:2007	5 <sup>th</sup> mile, chittar (p.o)
11:09:2007	Pulbunagaram, chittur (p.v)

The agents used for control measures, their preparation and applications are as follows

Kerosene - soap Emulsion

Finely sliced, 500g of ordinary bar soap dissolved in 4.5 liters of water by cooling. Cooled and added 9litres of kerosene oil under violent agitation till the oil is fully emulsified. The stock solution can be diluted with 15-20 times before spraying. Three batches of larvae consisting of ten each were taken in three Petri dishes. 5 ml of the diluted solution was sprayed on the larvae (Topical Application).

#### Tobacco decoction

This can be prepared by steeping 500g of tobacco wastes in 4.5 liters of water for 24 hours. Then 120g of ordinary bar soap dissolved in 1.5 litres of water separately in another vessel. The soap solution is added to Tobacco decoction under violent agitation. Three batches of larvae consisting of ten each were taken in three Petri dishes. 5ml of the above solution was sprayed on the larvae (Topical Application)

#### Neem kernel suspension (NKS)

The kernels should be ground in to a coarse powder. The effective Concentration of NKS ranges from 0.1 to 0.3 %. For obtaining 0.3% Concentration 3g of powered neem seed is required perlitre of water. The required quantity of the coarse powder should be put in a small bag of muslin Cloth and dipped in water contained in a bucket for about 12 hours. Thereafter Squeeze the cloth bag repeatedly after dipping in the fluid unit. The out flowing Fluid turns light brownish. The NKS is now ready to be sprayed. Three batches of larvae consisting of ten each were taken in Petri dishes. 5ml of the NKS solution was sprayed on the larvae.

#### Neem oil

The larvae hatched from the incubated eggs were reared. The Second instar larvae were used for the experiment. 5 ml of the concentrated neem oil was taken sprayed on them.

#### Common salt

50% stock solution was prepared by weighing 50g of salt and made up in to 100ml of distilled water. From the stock solution, 5 ml solution as taken and sprayed on the hatched larvae (first instar).

# **Result and Discussion**

It is observed that, in laboratory conditions the following agents caused high mortality rate.

Kerosene – soap emulsion: The treated seventh instar larvae showed sudden death. They wriggled very fast, coiled and died. Out of the three batches having ten each, nine died in the first batch, eight in the second batch and all the ten in the third batch. Percentage of mortality is 90%. Tobacco decoction: When 5 ml of the solution topically applied by spraying on the sixth instar larvae. They slowly

showed a knock down effect. Among three batches having ten each eight larvae died in first batch, six in the second batch and seven in the third batch. Live forms showed very least activity. Rate of mortality is 70%. Neem kernel suspension: When 5 ml of the solution topically applied by spraying on the forth instar larvae of each batch having ten each nine larvae died in each batch. A kind of liquid oozes out from the larval body. Live form showed very least activity. Rate of mortality is 80%. Neem oil (concentrated): When 5ml concentrated neem oil was sprayed on the 150 second instar larvae all showed sudden death. Rate of is a 100% mortality. Salt water: When 5 ml of the 50% stock solution was topically applied by spraying on the hatched larva that is the first instar larvae, all them died rapidly. There were about 211 larvae. It shows 100% mortality.

Table-2 Mortality rate of Asura conferta

ACENT USED	PREPARATION AND APPLICATION	NUMBER AND STAGE OF THE LARVA	NO OF LARVA DIED	940F MORTALITY
Karenene — esep Emuldon	Shing of ordinary harmony distanced in 4.5 litres, of water builted and cooled. Nitres of Kerosene ell anticed under violent agricultum (stock). Diffusion of stock reflection 14 times. Topical application by approxima.		• •	99%
Tebecco decoction	Steeping 50by of seburce waster in 4.5ktres water for 14 hours and 12by of ordinary bar soap is siteout and disselved in L58ktrs water. Topical application by spraying	(II) 10 (6 <sup>th</sup> laster)	5 6 7	79%
Neem karnet Suspension (NKS)	4.3% conevatated NKS put in the muslin cloth bug dipped in water containing backet (5lites) for 12 hours, sprayed readily.	(00) 10 (4 <sup>th</sup> laster)	•	20%
Norm vil (consentrated) Suit water	Smi concentrated neem all was approved an interact From the SMS-atock saintion, Smi enton, and approved on hatched larves		159 Ž11	100%

Larvae of *Asura conferta* walker is becoming a major problem nowadays. The nuisance created by the larva has been reported by many dailies. The public seeks helps from the researchers for controlling the larvae. The present project was taken upon the basis of this. In this work, a number of agents were used to control these larvae. In most cases high mortality was obtained.

The seventh instar larvae showed 90% mortality in the sixth instar larvae. Tobacco decoction showed 70% mortality in the sixth instar larvae. Neem Kernel suspension showed 80% mortality in the fourth instar larvae. The first instar and second instar larvae showed 100% mortality respectively.

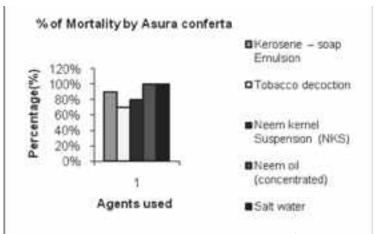


Fig.1 Percentage mortality rate of Asura conferta-larvae

It has become clear in this work that the agents such as Kerosene emulsion, Tobacco decoction, Neem Kernel Suspension, Neem oil(Concentrated) and Salt Water ,etc., are very much effective in controlling the larvae. All of them pollution free, low cost method and easy to handle. This is an added advantage also. All the agents tried in this work are biosafe and do not crate any health hazards to human or other domesticated animals. They are biodegradable also. This can be applied in public places as well as in houses. A complete eradication of the insect is a difficult task. Every year, the population size seems to increase and the larvae invade new areas also. More and more research work in this field is required before selecting a final solution for the pest.

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# Synthesis, characterization and antimicrobial study of mixed ligand complex of cobalt

Mercy C.I. and Teena James

Department of Chemistry, Mercy College

Palakkad, 678006, Kerala.

#### **Abstract**

Mixed Ligandcobalt (II)complex, formulated as Na<sub>2</sub>[Co{C<sub>6</sub>H<sub>6</sub>H<sub>4</sub>(OH)COO} (C<sub>6</sub>H<sub>5</sub>CONHCH<sub>2</sub>COO).2H<sub>2</sub>O]. 8H<sub>2</sub>O formed with salicylic acid and hippuric acid have been synthesized and characterized by their infra-red spectra and electronic spectra. Salicylic acid shows a bidentate behaviour with coordination occurring through carboxylate oxygen atom and oxygen atom of the hydroxyl group. Hippuric acid also acts as a bidentate ligand coordinating through the carboxylate oxygen atom and the nitrogen of the amido group. The fifth and sixth positions are satisfied by the water molecules. Antimicrobial studies of the complex were done using gram negative and gram positive bacteria. It is the compared with various standard antibiotic discs. The Co (II) complexes have been suggested to show six fold octahedral structures.

Key words: Mixed Ligand cobalt (II) complex, Salicylic acid, Hippuric acid, Antimicrobial

#### Introduction

It is well known that mixed ligand coordination complexes play an important role in biological systems. Hippuric acid (N-benzoylglycine) is a  $\,\acute{a}$ -amido acid containing an acidic COOH, basic NH and a substituent benzoyl group. It is thus capable of forming metal chelates. It has a special biological significance as it is found in the urine of camel. Its alkali, calcium and magnesium salts have also been used as solubilizers in the preparation of aqueous solutions of some sparingly soluble medicinal compounds. Several binary and ternary metal complexes of hippuric acid and its derivatives have been reported. It can coordinate with metal ions through the only carboxylate oxygen atom acting as a monodentate ligand or bidentate; or it may also coordinate through both the carboxylate oxygen atom and the nitrogen of the amido group and thus acts as a bidentate ligand. Salicylic acid (derived from the Latin word for the willow tree, Salix alba, from whose bark it can be obtained) is a beta hydroxyl acid (BHA) with the formula  $C_6H_4(OH)$  COOH, where the OH group is adjacent to the carboxyl group. This colourless crystalline organic acid functions as a plant hormone.

#### **Materials and Methods**

#### Preparation of mixed ligand complex of cobalt

Solution of salicylic acid and hippuric acid were prepared by dissolving them in one equivalent of sodium hydroxide. The cobalt chloride solution was prepared in one equivalent of hydrochloric acid. To prepare the metal complex, the two ligands are mixed with 0.1M metal ion solution in 1:1:1 molar ratio at room temperature. At first salicylic acid solution was mixed with cobalt (II) chloride solution followed by hippuric acid solution and the P<sup>H</sup> of the resulting solution was adjusted to 4.38. The solution was then concentrated over a steam bath and allowed to crystallize. The crystalline precipitate was then filtered and washed with 50% ethanol water mixture. It is then dried in an air oven and heated at 100-110° C for two hours, it loses water and the colour of the complex changes from pale pink to purple colour.

The IR spectra of ligands and the complex were recorded on a FTIR-8400S spectrometer in the range 4000-400cm<sup>-1</sup> in KBr disc. Systronics UV-VIS spectrometer- 117 was used to record the electronic spectra of mixed ligand cobalt complex of salicylic acid and hippuric acid in methyl alcohol in 200-800 nm range.

#### Antimicrobial studies of the complex

#### Preparation of nutrient agar media

Nutrient agar media constituents: Peptone-5g,Beefextract-3g,NaCl-5g,Agar-18gOther Requirements: Sterilized conical fask, glass beaker, measuring cylinder, glass rod, spatula, cotton plug, weighing balance, P<sup>H</sup> meter, autoclave or pressure cooker.

# Procedure

The conical flask, glass beaker, spatula, glass rod etc are washed clean with distilled water and kept in hot air oven for sterilization. Weighed out the media ingredients one by one into the sterile glass beaker using spatula and dissolved well in distilled water. Media constituents for 100ml is calculated, weighed and dissolved and made upto a little less than 100ml with distilled water.P<sup>H</sup> meter is standardized and P<sup>H</sup> of the media is adjusted to 7.0 by adding required amount of 1 N NaOH. The media is then made up to 100ml using distilled water and then transferred to sterile conical flask. Agar is weighed out and added to the media. After closing the conical flask tightly with cotton plug the agar in the media is melted by heating. This media is then kept for sterilization in pressure cooker for 20 minutes. After sterilization, media is left to cool and solidify. This solidified media can be used further for microbial isolation process.

#### **Antimicrobial Activity Test**

The sterilized Petri plates are taken and then shown into the flame. The media is then poured into different Petri plates and allowed to solidify. Using sterilized cotton swabs, the bacterial culture was spread evenly over the media in these different plates. Three holes were created at proper positions of the Petri plates using gel documentation system such that the zones of clearance do not overlap. The two ligands, SA & HA and the cobalt complex were introduced into the three holes of the two Petri plates, one containing bacteria E.coli and the other containing bacteria Bacillus Subtillis. The same experiment was repeated with a various standard Antibiotic discs such as Cefazolin, Gentamicin, Erythromycin etc. Rather than creating a hole on the Petri plate, placed the discs on the swab culture and pressed them gently. The Petri plates are then kept for incubation at 37° C for 24 hours. After incubation, the results are recorded. From the above results a comparative study of the activity of the complex and the antibiotic discs were done.

#### Results and discussion

#### IR studies

Mixed ligand cobalt complex of salicylic acid and hippuric acid

Table-1.IR frequencies of hippuric acid, salicylic acid and their cobalt complex

Hippuns soid (unit)	Salicyte wid	$Nadj(cos,A-8N2H,OJoH+O/cm^3) =$	Bare, assignment
		5/60	· (0°F) was:=
		5623.0%	nin Jis
		583£ <u>22</u>	
1424	-	3116.75	6(NII)
747	10 2445	•	o(C n) ca broyle acid
	-	161131	o <sub>a</sub> 000
466.10	-	1405.87	o <sub>p</sub> COO o COO - unide " lamé
760 1407 9		1973	Amazili (cMIFOCN)
.340	12 76	1321.15 977.34	Basicard bureausing ngCNP and smide III (1675) aNC I) band ngC Chiet passes.
	38 67	2750	1009Oyef prome
	74 70 (0)		8.3 K yafph and
		665.51	jal (Hijmy) il mor valla
		167 +6	

Hippuric acid and Salicylic acid show the characteristic U(C=O) absorption band for carbonyl group at 1747 cm<sup>-1</sup> and 1664.45 cm<sup>-1</sup> respectively, which vanishes in the case of metal complex. Instead, the saymmetric and symmetric stretching frequencies are obtained. The metal complex shows u<sup>as</sup> COO frequencies at 1641.31cm<sup>-1</sup> and 1409.87cm<sup>-1</sup> respectively. In salicylic acid u(OH) absorption band is observed at 2867cm<sup>-1</sup> which reduces to 2750.30cm<sup>-1</sup> in the metal complex. The u(NH) vibration in hippuric acid observed at 3420cm-1, which in the metal complex appears ~3116.75cm<sup>-1</sup>. Hippuric acid shows amide I u(C=O) band at 1606.59cm<sup>-1</sup>, amide II (dNH+uCN) band and benzene ring vibration is in 1560-1407.9cm range and amide III (uCN+dNH) band at 1340cm. In metal compex, amide I band, amide II band, benzene ring and  $u_{as}$  COO vibrations are mixed together to give a broad band at 1552cm<sup>-1</sup>. The amide III band is mixed together to give a broad band at 1552cm<sup>-1</sup>. Salicylic acid shows u(C-O) vibrations at 1276.79cm<sup>-1</sup> which reduces to 977. 84cm<sup>-1 in</sup> the metal complex. Salicylic acid also shows d(OH) vibration at 1460.01 cm<sup>-1</sup> which vanishes in the metal complex. The appearance of rocking r<sub>c</sub> (HOH) frequencies at 686.61cm<sup>-1</sup> and 487.96cm<sup>-1</sup> in the metal complex shows the presence of coordinated water molecules. The metal complex also shows some additional bands in the range 3622.07-3944.16cm<sup>-1</sup> attributable to water molecules. The IR frequencies of the ligands and the metal complex are given in the table-1. It may be then inferred that in the mixed ligand cobalt complex, both salicylic acid and hippuric acid acts as bidentate ligand in which the salicylic acid coordinates through carboxylate oxygen atom and the oxygen atom of the hydroxyl group, whereas hippuric acid coordinates through its carboxylate oxygen atom and the nitrogen atom of the amido group. The fifth and sixth positions are satisfied by water molecules.

#### Antimicrobial study

Antimicrobial test for salicylic acid, hippuric acid and complex are given in table 2 and 3.

Table-2 Antibiotic Sensitivity of the complex

Organism	Antibiolic disc used	Lhsc	Zone	Remark
		Conten	diameter	
		ന്ത്രാളു	(mm)	
Bacillus	Cefazolia (CZ)	30	10	Registant
	Gentamicin(GM)	10	2.5	Sensitive
Stabtillis	Pryther yan(UR)	H	20	In.errechate
	Na <sub>2</sub> [(Co-HA-SA)	. <b>-</b> 0	24	Sensitive
	2H <sub>2</sub> O[/8H <sub>2</sub> O			
	Cefazolia (CZ)	30	8	Resistant
	Gentamicin(GM)	10	20	Sensitive
E.coil	Frythomygin(ER)	11)	No 2006	Registant
	Na:[(Co-HA-SA)	~-10	30	Sensitive
	211-01.811:0			

From the above results, it is inferred that the complex show high antimicrobial activity when compared to the two ligands. It means that the complex is very sensitive to the microorganisms that is, for both Gram negative and Gram positive bacteria. Comparison of the Antimicrobial activity of the complex with various standard antibiotic discs are shown in the table -2.

Table-3 Antimicrobial Activity of the complex

Organism	Comparand	Content (ineg)	Zone d'ameter (ronu
Bacillus	Hippuric acid	-30	.6
Subtitis			
	Salicyte acid	·- jiJ	22
	Nas[(Co-HA-SA) 21150],81150	<b>-30</b>	33
	Hipperic seid	×30	12
lyroit	Salicylic soid	~30	.9.5
	Nagl(Co HA SA) 2H <sub>2</sub> OJ,8H <sub>2</sub> O	30	v

In the Petri plates swabbed with microorganism Bacillus Subtilis: The antibiotic disc cefazolin (CZ) show no antimicrobial activity. Hence cefazolin was resistant to the bacillus Subtills. The antibiotic disc Erthromycin (ER) show a little antimicrobial activity and it was having an On the other hand, the antibiotic disc Gentamicin show a high antimicrobial activity when compared to CZ and ER and it is very sensitive to Bacillus Subtilis. The cobalt complex of salicylic acid and hippuric acid also show a high antimicrobial activity comaparable to that of Gentamicin and hence it is very sensitive to intermediate sensitivity to Bacillus Subtilis. Bacillus Subtilis. In the Petri plates swabbed with microorganism E.coil: The antibiotic disc Cefazolin (CZ) shows little antimicrobial activity and was resistant to E.coil. The antibiotic disc Erthromycin (ER) was also resistant to E.coil, since it has no zone of clearance (ie, no antimicrobial activity). Gentamicin shows high antimicrobial activity on E.coil when compared to CZ and ER and was highly sensitive to E.coil. The cobalt complex of salicylic acid and hippuric acid shows very high antimicrobial activity on E.coil. Hence it is also very sensitive to that organism. In general, the cobalt complex of salicylic acid and hippuric acid shows high antimicrobial activity both on Bacillus Subtilis and E.coil. Hence it was very sensitive to both microorganisms.

The Co(II) complex of hippuric acid and salicylic acid can be synthesized and was characterized using FTIR and UV-VIS spectra. The evidence obtained from IR spectra and electronic spectra suggest a six fold octahedral structure for the Co(II) complex in which both hippuric acid and salicylic acid acts as bidentate ligands. The fifth and sixth positions are satisfied by the water molecules.

The Antimicrobial study results shows that the cobalt complex of hippuric acid and salicylic acid shows high antimicrobial activity on the microorganisms, bacillus Subtilis and E.coil when compared to that of the individual ligands. Its antimicrobial activity was then compared with the activity of standard antibiotic discs such as Cefazolin, Gentamicin and Erythromycin and was found that the complex was having very high antimicrobial activity that the standard antibiotic discs and it was very sensitive to these organisms.

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# Amylase activity in stress tolerant landraces of *Rice*- Kuttadan and Karimodan

Thushara Parameswaran

Department of Botany, Mercy College, Palakkad-678006

#### **Abstract**

Rice is rated as an especially salt sensitive crop. Extensive studies have been made to determine the effects of salt and stress duration to identify the most important yield components. The present characterization study is a pioneer attempt to have a better understanding of the performance of the rice landraces in stressed circumstances. The efficacy of the two rice landraces i.e. Karimodan and Kuttadan were tested for the tolerance to salt stress by treating the grains to varying salt concentrations along with the control. The amylase activity was perceived in both these landraces which had a direct relation with the depleting starch content. Critical analysis of the above said facet showed that Kuttadan is preferably resistant than Karimodan. Supportive evidences to complement this result could be obtained from morphological, physiological and biochemical studies.

Key words: Amylase, Landraces, Starch, Salt stress

#### Introduction

Oryza belonging to the family Poaceae, sub-family Oryzoideae and tribe Oryzinae is one of the few crop species endowed with rich genetic diversity. It is widely accepted that rice comes from Tamil word Arisi. According to Microsoft Encarta dictionary (2004) & Chambers Dictionary of Etymology (1988), the word "Rice" has an Indo-Iranian origin. It came to English from Greek Oryza via Latin Oriza; Italian riso and finally French riz. In the beginning, rice grew wild, but today most countries cultivate varieties belonging to Oryza type, which has around 24 different species. Only two of them offer an agricultural interest for humans: O.sativa and O.glaberrima. It has been contemplated that there are various parameters like drought, soil conditions, salinity, temperature etc. that would determine the degree of fruitfulness. Rice is sensitive to salinity and water stress. But, because of its ability to grow well in standing water, it is recommended as desalinization crop. Salt stress substantially reduced the grain yield of sensitive cultivars by affecting all yield attributes, though to variable extent (Moradi et al, 2003). Paliwal (1972) has recently reviewed the effect of salinity on crop plants. Rice is sensitive to salinity at the seedling stage (Pearson and Ayers, 1960; Yeo and Flowers, 1990). It becomes relative tolerant during active tillering and again becomes very sensitive at the reproductive phase, in which salt stress greatly

affect grain yield (Pearson and Bernstein, 1959; Maas and Hoffman1976). Plants accumulate osmotically active compounds when they grow in stress conditions, which allow cells to regulate turgor and to extract additional water from soil (Morgan, 1984). Moreover, carbohydrates in the cells subjected to high salt concentrations are built as a part of osmotic adjustments (Flowers et al 1973). Janardhanan and Murthy (1970) attributed salt tolerance to higher moisture and sugar concentration. The decrease in sugars and increase in starch and amino acid levels under saline conditions were correlated with tolerance and susceptibility of genotypes (Mandal and Singh, 2000).

Amylase refers to a group of enzymes whose catalytic function is to hydrolyze (breakdown) sugar and starch. During cereal seed germination, alpha amylase in the aleurone layer plays an important role in hydrolyzing the endosperm starch into metabolizable sugars, which provide the energy for the growth of roots and shoots (Akazawa and Hara-Mishimura, 1985; Beck and Ziegler, 1989). Previous physiological and biochemical studies have revealed that -amylase expression in the aleurone layer occurs as follows. First, active gibberellin (GA) biosynthesis commences in the embryo, and the GAs is transported from the embryo to the aleurone layer (Fincher, 1989). Then, -amylase is secreted from the aleurone layer into the endosperm to catalyze the hydrating reaction of stored starch. Although the major soluble carbohydrate in the dry seed is sucrose, a marked increase in the production of glucose and maltooligosaccharides accompanies the breakdown of starch. We propose the predominant formation of glucose from starch reserves in the endosperm by the action of -amylase and accompanying hydrolytic enzyme(s) and that this sugar is eventually mobilized to the growing tissues, shoots or roots.

#### Materials and method

Fresh paddy grains of 21 different landraces were collected and then screened for the salt tolerance by germinating them in varying salt concentrations. Out of the best performing landraces, Kuttadan and Karimodan were chosen for further morphological, physiological and biochemical studies. As there was a direct correlation between the nutritional aspects and the amylase activity, the levels of change in the enzyme was studied.

# Estimation of amylase activity:

The rice grains are starchy and contain amylase splitting starch to maltose during germination. This enzyme activity was estimated by the method of Bernfield(1959).

#### Preparation of buffers:

Acetate buffer was prepared by mixing 10.5 ml of acetic acid and 39.5 ml of sodium acetate. Similarly, 0.1M monosodium dihydrogen phosphate and disodium hydrogen phosphate was prepared and

stored in refrigerator. 39ml of monosodium dihydrogen phosphate and 61ml of disodium hydrogen phosphate was mixed during homogenization for preparing phosphate buffer. 2% starch solution (substrate) was prepared in 50mM acetate buffer and it was warmed slightly for complete dissolution.

## Preparation of DNS

5g DNS was dissolved in 100ml of 2M sodium hydroxide. 150g sodium potassium tartarate was dissolved in 250ml of distilled water. They were separately dissolved by warming. It was then mixed and made up to 500ml with distilled water.

#### Quantification of amylase

1g of seeds germinated in different concentrations of NaCl along with control were weighed and homogenized with 0.1M-phosphate buffer (pH-7) in ice-cold condition. It is then filtered through two layers of cheesecloth and centrifuged at 5000rpm for 30 minutes. The supernatant was made up to 10ml and stored as enzyme source. For the assay 1ml each of the enzyme and substrate was taken and incubated at room temperature for 20 minutes. Adding 2ml of DNS reagent terminated the reaction. It was heated for 5 minutes and then cooled. It was then made up to 10ml. The absorbance was recorded spectrometrically at 560nm. The graph was plotted with enzyme activity against hours of germination. The enzyme activity was calculated by the formula:

#### Preparation of standard graph

10mg of maltose was dissolved in 10ml of distilled water. A series of solutions of increasing concentrations (200mg, 300mg, 400mg, 500mg) were prepared and an aliquot of 1ml was pipetted out separately into boiling tubes. 2ml of DNS was added and shaken well. It was heated for 10 minutes and made up to 10ml. The OD was read at 560nm and plotted against concentration.

#### Enzyme activity

Difference in OD/volume of enzyme pipetted x Total volume/wt of tissue

# **Results and Discussion**

Both the Rice landraces showed variations in enzyme levels with an increase in salt concentration and in duration. The following table shows amylase activities in Kuttadan and Karimodan respectively.

Table 1. Comparison of amylase activity in salt treated seeds against seeds of control of Kuttadan

KUTTADAN					
Concentration of salt solution	Duration (				
	48 hrs	72 hrs	96 hrs	120 hrs	
Control	0.84	3.4	5.08	5.72	
M10.0	0.84	3.6	4.80	5.04	
0.02M	0.56	1.88	4.36	4.92	
0.03M	0.44	1.48	6.00	7.44	
0.04M	1.36	2.52	5.40	5.48	

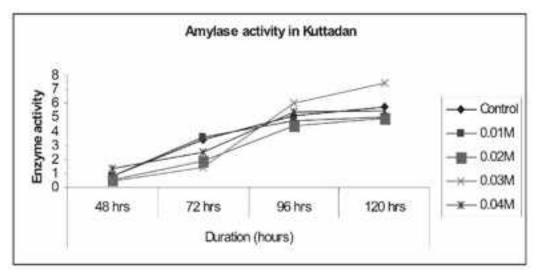


Fig.1. Amylase activity in treated seeds of Kuttadan

Table 2. Comparison of amylase activity in salt treatedseeds against seeds of control of Karimodan

KARIMODAN						
Concentration of salt solution	Duration (hours) of salt treatment.					
	48hrs	72hrs	96 ars	120 hrs		
Control	0.64	2.24	5.76	6.12		
0.01M	0.48	0.96	4.92	5.01		
0,02M	0.68	0.56	5,40	6 11		
0.03M	0.12	1.60	4.60	4.88		
0.04 <b>M</b>	0.60	1.16	5.92	5.92		

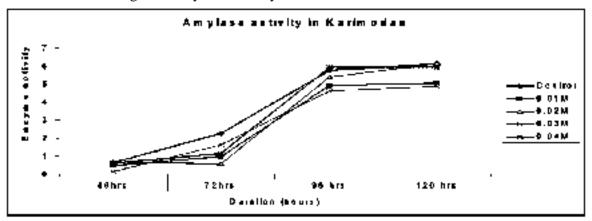


Fig 2. Amylase activity in treated seeds of Karimodan

It is evident in Kuttadan that the rate of amylase activity was highest in 0.03M NaCl concentrations, though the activity was minimum during 48 and 72 hours of germination. There was a four-fold increase in the activity during 96 hours of germination that goes on increasing during 120 hours of germination. Generally, there was an increase in the activity of all treatments at each stage, but except in 0.01M and 0.04M, the activity showed a comparative decline after 96 hours of germination. The amylase activity of seeds in all treatments more or less reached the same level, except in 0.03M, which showed a further increase in its activity. Even though, in Karimodan, the activity was less during the initial stages of growth, the maximum activity is shown in 0.02M NaCl where there is a four-time increase in the activity from 72 to 96 hours of germination. Seeds in 0.03M showed the least activity than that of seeds of other treatments at initial stages, but the rate increased as the duration of germination increased.

The enzyme commonly called as carbohydrases is the key enzyme that degrades starch and releases maltose as the product. It showed a differential distribution and a constant increase in its activity. It depicts a positive correlation with that of growth of the seedlings where the accessibility determines the enzyme action. Thus, this increase can be correlated with the starch depletion.

Rice provides more than 1/5 of the calories consumed by humans in their global diets. The rice landraces have varying amounts of carbohydrates, proteins, free amino acids, etc. Substantial quantity of carbohydrates is accumulated in different parts, which are of paramount importance when plant experiences deficit. Stored carbohydrates (reserve carbohydrates) play an important role in metabolism, growth, and development or stress tolerance. More over, the levels of the accumulating enzyme and its consequent activity on the starch explain their role in starch metabolism. It thus emphasizes on the nutritional value in spite of their growth in highly salt stressed conditions. Although there are extensive studies of salinity effects on rice, our understanding of the quantitative effects of salinity on rice and

critical thresholds of responses, especially with respect to modern, commonly used cultivars is still limited. This biochemical study shows that Kuttadan is better resistant to salt stress than Karimodan variety. It has a direct influence on the nutritional value. In order to effectively control calorie-protein malnutrition, it is essential to know the nutritional quality and also that deficiency of one or more nutrients affects utilization of other nutrients from food. Now, the nutritional problem is increasing by presence of various anti-nutritional factors. The evaluation of physiological activity of rice varieties including the viability & germination under induced drought & salt stress helps in manipulation of performances in selected regions for cultivation. From this study it can be concluded that effective interpretations regarding the unknown traits of the accessions can be made by the characterization studies and proper analysis of the data generated. Hence further studies are essential to screen the unknown treasures of genes located in our traditional unadulterated gene pool of our landraces.

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