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# The Journal of American Science

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## Lu Xun, Social Darwinism and Mahatma Gandhi

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**Abstract:** Lu Xun (1881-1936) was one of the most influential Chinese intellectuals of the 20th century. Some of his important ideas were that Darwinian evolution implies moral progress and that the Chinese should therefore no longer behave politically like cannibals, and yet that after the viciously reactionary human “dogs” have been kicked out of power, it is necessary to prevent their resurgence by “beating dogs in the water.” This latter idea is contrary to Mahatma Gandhi’s radical political precept that “win-win” works better than “win-lose.” [The Journal of American Science. 2008;4(3):1-10]. (ISSN: 1545-1003).

**Keywords:** Lu Xun, Mahatma Gandhi; anti-imperialism; social Darwinism; modern Chinese history; “beat dogs.”

Lu Xun, though little known in the West, was a brilliant writer of such importance in 20th-century China that anyone who considers it worthwhile to know about the most important 20th-century Asian political leaders such as Mao Zedong and Mahatma Gandhi may find it worthwhile to know about him as well. (“Lu Xun” was his pen name. It is pronounced rather like the last two syllables of “evolution.”) This essay provides an introductory sketch of his life, a critical assessment of some of his ideas and of certain aspects of his reception in modern China, and a fresh translation of a famous example of his art.

He was born in 1881 into a highly educated family whose fortunes declined when, during his childhood, his grandfather was arrested for trying to procure by bribery an office for his father (who then became chronically ill — maybe due to alcoholism — and died during Lu Xun's adolescence). Lu Xun's formal education included a year at a naval academy and three years at a school of mining and railways, during which he read translations of T. H. Huxley's *Evolution and Ethics*, which profoundly impressed him, J. S. Mill's *On Liberty*, and some novels including *Ivanhoe* and *Uncle Tom's Cabin*.

He spent most of 1902-09 in Japan, where, after studying modern Western medicine for two years, he decided to become a writer instead of a doctor, because a journalistic picture of a contemporary crowd of healthy-looking Chinese folks attending a public execution — and the disgustingly enthusiastic reaction of his healthy classmates to the same picture — convinced him that the Chinese needed to cure their spiritual callousness more than they needed medical care. But he was in those days an ineffective writer because he cultivated an arcane kind of Chinese rhetoric that few people would bother to read.

He stopped writing for a while and, upon returning to China in 1909, became a schoolteacher. But then in 1918 the first of his many masterworks was published, a short story entitled “A Madman's Diary” (implicitly in homage to Gogol) and employing an influential new kind of “plain-language” rhetoric and vocabulary.

Readers with access to the internet can find further bionotes at Wikipedia etc. Suffice it here to mention that in 1927 Lu Xun moved to Shanghai, where he co-founded a “League of Left-Wing Writers” and died in 1936; he never joined the Communist Party, but when the Long March succeeded in reaching northwest China in 1935 he telegraphed congratulations to the Party's Central Committee, saying, “In you lies the hope of China and all humanity.” I will describe here certain aspects of his significance by means of a some extracts adapted very freely (with permission) from a book by James R. Pusey, *Lu Xun and Evolution* (State University of New York Press, Albany 1998; if you wish to cite from this material, please revert to Pusey's text):

“From 1949 to 1966, more than 90 books were published about Lu Xun. And later, after the fall of the Gang of Four, the Academy of Social Sciences initiated an unprecedented national research effort, with research units organized in every province. In 1981, the centenary of his birth, in addition to a new edition of the (ever more) *Complete Works of Lu Xun*, at least 119 major books were published about him, and over 3000 scholarly articles, not to mention the thousands of newspaper articles. And then 1986, the 50th anniversary of his death, was another such ‘big year’.” (See pp.146-47.)

“‘Mankind’, Lu Xun said, ‘has not yet grown up.’ Most people were somewhere in between beasts and ‘true humans.’ Some were more ‘human’ than others; some more ‘brutal’.” (See p.85.)

“Never asking how evolution could mean *progress*, he echoed the great confusion of the day:

‘Mankind had made daily progress without end.’

‘The fact of mankind’s progressive evolution is clear beyond a shadow of a doubt.’” (See p.71.)

“In 1908 (while still in Japan) he expressed great hope: ‘I listen for the true voice of the knowing, and look for his inner light. Inner light is what will break the darkness. A true voice will eschew deceit. For a people, these two things will be like thunder in the early spring. A hundred grasses will begin to sprout, the color of dawn will light the east, and the dark night shall pass.’” (See p.88.)

“In an exceptionally patriotic moment during that same year, he called his own people very nearly ‘human’. The imperialists were beasts, but the Chinese ‘treasured peace, as few others on earth’:

‘Hating to spill blood, hating to kill people, abhorring separation, finding contentment in labor — *human* nature is like that. If only the whole world behaved as China did, then what Tolstoy said could be true: All the races of this earth, all the different states, would respect each other’s borders and not invade each other, and order would reign for ten thousand generations.’

“But he would rarely again call his people good-natured. After the tragedy of the successful Revolution of 1911, he spent the rest of his life exposing his own people’s ‘national nature’, which, though different, was as brutal and barbaric in his eyes as that of the imperialists. In 1918 he wrote, in *A Madman’s Diary*:

‘I opened a history book and found, scrawled over every page, the words *ren-yi daode* (benevolence, righteousness, or morality). I perused the book half the night before I made out the words between the words — two words that filled the book: *chi ren* [eat people].’” (See pp.85-86.)

“He said the Revolution of 1911 had failed because it had not wrested power from the ruling, people-eating, gentry class. When that revolution was over, the Manchus were gone but the Chinese who had held local power under the Manchus held power still — and still ate people. He wrote (in 1925):

‘When the revolution [of 1911] finally began, the whole pack of gentry with their stinking pretensions immediately became as scared as stray dogs, and coiled their queues on top of their heads. The revolutionaries behaved in a “civilized” manner, saying, “All shall be reformed. We do not beat dogs in the water.<sup>1</sup> Let them climb out.” So they climbed out, they lay low until the latter half of 1913, the time of the Second Revolution, and then they burst out to help Yuan Shikai bite a host of revolutionaries to death, and China once again sank day by day into darkness. And so it is to this day.’

“He said the Chinese revolutionaries should therefore ‘postpone “fair play”’, otherwise ‘this present state of chaos could last forever’.” (See pp.111-112.)

“He said that *future* human beings, ‘true human beings’, would be completely humane — but also that it would not be inhumane to be inhumane to the inhumane, because the inhumane were not truly human. He did not see the danger: that those who dehumanize their enemies dehumanize themselves.” (See pp.127 & 124.)

“During ‘The Decade of Disaster’ (1966-76), Chairman Mao and the Gang of Four used ‘Lu Xun’s revolutionary spirit’ of ‘beating dogs in the water’ to justify the mental or physical beating of millions of people. This explains Han Shaohua’s lament (1982):

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\* In a message kindly accepting my unusual way of drawing on his book, Prof. Pusey has mentioned that Lu Xun did not invent the metaphor of “beating dogs in the water”; it was already a known Chinese-language counterpart to the Western concept of “kicking a man when he is down.”

'I remember a few years ago, when I spoke with some young people in their early twenties about Lu Xun, they either said not a word (I do not know whether they had nothing to say or whether they had other reservations), or else they said such things as "Lu Xun was pretty brazen".' (See pp.145-46.)

"Lu Xun would have rejoiced in his [posthumous] gradual liberation throughout the 'liberal' decade from 1979 until 1989. But then June 4th 1989 [the Tienanmen-Square massacre] confirmed his most pessimistic views." (See pp. xiii-xiv.)

"Thumb through his works and you can hear him speak:

'China is especially ferocious towards its own.'

'The tears have been wiped away,

The blood has been washed away,

And the butchers go free and take their ease.'" (See p.168.)

"In 1988 one of the most promising of the younger Lu-Xun scholars, Wang Hui, wrote an important, and controversial, article, entitled 'An Historical Criticism of Lu Xun Research'. Among other things he said:

'In the development of human thought all unified theories that explain everything, all normative ideological systems, are necessarily coercive. They necessarily rely on religious or political authority. Under such an ideology the individual can no longer think freely, but can only explain anything he encounters according to the ideology's prescribed concepts.'" (See pp.148-150.)

"In the current ferment in China, officials and the disenchanteds both tell people, on occasion, to 'study Lu Xun'. But study what? What, if they actually listened to him, would they hear? Would officials hear 'You can change' or 'Beat dogs in the water'? Would the disenchanteds hear 'To rebel is justified' or 'Eating people is wrong'? The way is not clear; the dilemma is still there; but who can look at China's century of civil strife and wish for more? Who would not say 'amen' to the prayer (in the ancient Greek play, *The Eumenides*, by Aeschylus):

'I pray that civil strife,

Which knows no end of evil,

Shall never [again] roar within this city.

And may the dust

That drinks the black blood of its people

Wreak not havoc on the state,

In rage demanding recompense,

Life for life.'" (See pp.129-30.)

"Lu Xun wrote about Chinese, because he was most worried about Chinese, but he is great because, without trying to, he wrote about us all. The dog in 'The Dog's Retort', the one dog in all Lu Xun's works who puts people in their place, puts *all* people in their place:

'I dreamt that I was walking in a narrow lane, wearing tattered clothes and worn-out shoes, looking like a beggar.

'A dog started barking behind my back.

'I haughtily turned my head and cursed him, saying "Hey! Shut up! You snobbish cur!"

"Hee, hee," he laughed, and then said, "I dare not so presume. To my shame, I'm no match for you people."

"What!?" I was furious, thinking this an intolerable insult.

"I'm ashamed to say I still can't distinguish copper and silver. I still can't distinguish cotton and silk. I still can't distinguish officials and people. I still can't distinguish masters and slaves. I still can't distinguish..."

'I fled.

"But wait! Let us talk some more...": He loudly tried to hold me back.

'I fled all the way, as fast as I could, until I fled out of my dream, and back to my bed.'" (See pp.169-170.)

"'Marxists believe', said Yi Zhuxian, 'that Darwin's scientific theory of evolution "can be used as the basis in natural science of the class struggle in history".' That was Marx's view; and Lenin's view was that 'Marx's dialectic is the newest scientific theory of evolution.' But Marx and Lenin never distinguished themselves less as scientists than when they made such claims. How could their hopelessly muddled mixture of idealism, materialism, determinism, voluntarism, moral indignation, moral exhortation, and faith in the inevitable triumph of the forces of history, 'independent of human will,' over the evil of human alienation, have a basis in [valid] Darwinian science? Marx and Lenin, [Herbert] Spencer and [William Graham] Sumner, all were Social Darwinists. Yet [orthodox Communist] Chinese scholars insist that Lu Xun was not a Social Darwinist since 'he stood on the side of the weak'.'" (See p.158.)

"In modern China the term *jinhua lun* (literally the theory of progressive change) has been used indiscriminately to refer to Darwin's theory of evolution (understood or misunderstood) and to all sorts of theories of progress. No term in 2500 years of Chinese intellectual history has done more to confirm Confucius' famous dictum: 'When names are not correct, discourse is difficult.'" (See p.157.)

"Would Lu Xun have accepted *all* the violence of the [Communist] Revolution as necessary dog-beating? Would he have sat silent through thought-reform? It is not only infidels who speculate. Here is a translation of a poem written by Zhang Yu'an in 1980:

If he were still living, I do not know  
What people would call him.  
If he were still living, I do not know  
What he would urge people to do.  
Perhaps he would hold high position,  
But perhaps he would be only a soldier.  
In high office, he would not forget his  
    promise to be an ox for the young.  
In low estate, he would not act the fawning slave!  
Perhaps he would already have received many  
    honors.  
But perhaps he would just have been let out of  
jail.  
Honored, he would cry out and pace back and  
    forth anew.  
In jail, he would rewrite his *Permitted Dis-*  
    *cussions of Wind and Moon* and  
    *On False Liberty*.  
Perhaps he would no longer carry his notes in  
    that patterned paper bag.  
But surely he would not disdainfully walk about  
    with his nose in the air.  
Perhaps he would attend important meetings,  
But not followed by two secretaries and three  
    bodyguards.  
Perhaps he would ride in a modern sedan,  
But surely he would not use curtains to shut out  
    the outside.  
He would reach out to the destitute.  
He would quietly read the complaints of the many  
    young still waiting to be employed.  
Perhaps he would be spilling ink in hymns to



“the new life.”  
But perhaps he would be lancing with his pen  
the ills of the age.  
Perhaps he would enjoy more joy and laughter.  
But perhaps he would feel new uneasiness  
and rage.” (See pp.163-64.)

This concludes my notes adapted from Pusey's book. Much information could be added to outline more amply Lu Xun's work and its historical context; but instead of undertaking to do that here, I would like to comment on (1) one of the most salient points in Pusey's account, (2) a Western analogy to Lu Xun's precept of beating counter-revolutionary dogs in the water, and (3) the conclusion reached at the end of the poem cited just above. In connection with each of these three comments I will cite some relevant remarks made by the greatest of the Indian anti-imperialists, Mahatma Gandhi.

(1) Pusey acknowledges (p.200) that Lu Xun used the phrase “eat people” in “‘a large and metaphorical sense’ — as Darwin said of his famous phrase ‘Struggle for Existence’.” But it seems to me that Pusey's way of drawing the contrast between “eating people” and “beating dogs” might cause some readers to lose sight of the fact that people cowed by the mere threat of force may be “eaten” metaphorically — exploited very unfairly — without a constant use of force such as evoked by the phrase “beat dogs in the water.” It is important to understand that a fair and hence *genuinely* non-violent socio-economic order would differ substantially from an ostensibly non-violent one in which people are exploited very unfairly. Gandhi's perspective in this regard was clear (and can explain why, in India today, the “Naxalite” peasant revolutionaries are achieving a remarkable expansion of their sway):

“A violent and bloody revolution is a certainty one day unless there is a voluntary abdication of... the power that riches give, and sharing them for the common good.”<sup>1</sup>

(2) A salient precept of the Jacobins in the French Revolution was Saint-Just's motto: “No liberty for the enemies of liberty.” The director of the revolutionary *Terreur*, Robespierre, spoke out against the “treacherous insinuations” of those citizens who questioned the “severity of measures prescribed by the public interest.” He said: “This severity is alarming only for the conspirators, only for the enemies of liberty.” In Western history, Robespierre's directorship is a *locus classicus* of governmental use of terror ostensibly in the public interest. Gandhi doubted the benefit; he said, in 1909:

“There is a forceful book by Carlyle on the French Revolution. I realized after reading it that it is not from the white nations that India can learn the way out of her present degradation. It is my belief that the French people have gained nothing of value through the Revolution.”<sup>2</sup>

—And, in 1920 (in a passage just after another allusion to the French Revolution):

“[O]rder established by a tyrant in order to get hold of the tyrannical reins of Government... is no order for me but it is disorder. I want to evolve justice out of this injustice.”<sup>3</sup>

—And, in 1942:

“I believe that in the history of the world there has not been a more genuinely democratic struggle for freedom than ours [in colonial India]. I read Carlyle's *History of the French Revolution* while I was in prison, and Pandit Jawaharlal [Nehru] has told me something about the Russian revolution. But it is my conviction that inasmuch as these struggles were fought with the weapon of violence they failed to realize the democratic ideal. In the democracy which I have envisaged, a democracy established by non-violence, there will be equal freedom for all. Everybody will be his own master. It is to join a struggle for such democracy that I invite you today.”<sup>4</sup>

Here the phrase “everybody... his own master” is a call to self-discipline among the citizens of free India. Only such inner moral strength, so widespread as to be culturally predominant, could compensate adequately for the absence of tyrannical or quasi-tyrannical authority imposed by Government. I detect, in

Gandhi's call for universal self-discipline, an idealism that may be nearly as extreme (given modern capitalism's massive investment in persuading us to be idiotically ruthless consumers) than the kind of social Darwinism that Pusey sees in Lu Xun. There is always *some* kind of dilemma. However:

(3) When Gandhi felt outraged (which was often the case) he normally did not express it directly as outspoken rage, but instead took up certain forms of vigorous dialogue, political activism and constructive work. It seems to me that Mao's concept of political common sense:

“We cannot love our enemies. We cannot love the ugly things in society; our goal is to wipe out such things; that is human common sense”<sup>5</sup>

—is nowadays gradually being superseded in some quarters by Gandhi's common-sense view that win-win solutions to social conflicts are better than win-lose. I don't mean that a nonviolent approach to socially pathological people willing to devour other people (in one way and another) is so problem-free as to be a simple panacea. It was, in Gandhi's opinion, a necessary but insufficient precept of effective liberation. This can be a mere platitude but I would suggest that it is better than a likewise simple precept of “beating dogs in the water.”

### *Appendix:*

#### **Translation of a story by Lu Xun**

Several anthologies of writings by Lu Xun are available in standard English translations, which are, however, nowadays a little outdated in style.<sup>6</sup> The following is a fresh translation, prepared in collaboration with a young Chinese student,<sup>7</sup> of one of the best known short stories, a tale of parental love and desperate superstition, of a blood-thirsty mob, of callous men, of a heroic rebel with a democratic ideal in opposition to imperial authoritarianism, and of ordinary people's failure (even that of the hero's mother) to understand. The story was written and published in 1919 but refers back to the era of the last Chinese dynasty (i.e. before 1911).

### *MEDICINE*

#### I

It was the start of an autumn day. The moon had set but the sun had not yet begun to rise; the sky was dark. Every creature (except some nocturnal animals) was deep asleep.

Old Shuan Hua suddenly sat up, ignited a match and lit a dirty oil-lamp which filled the tea house's rooms with a feeble light.

“So you're going now, Papà?” It was the voice of an elderly woman; and meanwhile there came from the inner room a sound of coughing.

“Uh-huh,” Old Shuan nodded as he buttoned up his clothes; “Give it to me,” he murmured, and stretched out his hand. His wife reached beneath the pillow, probed, and pulled out a bag of silver coins which she handed him. He tipped the bag for a moment and put it in his pocket and secured it there. He lit a portable lantern, put out the tiny flame of the indoor lamp, and, holding the lantern, went into the inner room. There was a rustling noise, and more coughing; he waited till it died down, then murmured: “Little Shuan my son,... stay still, don't sit up.... Your mother will look after the tea-house.”

There was no reply from the boy, so Old Shuan, presuming that he had gone back to sleep, stepped outside and walked along the street. It was dark; all that could be seen was the grey road. His feet went ahead by the light of the lantern. Some dogs were about; but they were not barking on this unusual morning.

It was much colder out on the street than it had been indoors, but Old Shuan felt encouraged by it, as if suddenly rejuvenated: like a teenager with some kind of religious inspiration.

The darkness gradually faded, and as he walked on ahead, he was suddenly taken aback to see the T-crossroad just a bit further on. He stopped in his tracks for a moment, went over to a shop that hadn't yet opened, hid himself under the eaves and leaned against the door. He began to feel the chill.

"Some old guy." — "Satisfied this time, eh?" — Old Shuan was disconcerted again as a few people passed by and one of them gazed back at him. Their faces were too dim to recognize, but he could tell that their eyes were glinting with a strange, greedy lust. He looked down at his lantern, and saw that it had run out of oil. He tapped his pocket to make sure that the coins still there. He looked out again — and saw quite a few people. They seemed like ghosts, but as he looked harder he could see that they were real.

Some soldiers came by. Their uniforms were easy to recognize: a white circle was stitched on the chest and on the back, and as they came closer he could see the crimson-lace ornaments on the uniforms.

And now a throng of people came along, like a tide, but suddenly stopped at the T-crossroad and gathered into a half-circle. Old Shuan stared in their direction but the only thing he could see was the backs of the crowd. They were all standing on their toes and stretching their necks so much that they looked like ducks hanging from an invisible hand. There was a brief silence, and then a loud bang, and the crowd surged back past Old Shuan and almost knocked him over.

"OK, give me the money and take the thing you want!": a man dressed in black stood in front of Old Shuan. His eyes were like two daggers; Old Shuan cringed; one of the man's hands reached out for the money; the other one held a small, red, steamed bun from which blood was dripping. Old Shuan quickly got the money from his pocket, but quivered for a moment, not daring to take the blood-red thing. The man shouted impatiently, "What are you afraid of? — Take it!" Old Shuan still hesitated, so the man grabbed his lantern and tore off its paper cover, wrapped the bun in it and jammed the packet into Old Shuan's hand.

"Who is sick in your family?", Old Shuan heard someone ask him. But he didn't reply: his mind was focused on the red steamed-bun, and all his thoughts now were of infusing a new vitality from it into his home and bringing happiness to his family.

The sun had risen. The broad road, leading straight home, stretched out ahead. Behind him was the crossroad, with its faded sign: "Execution Square."

## II

By the time Old Shuan got home the tea house had already been cleaned up and the rows of tea tables were set out and shining with reflected morning sunlight; but no customers had yet arrived. Little Shuan was seated at a table near the wall, eating his breakfast quietly. Beads of sweat dripped from his head, dampening his shirt and making it stick to his spine so that his shoulder blades stuck out and made an inverted V. Looking at the boy, Old Shuan contracted his brows. His wife hurried out of the kitchen and asked, tremulously: "Did you get it?"

"Yes." The two of them went into the kitchen. They talked briefly; then she went out and in a few moments came back with a big lotus-leaf which she spread out on the kitchen table. Old Shuan took out the red bun and wrapped it in the leaf. Little Shuan had finished his breakfast by now; Madam Hua called out from kitchen, "Stay there, Little Shuan, don't come in." Old Shuan put the wrapped bun in the stove to cook. The fire flared up, and the tea house was filled with the aroma.

"What a delicious smell! What are you guys eating?" It was the hunchback who took tea here every day — usually the first to come in the morning and last to leave in afternoon. No one answered him, so he persisted: "Is it porridge?" There was still no reply, but Old Shuan hurried out of the kitchen and served him his morning tea.

"Little Shuan, come in now," Madam Hua called from the kitchen. The boy came and sat on a chair that was set out for him. His mother gave him a plate with a ball of black food on it and whispered to him:

“Eat it up — you’re going to get well now!” Little Shuan picked up the black thing with care and looked at it. He had a strange feeling, as if he was holding his life in his own hands. He tore the bun slowly in two; the smell of white-flour steam flowed out from inside the charred crust; he looked at the stuff inside and realized that it was just a rice-meal bun. Soon he had eaten it, and forgotten the flavor, and left the plate empty in front of him. His parents were standing next to him and watched anxiously as he chewed. Their gaze seemed to pour something into him, and pull something out. His heart beat faster; he patted his chest gently and resumed coughing. “Go to sleep, you’ll soon be well.” Little Shuan obeyed his mother and went to bed, coughing. She waited until it stopped, and then covered him with a patched blanket.

### III

The tea house was crowded with customers and Old Shuan was busy serving tea from a big copper kettle.

There were dark circles under his eyes. “Old Shuan, are you OK? What’s wrong?” mumbled an old man with white beard. — “Nothing!” — “Nothing? Oh, OK, then maybe I was wrong,” the bearded man said. The hunchback spoke up: “Old Shuan is just busy and tired. If his son...” — but here he was interrupted by a heavy-jowled man wearing a dark brown, unbuttoned shirt and a brown girdle at his waist. He had just now come into the tea-house, and he shouted to Old Shuan: “Has your son eaten it? Does he feel better? You were lucky, Old Shuan! If I hadn’t told you...”

Old Shuan, still holding the big kettle with one hand, put his other arm straight down (as a sign of respect) and listened to the heavy-jowled man quietly and with a smile on his face; and indeed all the tea-house customers listened respectfully, and Madam Hua — who also had deep circles under her eyes — hurried out from the kitchen and served him some specially brewed tea which Old Shuan topped up with steaming-hot water.

“A guaranteed cure! It’s not like other things. Just think! You bring it back and eat it while it’s still warm,” the heavy-jowled man shouted. — “It’s true, and we couldn’t have done it without Mr. Kang’s help,” Madam Hua said with gratitude. — “A sure cure, a sure cure! You eat it while it’s still warm! A rice-bun drenched in human blood will cure *any* kind of consumption,” Kang went on barking. At the word “consumption” Madam Hua registered dismay, but still forced a smile on her face, and then left quietly. Kang took no notice but went on speaking loudly. Little Shuan in the inner room was awakened by the noise and started coughing.

“So, Little Shuan is in luck — and of course his sickness will be cured — and that’s why Old Shuan has been smiling all this time,” the white-bearded man beard mused. He went over to Kang and asked him, in a low voice: “Mr. Kang, I heard that a man was executed this morning, and that he was from the Shia family. Who the devil was he, and why was he executed?” — “You ask me who it was? It was Madame Shia’s son, of course!” Mr. Kang replied. Everyone turned quiet and concentrated on hearing him, so he raised his voice even higher (and by now his jowls were quivering): “That crazy guy didn’t want to live, you know, he just didn’t *want* to live! *I* got nothing! Even his clothes were ripped off by the jailer before the execution. Old Shuan’s the lucky one — and that crazy bastard’s uncle was lucky too: *he* informed on him and got the reward — twenty-five thousand! *He* was lucky....”

Little Shuan came out from the inner room, coughing and with his two hands on his chest. He went to the kitchen, filled a bowl with cold rice and hot water, and started eating. Madam Hua followed and asked him: “Little Shuan, are you better now?... Are you still hungry?...” Kang glanced at him — “A sure cure!” — and turned back to the others: “That Uncle Shia is a smart man, you know. If he hadn’t informed on his crazy nephew, *his* family would have been executed; but now, you see, he got the reward, he got the money! The nephew was so crazy! In prison he tried to persuade the jailer to join him in the rebellion!” — “The bastard! He *had* to be executed!”, an angry young man seated in the back of the tea house growled. — “He told the jailer the Empire doesn’t belong to the Emperor but to the people! It’s evil!... The jailer knew he lived with his mother but had never imagined the crazy sonofabitch was so poor; he simply couldn’t get any money out of him. He was so mad he worked him over when he come out with that idea of his.” — “Yeah, I hear the jailer used to be a boxer. He must have punched him out pretty good!”, the hunchback

chimed in. — “But that crazy bastard wasn't scared; he told the jailer how *sorry* he felt!” — “Who would feel sorry for a sonofabitch like that?”, the bearded man said. Kang looked at him superciliously: “You don't get it: the crazy sonofabitch meant he was sorry *for the jailer!*” Everyone was dumbfounded by this. (By now Little Shuan had finished his meal and his head was steaming with sweat.) — “Sorry for the jailer?! He was loony, he must have been out of his mind,” the bearded man said, suddenly seeing the light. — “That's it, he must have gone loony,” a young man agreed. And conversation began to pick up again among the customers. Little Shuan started coughing. Kang walked over to him and clapped his shoulder and said: “A sure cure! Stop coughing! It's a guaranteed cure!” — “A loony!”, the hunchback muttered, shaking his head gently.

#### IV

The land just outside the city's west wall had originally been government property. A small path worn down out by passers-by meandered through it and had become a kind of boundary. Convicts who had been executed were buried on the left side; the land to the right was for paupers' graves. Both sides were crowded with countless grave mounds looking like steamed-rice buns in a rich family's birthday celebration.

This year the weather was unusually cold for the Commemoration Festival in April; the willow trees had tiny shoots no larger than grains.

It was early morning. Madam Hua laid four dishes and a bowl of rice in front of the fresh grave on the right side of the path. She wept, and burned some paper money, and sat, as if numb, on the ground. She felt like waiting for something, but didn't know what it was. A breeze rippled her hair; it was whiter now than a year ago.

Another woman, gray-haired and dressed in rags, came along. She held an old basket with paper money in it, and walked with staggering steps. Suddenly she noticed Madam Hua sitting on the ground, and hesitated for a while, embarrassed, but then after a brief silence forced herself to go on to a grave near where Madam Hua was sitting, but on the left side of the path. She put down her basket. This other grave was just opposite Little Shuan's on the other side of the path; Madam Hua saw the old woman put four dishes on the grave, burn paper money, and weep: no doubt she too was mourning a dead son. The grey-haired woman gazed ahead for a while, and then suddenly seemed to register shock, stepping backwards, her hands and legs quivering. Madam Hua thought sorrow had driven her delirious, so she stood up and crossed the path and said, gently, “Let's not grieve too much; let's go home.” The old woman nodded, but stared ahead with dull eyes and said, in a low voice: “Look at that!, what is that?” Madam Hua looked at where the old woman was pointing to on the grave-mound in front of them. It was covered with ugly patches of fresh soil, but as she looked harder she could make out, to her surprise, a wreath of red and white flowers on top of the mound. The old woman had suffered from poor eyesight for many years, but they could see the flowers clearly: not fresh, but set out in a neat circle. Madam Hua looked over at her own son's grave-mound and at the others nearby. Each one had a few small pale flowers growing on it like dots. She felt disappointed, and her curiosity about the red and white flowers began to fade; but now the other woman stepped closer to them, and murmured to herself: “They don't have roots. Someone must have put them there. But who would come here? Kids never play here. Our relatives haven't come for a long time. How can it be?” She thought for a while, then burst into tears: “Dear son! They have wronged you, and you can't forget, and you're still grieving in the other world, and you've done this to let me know — is that it?” She looked around and saw a crow perched on a leafless branch. “Yes, they murdered you; but someday, God knows, they'll get the punishment they deserve! Rest in peace now — and if you can hear what I'm saying, let that crow fly to the top of your grave-mound in front of my eyes!”

The breeze had stopped, and the dry grass stood straight like copper wires. A trembling sound vibrated in the air and faded away. There was a deathly stillness. The two women standing in the grass looked up at the crow perched on the bare branch.

Some time passed. Some other people came to visit graves — old people, holding children's hands and walking along the path. Madam Hua felt as if a burden had been lifted from her soul. She wanted to leave, and urged the old woman: "Let's go now." The old woman sighed, and put her dishes and bowl back in her basket, and after hesitating for a moment started to leave with slow steps and still muttering, "How can it be?"

When they had walked about twenty steps they heard a loud cawing, and they turned, startled, and saw the crow fan out its wings and soar like an arrow to the far sky.

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## Fuzzy Variable Model for Lake Eutrophication Evaluation and Sustainable Development Countermeasure

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**Abstract:** Considering that lake eutrophication evaluation involves many indexes, many classifications and interval values, fuzzy variable sets theory is applied. Making reference to eutrophication standard of Chinese lakes, Eutrophication degree of lake is divided into 6 classifications. Evaluation indexes include Chl-a, TP, TN and COD<sub>Mn</sub>. According to data measured, index feature value matrix of sample set evaluated can be built. Based on the premise that the same mathematics model is used, index weights are determined by means of entropy value method, equal weight method and pure threshold value method. Relative membership degree of each index to each classification can be calculated with relative difference function model. Then comprehensive relative membership degree of each sample to each classification can be obtained. And then feature value of classification of each sample can be got. At last, the stability of feature values of classification corresponding to three methods is analyzed and grade of each sample is determined. This model is applied to Dongchang Lake. Three lake regions all belong to the fifth classification eutrophic. It corresponds to reality. So it can be indicated that the proposed model is reasonable and practical. Finally, suggestions are made for the protection and sustainable development of Dongchang Lake. [The Journal of American Science. 2008;4(3):11-17]. (ISSN: 1545-1003).

**Keywords:** lake eutrophication evaluation; fuzzy variable sets; index weights; Dongchang Lake; features of classification; sustainable development

### 1 Introduction

Eutrophication is one of the most serious water quality problems in the world. The contradiction of supply and demand of water resources is getting more and more serious with the rapid development of economy and society. Eutrophication is a sharp problem to lakes. Lake eutrophication evaluation is such a problem that involves many indexes, many classifications and interval values. Fuzzy variable evaluation method can effectively deal with the influence of evaluation standard interval values and set up comprehensive evaluation model to fulfill the comprehensive evaluation to water environment. Based on the premise that the same mathematics model is used, this paper analyzes the influence of the change of index weights to evaluation result.

### 2 Method

#### 2.1 Fuzzy variable model for lake eutrophication

Suppose that there is a set that includes  $n$  water-quality samples whose features are showed by  $m$  features of indexes. Then index feature matrix of samples can be summarised as follows:

$$X = \begin{pmatrix} x_{ji} \end{pmatrix} \quad (1)$$

Where  $x_{ji}$  is the feature of index  $i$  of sample  $j$ , and  $i = 1, 2, \dots, m$ ;  $j = 1, 2, \dots, n$ .

Standard value interval matrix of indexes to classifications ( $I_{ab}$ ) can be determined by:

$$I_{ab} = \begin{pmatrix} [a, b]_{ih} \end{pmatrix} \quad (2)$$

Where  $h = 1, 2, \dots, c$ .

The main evaluation steps are as follows: first, referring to  $I_{ab}$ , range value matrix with variable intervals ( $I_{cd}$ ) can be structured by:

$$I_{cd} = ([c, d]_{ih}) \quad (3)$$

Secondly, according to physical analysis to index i, point value matrix M to index i and classification h can be determined by:

$$M = (M_{ih}) \quad (4)$$

Fig. 1 shows the relation of point x, point M, interval [a,b] and interval [c,d].

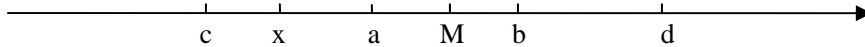


Fig.1 Relation of point x, point M, interval [a,b] and interval [c,d]

Thirdly, relative membership degree matrix ( $\mu_{\%}^A(u)$ ) can be summarized by

$$\mu_{\%}^A(u) = (\mu_{\%}^A(u)_{ih}) \quad (5)$$

Where  $\mu_{\%}^A(u)$  can be calculated using formula (6) to (8) and the corresponding data in matrix  $I_{ab}$ ,  $I_{cd}$  and M.

$$\begin{cases} D_{\%}^A(u) = \left(\frac{x-a}{M-a}\right)^{\beta} & x \in [a, M] \\ D_{\%}^A(u) = -\left(\frac{x-a}{c-a}\right)^{\beta} & x \in [c, a] \end{cases} \quad (6)$$

$$\begin{cases} D_{\%}^A(u) = \left(\frac{x-b}{M-b}\right)^{\beta} & x \in [M, b] \\ D_{\%}^A(u) = -\left(\frac{x-b}{d-b}\right)^{\beta} & x \in [b, d] \end{cases} \quad (7)$$

$$\mu_{\%}^A(u) = [1 + D_{\%}^A(u)]/2 \quad (8)$$

Among formula (6) and (7),  $\beta$  is a non-negative index and usually is endowed with 1. Forthly, index weight vector ( $w = (w_i)$ ) determined, comprehensive relative membership degree vector ( $v_{\%}^A(u)$ ) can be calculated using the following approach:

$$v_{\%}^A(u)_h = \frac{1}{1 + \frac{\left\{ \sum_{i=1}^m [w_i (1 - \mu_{\%}^A(u)_{ih})]^p \right\}^{\frac{\alpha}{p}}}{\sum_{i=1}^m (w_i \mu_{\%}^A(u)_{ih})^p}} \quad (9)$$

Making both  $\alpha$  and  $p$  equal 1 and normalizing ( $v_{\%}^A(u)$ ), comprehensive relative membership degree vector can be got as follows:



$$v_{\frac{A}{\%}}^o(u) = \left( v_{\frac{A}{\%}}^o(u)_h \right) \quad (10)$$

Fifthly, eutrophication degree of each sample can be evaluated. If  $v_{\frac{A}{\%}}^o(u)_h > 0.5$ , then it belongs to classification h. If  $v_{\frac{A}{\%}}^o(u)_h \leq 0.5$ , then it can be evaluated with feature of classification of the same sample (H), which is calculated:

$$H = (1 \quad 2 \quad \Lambda \quad c) \cdot v_{\frac{A}{\%}}^o(u)^T \quad (11)$$

And then we can differentiate classification of sample j according to its classification features. The method is as follows. If  $c - 0.5 < H_j \leq c$ , sample j can be differentiate to classification c. If  $1 < H_j \leq 1.5$ , it can be differentiate to classification 1. If  $h - 0.5 < H_j \leq h + 0.5$ , it can be differentiate to classification h ( $h = 2, 3, \dots, c - 1$ ). Sixthly, duplicating step 4 and 5, classification features corresponding to different weight are calculated. Analyzing their stability, eutrophication classification of each sample can be determined.

## 2.2 Index weight determination

Index weight determination is focal and difficult point in multi-index evaluation. At present, there are many methods, such as entropy value method, equal weight method and pure threshold value method. Among them, the former two belong objective method and the latter subjective. This paper combines them to improve rationality and reliability of evaluation results.

### 2.2.1 Entropy value method

Calculation steps are as follows. First, judging matrix of m indexes corresponding to n samples is constructed ( $R = (x_{ji})_{n \times m}$ ). Secondly, judging matrix is normalized to matrix B, whose arbitrary element is calculated from:

$$b_{ji} = \frac{x_{ji} - x_{\min}}{x_{\max} - x_{\min}} \quad (12)$$

where  $x_{\max}$  and  $x_{\min}$  are separately the most satisfied and the most unsatisfied to the same index of different samples. Thirdly, entropy of index i is calculated from:

$$H_i = -\frac{1}{\ln n} \left( \sum_{j=1}^n f_{ji} \ln f_{ji} \right) \quad (13)$$

where  $f_{ji} = \frac{1 + b_{ji}}{\sum_{j=1}^n (1 + b_{ji})}$ . Forthly, entropy weight of index i is calculated from:

$$w_i = \frac{1 - H_i}{m - \sum_{i=1}^m H_i} \quad (14)$$

where  $\sum_{i=1}^m w_i = 1$  should be satisfied.

**2.2.2 Equal weight method**

Weight of each index is thought as equal. That is to say:

$$w_i = \frac{1}{m} \quad (i = 1, 2, L, m) \tag{15}$$

**2.2.3 Pure threshold value method**

Reciprocal method reflects the idea that the bigger the threshold value is, the smaller the relative weight is. Weight is calculated from:

$$w_{ih} = \frac{1}{y_{ih}} \bigg/ \sum_{i=1}^m \frac{1}{y_{ih}} \tag{16}$$

where  $y_{ih}$  is the value non-dimensioned.

**3 Applied Example**

Dongchang Lake lies in southwest of Liaocheng, shandong, China. Its area is 4.2 square kilometers and its depth is 2-3 meters. With the rapid development of economy and society of Liaocheng City, eutrophication occurs to a certain extent for Dongchang Lake. So eutrophication degree should be evaluated for it in order to supply basis for pollution control and ecology environment management.

The measured data for water quality to Dongchang Lake are provided in table 1. Chl-a,TP,TN and COD<sub>Mn</sub> are chosen as evaluation indexes to evaluate nutrient states of its three regions.

Table 1. Eutrophication measured data of Dongchang Lake

Evaluation indexes	Northwest region	Southwest region	Southeast region
Chl-a(mg/m <sup>3</sup> )	21.25	22.02	22.54
TP(mg/m <sup>3</sup> )	180	161	173
TN(mg/m <sup>3</sup> )	1980	1760	1840
COD <sub>Mn</sub> (mg/l)	14.10	13.48	13.36

According to eutrophication classification standard of lakes and reseriors in China, that with interval values is given in table 2.

Table 2. Eutrophication classification standard of lakes and reseriors in China

Evaluation indexes	Oligotrophic	Lower-mesotrophic	Mesotrophic	Upper-mesotrophic	Eutrophic	Hypereutrophic
Chla(mg/m <sup>3</sup> )	0-1.0	1.0-2.0	2.0-4.0	4.0-10.0	10.0-65	65-160
TP(mg/m <sup>3</sup> )	0-2.5	2.5-5.0	5.0-25.0	25.0-50.0	50.0-200	200-600
TN(mg/m <sup>3</sup> )	0-30	30-50	50-300	300-500	500-2000	2000-6000
COD <sub>Mn</sub> (mg/l)	0-0.3	0.3-0.4	0.4-2.0	2.0-4.0	4.0-10	10-25

Then matrix  $I_{ab}$ ,  $I_{cd}$  and  $M$  can be expressed as follows:

$$I_{ab} = \begin{bmatrix} [0,1.0] & [1.0,2.0] & [2.0,4.0] & [4.0,10.0] & [10.0,65] & [65,160] \\ [0,2.5] & [2.5,5.0] & [5.0,25] & [25,50] & [50,200] & [200,600] \\ [0,30] & [30,50] & [50,300] & [300,500] & [500,2000] & [2000,6000] \\ [0,0.3] & [0.3,0.4] & [0.4,2.0] & [2.0,4.0] & [4.0,10] & [10,25] \end{bmatrix} = ([a,b]_{ih})$$

$$I_{cd} = \begin{bmatrix} [0,2.0] & [0,4.0] & [1.0,10] & [2.0,65] & [4.0,160] & [10,160] \\ [0,5.0] & [0,25] & [2.5,50] & [5.0,200] & [25,600] & [50,600] \\ [0,50] & [0,300] & [30,500] & [50,2000] & [300,6000] & [500,6000] \\ [0,0.4] & [0,2.0] & [0.3,4.0] & [0.4,10] & [2.0,25] & [4.0,25] \end{bmatrix} = ([c,d]_{ih})$$

$$M = \begin{bmatrix} 0 & 1.0 & 3.0 & 7.0 & 65 & 160 \\ 0 & 2.5 & 15 & 37.5 & 200 & 600 \\ 0 & 30 & 175 & 400 & 2000 & 6000 \\ 0 & 0.3 & 1.2 & 3.0 & 10 & 25 \end{bmatrix} = (M_{ih})$$

Northwest region taken as an example, calculation process are as follows. According to the upper matrixes and formula (6) to (8),  $\mu_{\%}^A(u)$  can be calculated.

$$\mu_{\%}^A(u)_{ih4 \times 6} = \begin{bmatrix} 0 & 0 & 0 & 0.398 & 0.602 & 0.102 \\ 0 & 0 & 0 & 0.067 & 0.933 & 0.433 \\ 0 & 0 & 0 & 0.007 & 0.993 & 0.493 \\ 0 & 0 & 0 & 0 & 0.363 & 0.637 \end{bmatrix} \quad (17)$$

### 3.1 Classification feature corresponding to entropy weight

Entropy weight is calculated using formula (12) to (14):

$$w = (0.23 \quad 0.231 \quad 0.246 \quad 0.293) \quad (18)$$

Formula (17) to (18) substituted into formula (9) and normalized, then comprehensive relative membership degree vector ( $u = (0 \quad 0 \quad 0 \quad 0.087 \quad 0.566 \quad 0.347)$ ) is got. The classification feature of the sample can be calculated using formula (11):  $H_1 = 5.259$ .

Similarly, the classification features of the southwest and southeast regions are separately 5.210 and 5.234.

### 3.2 Classification feature corresponding to equal weight

Weight vector is given by:

$$w = (0.25 \quad 0.25 \quad 0.25 \quad 0.25) \quad (19)$$

Formula (17) to (19) substituted into formula (9) and normalized, then comprehensive relative membership degree vector ( $u = (0 \ 0 \ 0 \ 0.094 \ 0.575 \ 0.331)$ ) is got. The classification feature of the sample can be calculated using formula (11):  $H_1 = 5.237$ .

Similarly, the classification features of the southwest and southeast regions are separately 5.187 and 5.212.

### 3.3 Classification feature corresponding to weight determined by pure threshold value method

Weight is calculated using formula (16):

$$w_{ih} = \begin{bmatrix} 0.232 & 0.209 & 0.413 & 0.345 & 0.222 & 0.247 \\ 0.347 & 0.306 & 0.241 & 0.253 & 0.264 & 0.240 \\ 0.286 & 0.306 & 0.203 & 0.253 & 0.265 & 0.241 \\ 0.135 & 0.179 & 0.143 & 0.149 & 0.249 & 0.272 \end{bmatrix} \quad (20)$$

Formula (17) to (20) substituted into formula (21),

$$v_{\%}^A(u)_h = \frac{1}{1 + \left\{ \frac{\sum_{i=1}^m [w_{ih} (1 - \mu_{\%}^A(u)_{ih})]^p}{\sum_{i=1}^m (w_{ih} \mu_{\%}^A(u)_{ih})^p} \right\}^{\frac{\alpha}{p}}} \quad (21)$$

Making both  $\alpha$  and  $p$  equal 1 and normalizing, comprehensive relative membership degree vector ( $u = (0 \ 0 \ 0 \ 0.119 \ 0.559 \ 0.321)$ ) is got. The classification feature of the sample can be calculated using formula (11):  $H_1 = 5.202$ .

Similarly, the classification features of the southwest and southeast regions are separately 5.166 and 5.185.

## 4 Conclusions and Countermeasure for Sustainable Development

To sum up, the change of the value of H is very small when index weights change. So we use the mean value of H calculated by the three methods as the ultimate value. And we can get the evaluation result that the three lake regions all belong to classification 5(eutrophic).

Combine with the reality of Dongchang Lake, we can promote its sustainable development mainly by water diversion, control of pollution source and restoration and reconstruction of aquatic vegetation.

(1) The degree of eutrophication of Dongchang Lake is closely relevant to water diversion from Tanzhuang Reservoir. So the quantity and time of water diversion should be studied with the aim of guaranteeing good cycle of the water in Dongchang Lake.

(2) The improvement degree of water environment quality in Dongchang Lake also relates to the site and number of entrances of water diversion, which should be determined by calculating scientifically on the premise of feasibility to construction and economy in order to make the yield of limit water resources better.

(3) To improve the water quality, we should control the discharge of domestic wastewater, retrieve tour garbage and decrease or eliminate nets for aquaculture.

(4) Ecological restoration is a way usually used to improve the water quality of city lake. Restoration and Reconstruction experiment of aquatic vegetation of Wuli Lake, East Lake in Wuhan and Dianchi Lake

produced a certain effect. It is thus clear that the perfect water ecosystem and the higher degree of hydrobiological diversity can be gradually set up.

By means of adopting measures and methods above mentioned, the water environment quality of Dongchang Lake will be obviously improved. Thus environment grade of Liaocheng City will be heightened.

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## Comparison Of Dac-Elisa And Dot-Blot-Elisa For The Detection Of Cucumber Mosaic And Banana Streak Viruses Infecting Banana

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**ABSTRACT:** This paper presents the details of direct antigen coating (DAC) - ELISA and Dot-blot-ELISA for the detection of banana streak badnavirus (BSV) and cucumber mosaic cucumovirus (CMV) in banana leaf and pseudostem tissues. Suckers were collected from banana plants infected with BSV and CMV. The plants were indexed for presence of viruses by DAC-ELISA. The DAC form of indirect ELISA was adopted to detect viruses in plants. From the studies, it is observed that BSV and CMV induced similar interveinal chlorotic streaks of varied sizes in banana. In DAC-ELISA, BSV was detected up to  $10^{-2}$  dilutions of tissue extracts but it was detected by Dot-blot-ELISA up to  $10^{-3}$  dilution. Further, it is observed that among ten field collected samples, none were positive for CMV in DAC-ELISA, but three samples reacted positively in Dot-blot-ELISA. Out of ten field samples tested for BSV, only one weakly reacted. However, in Dot-blot-ELISA, none were found positive for BSV. Out of ten samples, none were found mixedly infected. Above findings indicate that Dot-blot-ELISA is relatively more sensitive for the detection of BSV and CMV in banana. [The Journal of American Science. 2008;4(3):18-27]. (ISSN: 1545-1003).

**Keywords:** Banana streak virus, Cucumber mosaic virus, DAC-ELISA, Dot-blot-ELISA

### 1. Introduction

Banana is one of the world's most important tropical fruit crop. It is grown both as a staple food and a major cash crop. It is propagated vegetatively through suckers. Asia accounts nearly 40% of world banana production. Bananas grown in South India can be broadly grouped into three types like deserts, culinary and dual purpose varieties. Successful cultivation of banana is varied, because it is influenced by abiotic and biotic factors. Among the biotic factors, several insect and nematode pests and fungal, bacterial and viral pathogens are known to limit the growth and fruit yield of banana (Jeger et al., 1995). Banana is a humid tropic plant coming up with a temperature range of  $10^{\circ}\text{C}$  to  $40^{\circ}\text{C}$  and average of  $23^{\circ}\text{C}$ . Four viruses known to naturally infect banana widely in different countries are BBTv, CMV, BBrMV and BSV ([Jeger et al., 1995). Table 1 shows the details of virus diseases of banana.

**Table 1. Virus diseases of banana**

Sl. No.	Disease	Causal virus
1	Bunchy top	Banana Bunchy top virus (BBTV)
2	Infectious chlorosis	Cucumber mosaic virus (CMV)
3	Bract mosaic	Banana bract mosaic virus (BBrMV)
4	Streak	Banana streak virus (BSV)
5	Mosaic	Tobacco mosaic virus (TMV)
6	Die-back	Nepovirus
7		Potexvirus
8	Abaca mosaic	Abaca mosaic virus (AbaMV)

So far TMV was reported to infect banana only in India (Singh, 1988). Abaca mosaic virus, nepovirus and potexvirus infecting banana in few countries have less significance (Brunt et al., 1996, Anonyms, 1997). BSV causing streak disease of banana has been reported from several countries. Streak symptoms of BSV infection of *Musa* spp. are sometimes similar to those caused by CMV and the two diseases have been confused (Lockhart et al., 1992). Causal virions are nonenveloped, bacilliform belongs to badnavirus. It is transmitted by the citrus mealy bug (*Planococcus citri*) and through suckers (Jones and Lockhart, 1993).

Harper et al., (1999) developed a PCR based strategy to detect episomal banana streak badnavirus (BSV) in banana and plantain plants that carry integrated BSV sequences. Antisera used in immuno capture polymerase chain reaction (IC-PCR) are capable of binding a large number of BSV serotypes. They found that IC-PCR is suitable for the large scale screening of *Musa* for episomal BSV which is necessary for germplasm movement. Geering et al., (2000) cloned and sequenced part of the genomes of four isolates of BSV and compared with those of other badnaviruses. Immunocapture polymerase chain reaction assays were developed allowing specific detection and differentiation of the four isolates of BSV. Helliot et al., (2003) reported that the anti retroviral and anti hepadnavirus molecules, adefovir, tenofovir and 9-(2-phosphonomethoxyethyl)-2, 6-diamino-purine (PMEDAP), efficiently eradicate the episomal form of BSV from banana plants. Harper et al., (2004) isolated BSV from infected plants sampled across the Uganda *Musa* growing area and the isolates were analysed using molecular and serological techniques. Their analyses showed that BSV is very highly variable in Uganda. Provost et al., (2006) developed a multiplex immunocapture PCR (M-IC-PCR) for the detection of BSV. *Musa* sequence tagged microsatellite site (STMS) primers were selected and used in combination with BSV species specific primers in order to monitor possible contamination by *Musa* genomic DNA, using multiplex PCR. Teycheney et al., (2007) adapted an existing polyvalent degenerate oligonucleotide Rt-PCR (PDO-RT-PCR) assay to the detection of banana mild mosaic virus (BanMMV) and banana virus X, two flexivaridae infecting *Musa* spp. PDO inosine containing primers were found to be well suited to the detection of BanMMV, despite its high molecular diversity, but not to that of the highly conserved BVX, for which species-specific primers were designed.

There is a need to detect these viruses for the selection of virus free planting material. Planting of virus free seed or other propagation material is a prime practice for effective disease control. Dot-blot-ELISA using nitrocellulose or nylon membrane as support has been used for the detection of potato viruses initially. Subsequently, this technique has been applied for the detection of several viruses in both plant tissues and insect vectors. It was reported that Dot-blot-ELISA is a relatively more sensitive and economical in using the different reagents when compared to conventional ELISA performed in plastic plates. Further, the test sample extracts can be blotted on the membrane at the field level and send them to laboratories for further processing. This indicates a wide potential application of the technique for the large scale detection of viruses.

In the present study, an attempt was made to compare the DAC-ELISA and Dot-blot-ELISA for the detection of BSV in banana leaf and pseudostem tissues using heterologous RTBV-polyclonal antiserum.

## 2. Materials and Methods

Suckers collected from banana plants infected with BSV and CMV from West Godavari district of Andhra Pradesh state, India were propagated in the garden of Virology Department, S.V. University, Tirupati. The plants were indexed for presence of viruses by DAC-ELISA. The direct antigen coating (DAC) form of indirect ELISA described by Hobbs et al., (1987) and Mowat and Dawson (1987) was adopted to detect viruses in plants.

Preparation of reagents:

- (a) Phosphate buffered saline (PBS), pH 7.4:
- |   |          |
|---|----------|
| NaCl  | = 8.0 g  |
| Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O | = 1.44 g |
| KH <sub>2</sub> PO <sub>4</sub>                     | = 0.2 g  |

- KCl = 0.2 g  
Distilled water = 1000.00 ml
- (b) Phosphate buffered saline – tween – 20 (PBS-T), pH 7.4:  
0.5 ml of Tween-20 was added to 1000 ml of PBS
- (c) PBS – TPO:  
Polyvinyl pyrrolidone (MW 40000) = 2 g  
Ovalbumin = 0.2 g  
PBS – T = 100.0 ml
- (d) Coating buffer, pH 9.6:  
Na<sub>2</sub>CO<sub>3</sub> = 1.59 g  
NaHCO<sub>3</sub> = 2.93 g  
DIECA = 2.25 g  
Distilled water to 1000 ml
- (e) Diethanolamine substrate buffer, pH 9.8:  
Diethanolamine = 97.0 ml  
Distilled water = 800.0 ml  
pH adjusted to 9.8 with IN HCl (about 67 ml), made up to 1000 ml with distilled water and stored at room temperature.
- (f) Alkaline phosphates (ALP) conjugate  
Goat antirabbit antibodies labelled with ALP (Genei, Bangalore) diluted (1:5000) with PBS-TPO was used
- (g) Substrate para-nitrophenylphosphate (PNP) solution  
Two 5 mg tablets of PNP (Sigma) were dissolved in 20 ml of substrate buffer
- (h) Antiserum  
RTBV and CMV-Banana antisera (Virology Department, S.V. University, Tirupati) were used at 1:5000 dilution in PBS-TPO respectively
- (i) Antigen Extraction Buffer  
For DAC-ELISA, virus infected and healthy leaf and pseudostem tissues were extracted in carbonate buffer containing 0.01 M DIECA

### Procedure:

Antigen samples prepared in carbonate buffer were added to wells of the plate and incubated for 90 min. at 37°C. The plate was washed three times with PBS-T. RTBV and CMV-Banana antisera were added to the wells. The plate was covered with a lid and incubated at 37°C for 90 min. Then the plate was washed 3 times with PBS-T with 3 min. gap between each wash. The goat antirabbit labelled with ALP diluted (1:5000) with PBS-TPO was added to wells. The plate was incubated at 37°C for 90 min. and washed with PBS-T three times as above. The enzyme substrate PNP (sigma no-104) added to wells and incubated at room temperature for 1 hour in dark for colour development. The reaction was terminated by adding 3N NaOH solution at 50 µl/well. The reactions were noted according to colour intensity. The plate was read at A<sub>405nm</sub> in ELISA plate reader.

### Dot-blot-ELISA

Dot-blot-ELISA was carried out according to the method described by Bantari and Goodwin (1985) and Hibi and Satio (1985).

Preparation of reagents:

- (a) Coating buffer, pH 9.6  
Na<sub>2</sub>CO<sub>3</sub> = 1.59 g  
NaHCO<sub>3</sub> = 2.93 g  
Dissolved in about 900 ml distilled. H<sub>2</sub>O, adjusted pH to 9.6 and made up to 1000 ml with distilled water.
- (b) Tris – buffered saline (TBS), pH 7.5:  
Tris (0.02 m) = 4.84 g



- NaCl (0.15 m) = 58.8 g  
Dissolved in 1900 ml distilled water, adjusted pH to 7.5 and made up the volume to 2000 ml with distilled water.
- (c) TBS-Tween  
TBS = 1000 ml  
Tween-20 = 0.5 ml
- (d) Blocking solution  
TBS = 100 ml  
Non fat dried Milk powder = 5g
- (e) Antibody buffer  
TBS-T = 100 ml  
Nonfat dried milk powder = 5 g
- (f) HRP labeled goat antirabbit IgG  
Diluted in antibody buffer (1:5000) just before use.
- (g) Substrate buffer (0.5M sodium citrate, pH 5.2)  
for HRP system:  
Trisodium citrate = 735 mg  
Dissolved in 30 ml distilled H<sub>2</sub>O adjusted pH to 5.2 with IN HCl and made up to 50 ml with distilled H<sub>2</sub>O
- (g) Substrate solution  
For HRP system  
Dissolved 6 mg DAB in 9 ml substrate buffer and added 1ml of 0.3% cobaltous chloride and 10 ml of 30% H<sub>2</sub>O<sub>2</sub>, mixed well and used it immediately
- (h) Antisera  
RTBV – heterologous antiserum and CMV banana antiserum were diluted (1:5000) and (1:500) using antibody buffer respectively.
- (i) Antigens  
For Dot-blot-ELISA, virus infected (BSV and CMV) and healthy leaf and pseudostem tissues were extracted in carbonate buffer containing 0.01 M DIECA, subsequent dilutions of the antigens was made in carbonate buffer.

Antigen samples with a micropipette were applied on to the nitrocellulose membrane according to labelling. The membrane was allowed for drying and then transferred to a petriplate and blocking solution added till the membrane was fully immersed. The membrane was kept constant in blocking solution for 3 hours at room temperature with intermittent shaking. The membrane was transferred from blocking solution to diluted antiserum in blocking buffer and kept at 37°C for 1 hour. The antibody solution was discarded and washed the membrane thrice with TBS-T at 5 min. interval. The goat antirabbit antibodies labelled with HRP were added to the antibody buffer and placed the membrane in it under constant shaking conditions. The conjugate solution was discarded and the membrane was washed thrice with TBS-T at 5 min. interval. The substrate solution specific to enzyme was added and kept in shaking till sufficient colour was developed. The membrane was washed with water and then it was treated with 1.05% sodium hypochlorite solution for decreasing the back ground colour.

Leaf and pseudostem samples from suspected banana (variety Robusta) plants in commercial gardens near Duvvur, Buchireddypalam Mandal, Nellore District, Andhra Pradesh, India were collected and tested for the presence of CMV and BSV by employing DAC-ELISA and Dot-blot-ELISA described above.

### Results and Discussion

In sucker propagated banana plants interveinal chlorotic streaks of varied sizes were noticed on fully expanded leaves (Figure 1). The distribution of the chlorotic streaks is not uniform throughout the leaf in certain plants. As the leaves matured, the chlorotic streaks were less prominent and in certain leaves necrosis was initiated in chlorotic streaks. Overall the symptoms induced by BSV are comparatively less severe as comparative to the symptoms induced by CMV.



**Figure 1. BSV infected banana leaf showing interveinal chlorotic streaks**

The sucker propagated banana plants exhibited bright yellow chlorotic speckles spindle shaped streaks and sometimes continuous interveinal chlorotic streaks. Like BSV, the symptoms induced by CMV are also not uniformly distributed throughout the leaves in infected plants. As the leaves aged, the severity of the symptoms reduced. The lateral veins appear prominent in diseased leaves compared to healthy leaves. Banana (variety Robusta) leaf samples collected from Nellore district also exhibited interveinal chlorotic specks and streaks of varied sizes (Figure 2).



(a) CMV infected banana leaf showing interveinal chlorotic specks and streaks

(b) Healthy banana leaf

Figure 2. Various types of banana leaves

DAC-ELISA and Dot-blot-ELISA were performed using homologous CMV and heterologous RTBV antisera. For this, two sets were first evaluated using the BSV and CMV infected samples of banana propagated in the garden of Virology Department. In DAC-ELISA, BSV was detected up to  $10^{-2}$  dilutions in leaf samples up to  $10^{-1}$  dilution in pseudostem samples of infected banana using RTBV antiserum (Figure 3 and Table 2). But in Dot-blot-ELISA, BSV was detected in both leaf and pseudostem samples up to  $10^{-3}$  dilution. However, background reaction was noticed with healthy samples in  $10^{-1}$  dilution (Figure 4).



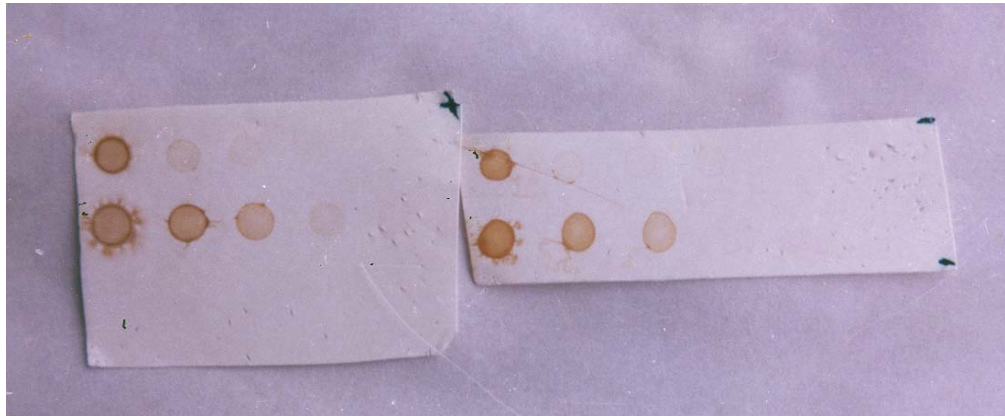
Figure 3. ELISA plate showing positive reactions (yellow colour) of BSV infected banana samples with RTBV antiserum

DAC-ELISA and Dot-blot-ELISA were used for the detection of CMV and BSV in banana (variety Robusta) collected from Duvvur area of Buchireddypalem of Nellore District. Among all the ten tested samples (with mild/faint-chlorotic streaks), none were found positively reacted with CMV-banana antiserum in DAC-ELISA. However, one sample reacted weakly with heterologous RTBV antiserum. Out of

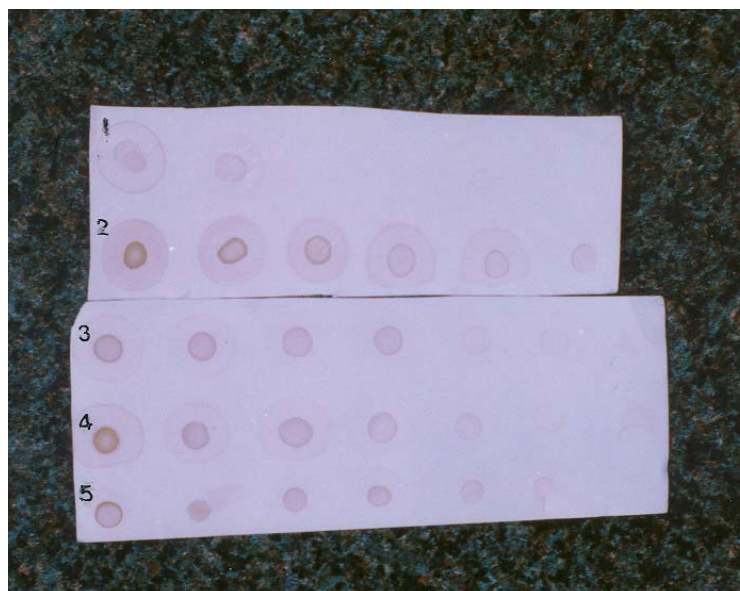
ten CMV negative samples, three samples were further tested by Dot-blot-ELISA. Three samples positively reacted with CMV antiserum up to  $10^{-3}$  to  $10^{-4}$  dilutions (Figure 5, Table 3). Light back ground reaction is also noticed in healthy samples up to  $10^{-1}$  dilution. However one sample positive for BSV in DAC-ELISA was turned out to be negative in Dot-blot-ELISA.

**Table 2. Detection of BSV in banana samples by DAC-ELISA using heterologous RTBV polyclonal antiserum**

Nature of the sample	Dilution	A <sub>405</sub> reading
Healthy rice leaf extract	$10^{-1}$	0.14
	$10^{-2}$	-0.07
	$10^{-3}$	-0.02
Infected rice leaf extract	$10^{-1}$	0.77
	$10^{-2}$	0.50
	$10^{-3}$	0.21
Healthy banana leaf extract	$10^{-1}$	0.12
	$10^{-2}$	-0.04
	$10^{-3}$	-0.04
Infected banana leaf extract	$10^{-1}$	0.48
	$10^{-2}$	0.34
	$10^{-3}$	0.06
Healthy banana Pseudostem extract	$10^{-1}$	0.12
	$10^{-2}$	-0.04
	$10^{-3}$	-0.04
Infected pseudostem extract	$10^{-1}$	0.44
	$10^{-2}$	0.22
	$10^{-3}$	0.041



**Figure 4. Detection of BSV in banana leaf and pseudostem samples using RTBV antiserum by dot-blot-ELISA**



**Figure 5.** Detection of CMV in banana leaf samples using CMV-banana antiserum by dot-blot-ELISA

**Table 3.** Comparison of DAC-ELISA and dot-blot-ELISA for detection of CMV and BSV in field collected banana samples

Sample No.	Virus	DAC-ELISA	Dot-blot-ELISA
1	CMV	0.04	+
2		0.33	+
3		0.04	+
4		0.03	-
5		0.18	-
6		0.11	-
7		0.27	-
8		0.23	-
9		0.14	-
10		0.27	-
Healthy banana leaf extract		0.30	-
CMV infected banana leaf extract		3.58	+
11		0.04	-
12		0.01	-
13		0.04	-
14		0.02	-
15		0.44	-
16		0.03	-
17		0.25	-
18		0.13	-

19		0.24	-
20		0.12	-
Healthy banana leaf extract		0.26	-
BSV infected banana leaf extract		0.48	+
Healthy rice leaf extract		0.13	-
RTBV infected rice leaf extract		1.40	+

### Summary and Concluding Remarks

An attempt was made to compare DAC-ELISA and Dot-blot-ELISA for the detection of BSV and CMV in banana leaf and pseudostem tissues. The significant observations are summarized below:

- BSV and CMV induced similar interveinal chlorotic streaks of varied sizes in banana and hence difficult to identify based on visual symptoms.
- In DAC-ELISA, BSV was detected up to  $10^{-2}$  dilutions of tissue extracts but it was detected by Dot-blot-ELISA up to  $10^{-3}$  dilution.
- Out of ten field collected samples, none were positive for CMV in DAC-ELISA, but three samples reacted positively in Dot-blot-ELISA.
- Out ten field samples tested for BSV, only one weakly reacted. However, in Dot-blot-ELISA, none were found positive for BSV.
- Out of ten samples, none were found mixedly infected.

Above findings indicate that Dot-blot-ELISA is relatively more sensitive for the detection of BSV and CMV in banana.

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**Germination and Seedling growth of Field Pea *Pisum sativum* Malviya Matar-15(HUDP-15) and Pusa Prabhat (DDR-23) under varying level of Copper and Chromium**

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**Abstract:** The heavy metal gradual impact on field pea (*Pisum sativum*) Var. Malviya Matar-15 (HUDP-15) and Pusa Prabhat (DDR-23) was assessed at germination, seedling growth and pigments concentration after having subjected it to different concentration of  $\text{CuSO}_4$  and  $\text{K}_2\text{CrO}_4$ . The germination percentage seedling growth and pigment concentrations were affected by elevating concentration, where Pusa Prabhat (DDR-23) is more sensitive to the elevated concentration of  $\text{CuSO}_4$  and  $\text{K}_2\text{CrO}_4$  solutions concentrations than Malviya Matar-15. But the negative stress of the non essential Cr is more vigorous than essential Cu. This justifies that the field pea variety Pusa Prabhat is less suitable for the cultivation under situation where water and soil suffer from intermittent and momentary metal pollution like Copper and Chromium. [The Journal of American Science. 2008;4(3):28-40]. (ISSN 1545-1003).

**Keywords:** Heavy Metals, Seed Germination, Chemical Treatments, Field Pea

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### 1. Introduction:

Heavy metals are the intrinsic component of the environment with essential and non essential both types. It is the unplanned municipal waste disposal, mining and use of extensive pesticides, other agro-chemicals uses are the significant cause of elevation in environment, its persistence is the cause of most concern. Copper is essential element required in trace amount to plants. Copper concentration increasing in the environment is the cause of disposal of copper containing waste water, sludge and combustion of fossil fuel. Its source may be mining, metal production, phosphate fertilizer production. It has been also incorporating by natural agencies like wind blown dust, decaying vegetation forest fire and sea spray. A very common practice sewage sludge amendment to agricultural soil about 1:20 ratio can expose >100 ppm of Cu to the plants, Singh, R.P.et.al. (2007). Since Cu does not break down in the environment and is continually accumulated by plants and animals thus Cu can influence plant diversity depending on acidity of soil and presence of organic matter, Anonymous b (2004). Cu is essential plant nutrients in trace in excess it is causing stunted growth, chlorosis and root malformation. Cook et.al. (1997) Foy et.al. (1978), Imaculada, Y. (2005).

Chromium is a non-essential and toxic element to plants; Chromium is found in all part of the environment, including air, water and soil naturally occurring in soil, normal range of Cr is from 10 to 50 mg/ kg depending on the parental material. In ultramafic soils (serpentine), it can reach up to 125 mg/ kg, Adriano (1986). The leather industry is the major cause for the high invasion of Cr to the biosphere, accounting for 40% of the total industrial use Barnhart (1997). In India, about 2000–32,000 tons of elemental Cr yearly escapes into the environment from tanning industries. Average 1:20 ratio of soil and sewage sludge blending can expose >20 ppm of Cr to the plants Singh,R.P et.al. (2007). Since seed germination is the first physiological process affected by Cr, the capability of a seed to germinate in a medium containing Cr would be indicative of its level of tolerance to this metal Peralta et al., (2001). High levels (500ppm) of hexavalent Cr in soil reduced germination up to 48% in the bush bean *Phaseolus vulgaris* Parr and Taylor,(1982). Peralta et al. (2001). The maximum quantity of element contaminant was always contained in roots and a minimum in the vegetative and reproductive organs. In bean, only 0.1% of the Cr accumulated was found in the seeds as against 98% in the roots Huffman and Allaway (1973). Decrease in root growth is a well-documented effect due to heavy metals in trees and crops Breckle, (1991); Goldboldand Kettner, (1991); Tang et al., (2001) Prasad et al.(2001) reported that the order of metal toxicity to new root primordia in *Salix viminalis* is  $\text{Cd} > \text{Cr} > \text{Pb}$ , whereas root length was more affected by Cr than by other heavy metals studied. Adverse effects of Cr on plant height and shoot growth have been reported (Rout et al., 1997), Shankar Arun,et. al. (2005).Barcelo,et.al.(1986) studied the chlorophyll-a, chlorophyll-b, total carotenoids and trace elements of initial and first trifoliolate leaves of *Phaseolus vulgaris*. Plants grown with or without Cr(IV) in the nutrient solutions showed negative linear relation between chlorophyll and carotenoid contents due to chromium. High correlation coefficient was also obtained between pigment contents and Iron and Zinc contents. Chromium induced inhibition of Fe and Zn transport was considered to cause chlorosis in plants. Rai et.al.(1990) studied the effect of Cr,Pb,Ni, & Ag on growth, pigments DNA,RNA,heterocysts,frequency uptake of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  nitrate reductase and glutamine synthetase activities of *Nostoc muscorum* and revealed a direct positive correlation between molal concentration and inhibition of different processes. The inhibition of pigments was recorded in the order as  $\text{Chl} < \text{Phycocyanin} < \text{Carotenoid}$ . No generalized trend for inhibition of macromolecules was



observed. Field peas are the most consumable pulses crops, Malviya Matar-15 and Pusa Prabhat DDR-23 are the recently developed high yielding varieties. The required attribute for plants are metal uptake avoidance and accumulation without toxic symptoms development, these are the symptoms of tolerant plants, meanwhile the sensitive plant shows more stress symptoms, To determine tolerance capabilities, the plants were tested by germination test and seedling growth, chlorophyll content these are the key issues for sustenance of plants in any pre-existing conditions. The present work is to assess the effect of increasing concentration of heavy metals like Cu and Cr their possible gradual impact on physiology, biochemistry and heavy metal tolerance of the plants and also to find out the correlation between elevated concentration of heavy metals to pigments concentration and intra pigments dependencies on different concentration of heavy metals.

## 2. Materials and Methods:

### 2.1 Study area

The experiment was conducted at Deptt. of Environmental Science, P.G.College, Ghazipur a suburban area of district head quarter, located in the eastern Gangetic plain of the Indian sub continent at 25°19' and 25°54' N latitude, 83°4' and 83°58' E longitude and 67.50 m above the sea level. The experiment was carried out between Sept.-Oct 2005. This period of the year is characterized by mean monthly maximum temperatures between 34.8 and 36.1 °C and mean monthly minimum temperatures between 16 and 23.5 °C. Maximum relative humidity varied from 95% to 100% and minimum from 69% to 71%.

### 2.2 Selection of Seed

Seed of Field Pea Malviya Matar-15 (HUDP-15) and Pusa Prabhat (DDR-23) was chosen for the experimental work has developed by B.H.U. Varanasi and Indian Agricultural Research Institute (IARI); New Delhi, both are resistant to PM, adoptive to the site of the study (Eastern U.P.) India. And a recently developed and exceedingly espouse high yield variety respectively.

### 2.3 Experimental Design and Set up

Forty seeds of uniform size for each variety were selected, the seeds were surface sterilized in 5% sodium hypochlorite solution to remove the microbial contamination then seed were thoroughly washed with di-ionized water. Water soluble salts of copper (CuSO<sub>4</sub>) and Chromium were (K<sub>2</sub>CrO<sub>4</sub>) were taken for making the solutions by the A.R. Grade reagents first stock solution for 1000 ppm were made and desirable 25,50 and 100 ppm obtained by diluting them by distilled water. Seeds were spread on sterilised petri dishes lined with filter paper whatman No.-1 . The seed were irrigated with equal volume (20ml.) of different concentration of solutions for each treatment , the petri dishes were arranged in a completely randomized block design with three replicates and in each replicates 40 seeds .The experiment was carried out in a growth chamber at 25°C , 12 hours dark and 12 hours light period with illumination of 2500 lux. Their germination were recorded on each day at fixed time and fixed intervals. The germination percentage was recorded in tabular form. After 15 days only 12 germinated seedlings of similar morphology and age groups of each variety were transferred in plastic trays with size 24``×10`` size with soil and irrigated on alternate days with 250 ml of respective concentration of 25, 50 and 100 % of Cu and Cr solutions. Each treatment having three replicates and each replicates contains 12 seedlings. The seedlings were harvested after 15 days from the date of seedling transferred in Trays.

### 2.4 Estimation of Germination %, Root length, Shoot length and No. of lateral roots

Germination percentage was calculated by dividing the seed germinated on each day by total no. of seed taken × 100. And finally adding the total percentage. Root, and shoot length measured by normal scale.

### 2.5 Estimation of Chlorophyll and Carotinoids

The chlorophyll and carotinoids contents of primary leaves were estimated with 80% acetone with help of spectrophotometer (Hitachi, Ltd. Tokyo) Jayaraman,J.(1981).

Chlorophyll a = (.0127) (OD663)-(0.0269) (OD 645) gm/lit.

Chlorophyll b = (.0229) (OD 645) – (.00488) (OD 638)gm/lit.

Carotenoids = (OD 490)-(0.114) (OD663) – (.638) (OD 645) gm/lit.

## 3. Results:

**Table 1 (a):** Effect of Copper and Chromium on percentage germination of Malviya Matar-15. (Mean±Stand.Error)

S.No.	Treatment	Concentration	% Germination	% Reduction in germination
1.	Control	-----	100±.00 <sup>a</sup>	-----
2.	CuSO <sub>4</sub>	25	88±3.0 <sup>b</sup>	12
		50	80±1.1 <sup>c</sup>	20
		100	70±1.5 <sup>d</sup>	30
3.	K <sub>2</sub> CrO <sub>4</sub>	25	83±1.5 <sup>b</sup>	17
		50	75±1.5 <sup>c</sup>	25
		100	66±1.8 <sup>d</sup>	44

Different letters in each group shows significant difference at P<0.05 levels.

**Table 1 (b):** Effect of Copper and Chromium on percentage germination of Pusa Prabhat (DDR-23). (Mean±Stand.Error).

S.No.	Treatment	Concentration	% Germination	% Reduction in germination
1.	Control	-----	100±.00 <sup>a</sup>	-----
2.	CuSO <sub>4</sub>	25	85±2.5 <sup>b</sup>	15
		50	78±2.5 <sup>b</sup>	22
		100	67±2.5 <sup>c</sup>	33
3.	K <sub>2</sub> CrO <sub>4</sub>	25	83±3.6 <sup>b</sup>	17
		50	72±1.5 <sup>c</sup>	28
		100	63±3.0 <sup>d</sup>	37

Different letters in each group shows significant difference at P<0.05 levels.

**Table-2(a):** Effect of Copper and Chromium on Root length, Shoot length and Number of lateral roots of Malviya Matar-15.(Mean±Stand.Error)

S.No.	Treatment	Concentration %	Root length (Cm.)	Shoot length (Cm.)	No. of lateral roots
1.	Control	-----	6.5±.40 <sup>a</sup>	9.9±.26 <sup>a</sup>	9.2±.10 <sup>a</sup>
2.	CuSO <sub>4</sub>	25	5.4±.01 <sup>b</sup>	6.2±.20 <sup>b</sup>	7.3±.40 <sup>b</sup>
		50	4.6±.20 <sup>bc</sup>	5.6±.35 <sup>b</sup>	6.2±.15 <sup>c</sup>
		100	3.2±.15 <sup>d</sup>	3.8±.11 <sup>c</sup>	4.2±.15 <sup>d</sup>
3.	K <sub>2</sub> CrO <sub>4</sub>	25	2.9±.45 <sup>b</sup>	3.6±.26 <sup>b</sup>	7±.40 <sup>b</sup>
		50	2.2±.15 <sup>bc</sup>	2.9±.26 <sup>bc</sup>	5±.20 <sup>c</sup>
		100	1.3±.15 <sup>c</sup>	1.6±.30 <sup>c</sup>	3.2±.20 <sup>d</sup>

Different letters in each group shows significant difference at P<0.05 levels.

**Table-2(b):** Effect of Copper and Chromium on Root length, Shoot length and Number of lateral roots of Pusa Prabha(DDR-23).(Mean±Stand.Error).

S.No.	Treatment	Concentration	Root length (Cm.)	Shoot length (Cm.)	No. of lateral roots
1.	Control	-----	6.8±.20 <sup>a</sup>	9.1±.005 <sup>a</sup>	8.7±.11 <sup>a</sup>
2.	CuSO <sub>4</sub>	25	5.0±.75 <sup>b</sup>	6.0±.30 <sup>b</sup>	7.0±.30 <sup>b</sup>
		50	4.2±.15 <sup>bc</sup>	5.2±.15 <sup>c</sup>	5.9±.005 <sup>c</sup>
		100	2.9±.23 <sup>c</sup>	3.7±.15 <sup>d</sup>	3.9±.008 <sup>d</sup>
3.	K <sub>2</sub> CrO <sub>4</sub>	25	2.5±.005 <sup>b</sup>	3.3±.01 <sup>b</sup>	6.6±.20 <sup>b</sup>
		50	1.8±.15 <sup>c</sup>	2.0±.25 <sup>c</sup>	4.7±.11 <sup>c</sup>
		100	1.1±.1 <sup>d</sup>	1.4±.26 <sup>c</sup>	2.9±.26 <sup>d</sup>

Different letters in each group shows significant difference at P<0.05 levels.

**Table-3(a):**Effect of Copper and Chromium on pigment content of Malviya Mater-15. (Mean±Stand.Error)

S.No.	Treatment	Concentration	Cholophyll 'a' (g/lit.)	Chlorophyll 'b' (g/lit.)	Carotenoids (g/lit.)
1.	Control	-----	.0042±.00002 <sup>a</sup>	.0056±.00003 <sup>a</sup>	.345±.00005 <sup>a</sup>
2.	CuSO <sub>4</sub>	25	.0036±.00005 <sup>a</sup>	.0052±.000015 <sup>a</sup>	.221±.00026 <sup>b</sup>
		50	.0029±.00002 <sup>b</sup>	.0049±.00002 <sup>b</sup>	.168±.00015 <sup>c</sup>
		100	.0021±.00002 <sup>c</sup>	.0038±.000015 <sup>c</sup>	.0978±.000046 <sup>d</sup>
3.	K <sub>2</sub> CrO <sub>4</sub>	25	.0028±.000015 <sup>b</sup>	.0050±.000050 <sup>a</sup>	.208±.000025 <sup>b</sup>
		50	.0019±.000057 <sup>c</sup>	.0037±.000020 <sup>b</sup>	.144±.00040 <sup>c</sup>
		100	.0012±.000015 <sup>d</sup>	.0025±.000015 <sup>c</sup>	.0975±.000028 <sup>d</sup>

Different letters in each group shows significant difference at P<0.05 levels.

**Table-3(b):** Effect of Copper and Chromium on pigment content of Pusa Prabhat (DDR-23). (Mean±Stand.Error).

S.No.	Treatment	Concentration	Cholophyll 'a' (g/lit.)	Chlorophyll 'b' (g/lit.)	Carotenoids (g/lit.)
1.	Control	-----	.0040±.000025 <sup>a</sup>	.0054±.000016 <sup>a</sup>	.339±.00011 <sup>a</sup>
2.	CuSO <sub>4</sub>	25	.0031±.000025 <sup>b</sup>	.0047±.000015 <sup>b</sup>	.218±.00011 <sup>b</sup>
		50	.0024±.000026 <sup>bc</sup>	.0040±.00002 <sup>b</sup>	.162±.00011 <sup>c</sup>
		100	.0019±.000015 <sup>c</sup>	.0036±.000013 <sup>c</sup>	.0973±.000017 <sup>d</sup>
3.	K <sub>2</sub> CrO <sub>4</sub>	25	.0022±.000030 <sup>b</sup>	.0050±.000026 <sup>a</sup>	.200±.0000 <sup>c</sup>
		50	.0020±.000015 <sup>b</sup>	.0031±.00001 <sup>b</sup>	.140±.00028 <sup>c</sup>
		100	.0011±.0000 <sup>c</sup>	.0022±.000015 <sup>c</sup>	.0965±.00010 <sup>d</sup>

Different letters in each group shows significant difference at P<0.05 levels.

Percentage germination of field pea (Malviya Matar-15) and Pusa Prabhat (DDR-23) has affected by the copper and chromium solutions. The rate of germination have highly retrograde in presence of higher concentration of solution than preceding lower concentration and control as mentioned in Table No. 1(a)and (b).

Root and Shoot length , No. of lateral roots and pigments of 15 days old seedlings at different concentrations of Cu and Cr are presented in Table No. 2(a),(b) and 3 (a),(b) respectively. The highest value of Root length, Shoot length and No. of lateral roots and amount of pigments reported in control condition for both species and treatments and least values were reported in case of 100 PPM concentration. There was considerable difference in root length shoot length, no. of lateral roots for both species treatments with Copper sulphate and Potassium chromate , the extent of decline was significantly greater in case of Pusa Prabhat than Malviya Matar-15. The root length was significantly influenced by varying concentration of copper sulphate and vigorously affected by potassium chromate solution. The comparative negative effect was higher in case of Pusa Prabhat than Malviya Matar-15. The two different metal treatments had a significant effect on shoot development. The shortest shoot length was observed at highest concentration 100 PPM of potassium chromate in Pussa Prabhat. However comparatively longer shoot length in case of Malviya Matar-15 reported in both chemical treatments as mentioned in Table No.- 2. Negative effect of chromate solution is more on both crops then copper sulphtae solution. Percentage germination and number of lateral roots have reduced with each elevation in concentration in all the treatments. Effect of metal treatment on pigments had been significantly seen as the copper sulphate solutions decreases in the pigment formation like Chlorophyll a, Chlorophyll b and formation of carotenoids. While increase the concentration potassium chromate causes a more adverse effect on the vegetations. As lesser formation of chlorophyll a, chlorophyll b and carotinoids. ANOVA analysis indicates the P<.05 level of significance in all the treatment cases compare to control.

**Table: 4a.** Co-relationship between varying level of copper concentration and pigments concentration in leaf of Malviya Matar-15.

**Correlations**

		CHLOROPH	CHLB	CARATINO
CHLOROPH	Pearson Correlation	1.000	.780**	.921**
	Sig. (2-tailed)	.	.003	.000
	N	12	12	12
CHLB	Pearson Correlation	.780**	1.000	.848**
	Sig. (2-tailed)	.003	.	.000
	N	12	12	12
CARATINO	Pearson Correlation	.921**	.848**	1.000
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

\*\* . Correlation is significant at the 0.01 level (2-tailed).

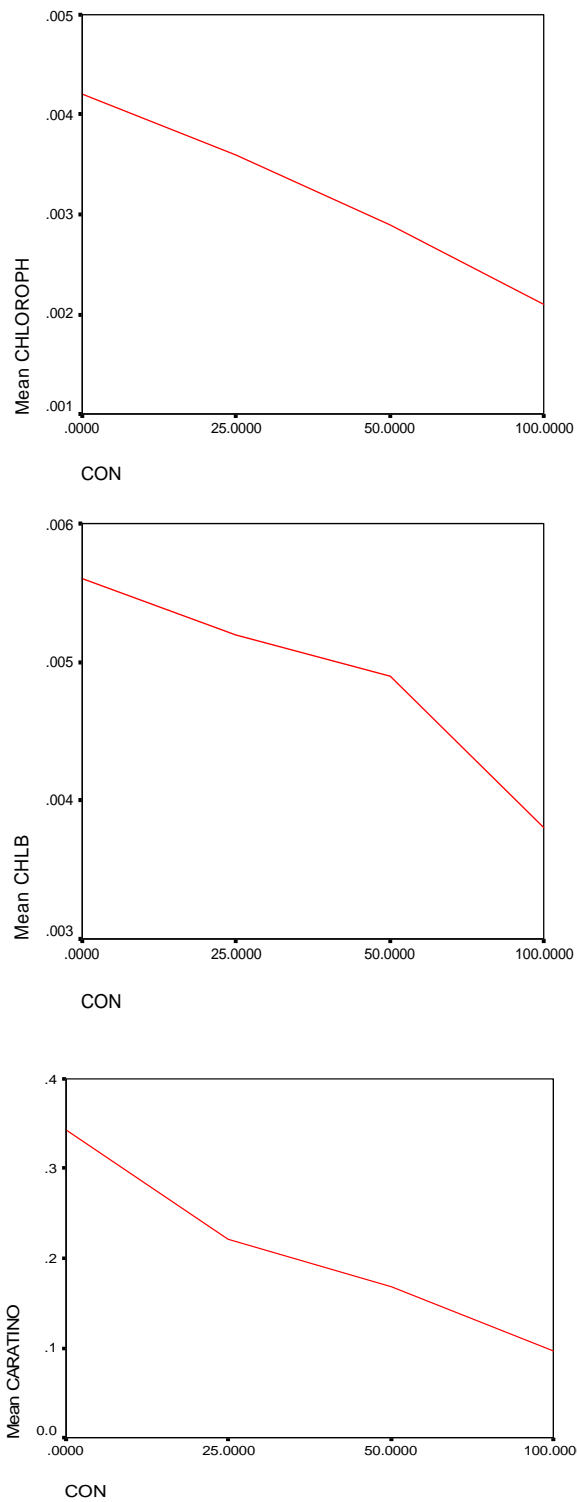


Fig.:1a. Relationship between varying level of copper concentration and pigments concentration in leaf of Malviya Matar-15.

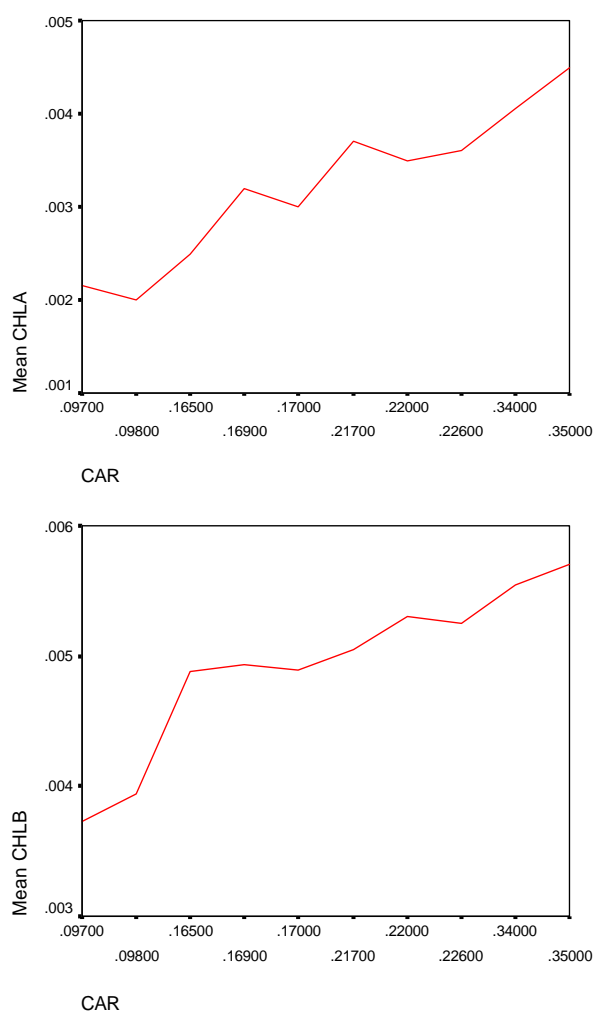


Fig.:1b. Rerelationship between varying level of carotinoids concentration with chlorophyll 'a' and 'b' concentration in leaf of Malviya Matar-15.

**Table: 4b.**Co-relationship between varying level of copper concentration and pigmentsconcentration in leaf of Pusa Prabhat.

**Correlations**

		CHLA	CHLB	CAR
CHLA	Pearson Correlation	1.000	.913**	.918**
	Sig. (2-tailed)	.	.000	.000
	N	12	12	12
CHLB	Pearson Correlation	.913**	1.000	.929**
	Sig. (2-tailed)	.000	.	.000
	N	12	12	12
CAR	Pearson Correlation	.918**	.929**	1.000
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

\*\* . Correlation is significant at the 0.01 level (2-tailed).

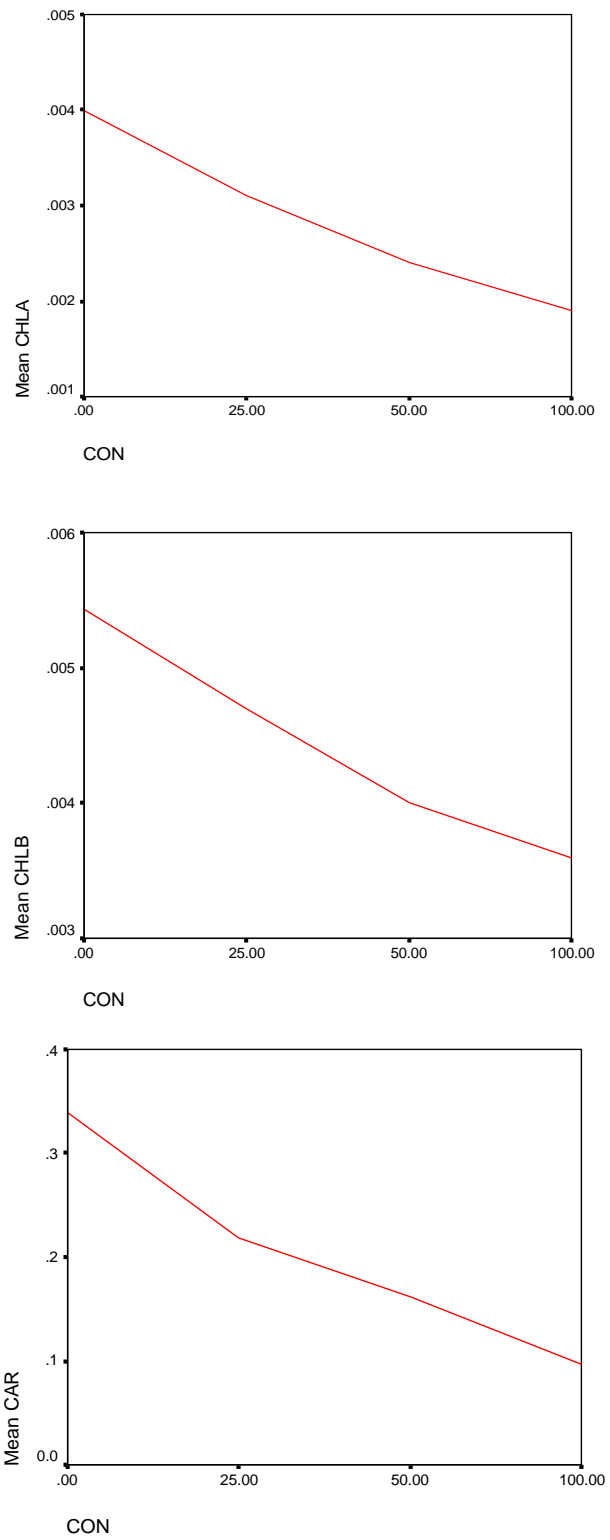


Fig.:2a. Relationship between varying level of copper concentration and pigments concentration in leaf of Pusa Prabhat.

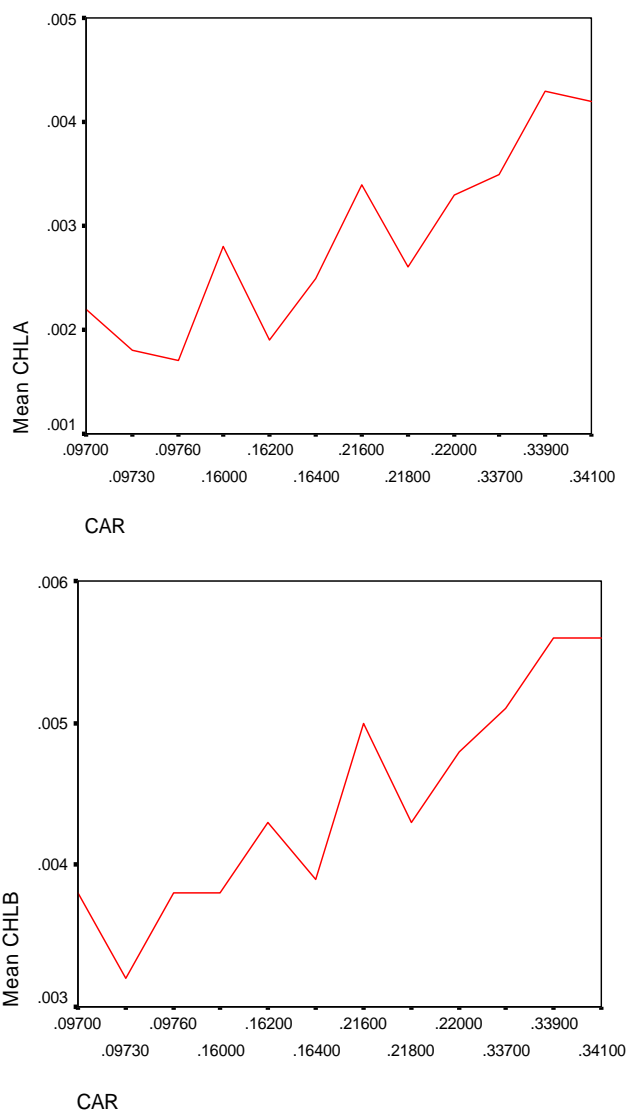


Fig.:2b. Rerelationship between varying level of carotinoids concentration with chlorophyll 'a' and 'b' concentration in leaf of Pusa Prabhat.

**Table:4c.**Co-relationship between varying level of Chromium concentration and pigments concentration in leaf of Malviya Matar-15.

**Correlations**

		CHLA	CHLB	CAR
CHLA	Pearson Correlation	1.000	.875**	.977**
	Sig. (2-tailed)	.	.000	.000
	N	12	12	12
CHLB	Pearson Correlation	.875**	1.000	.889**
	Sig. (2-tailed)	.000	.	.000
	N	12	12	12
CAR	Pearson Correlation	.977**	.889**	1.000
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

\*\* . Correlation is significant at the 0.01 level (2-tailed).

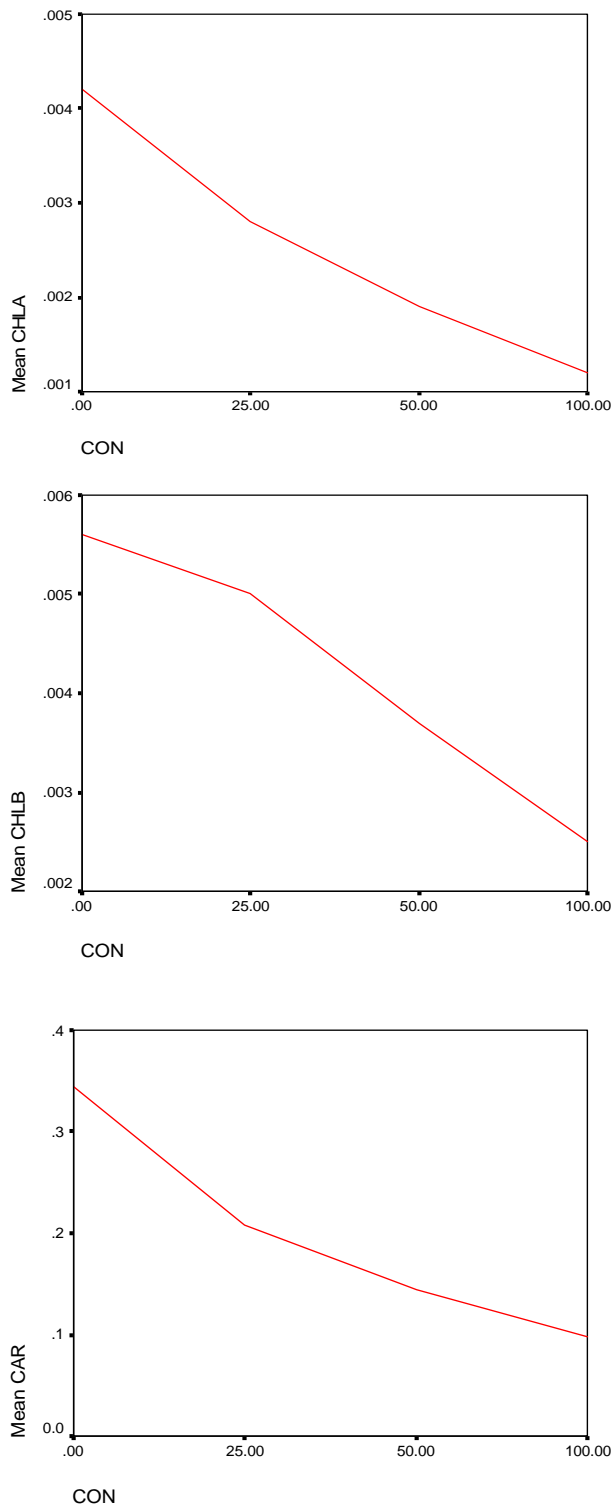


Fig.:3a. Relationship between varying level of Chromium concentration and pigments concentration in leaf of Malviya Matar-15.



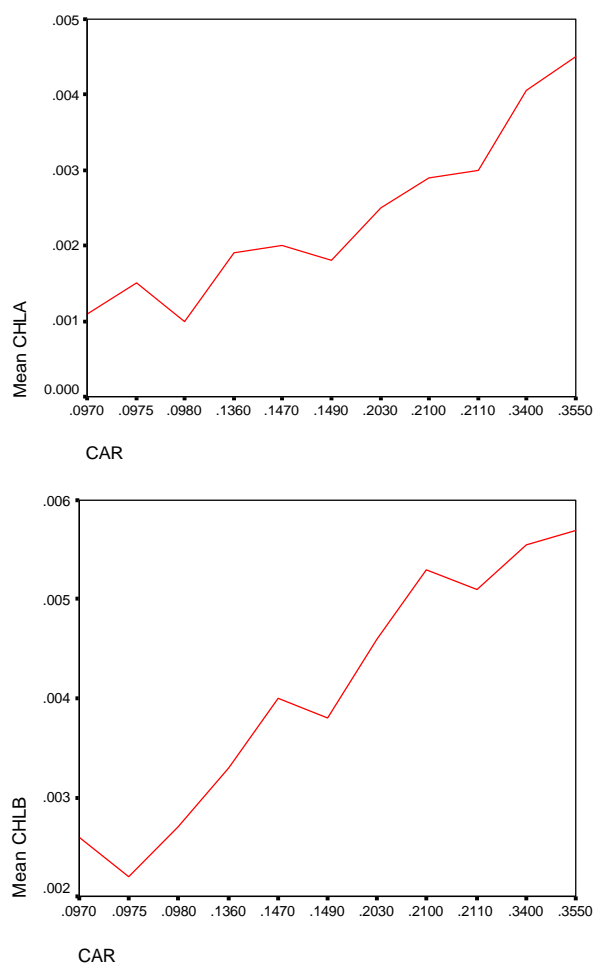


Fig.:3b. Rerelationship between varying level of carotinoids concentration with chlorophyll 'a' and 'b' concentration in leaf of Malviya Matar-15.

**Table:4d.**Co-relationship between varying level of Chromium concentration and pigments concentration in leaf of Pusa Prabhat.

**Correlations**

		CHLA	CHLB	CAR
CHLA	Pearson Correlation	1.000	.787**	.948**
	Sig. (2-tailed)	.	.002	.000
	N	12	12	12
CHLB	Pearson Correlation	.787**	1.000	.882**
	Sig. (2-tailed)	.002	.	.000
	N	12	12	12
CAR	Pearson Correlation	.948**	.882**	1.000
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

\*\* . Correlation is significant at the 0.01 level (2-tailed).

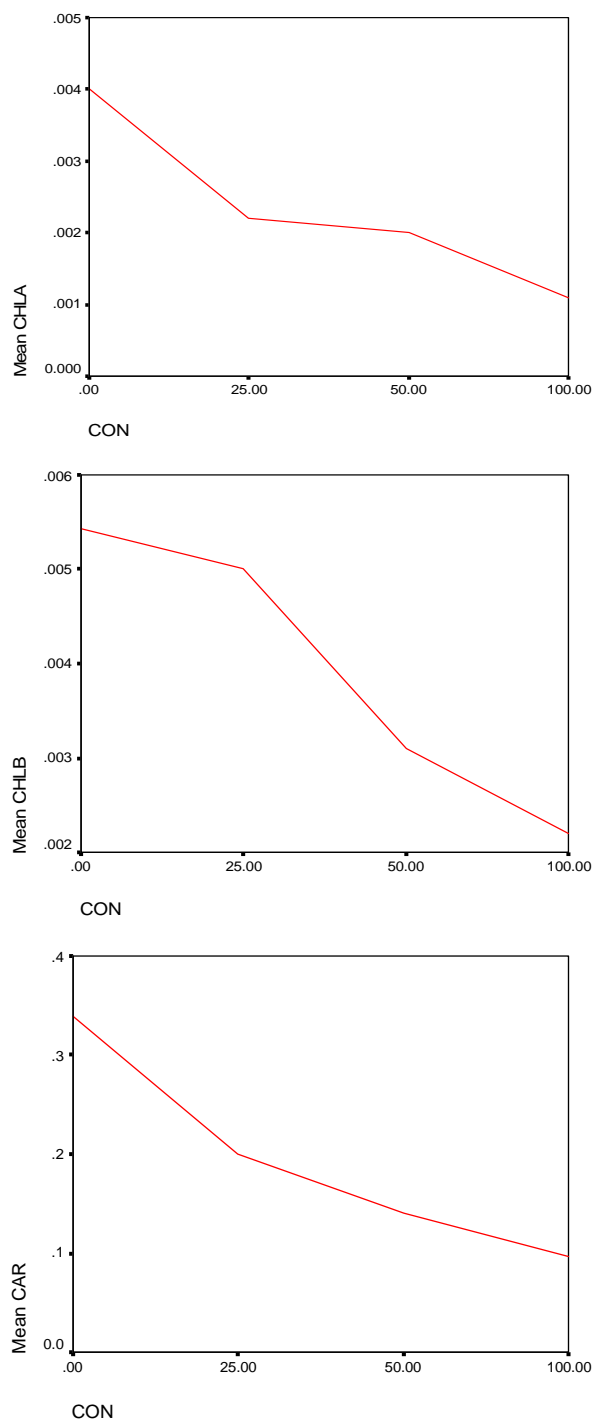


Fig.:4a. Relationship between varying level of Chromium concentration and pigments concentration in leaf of Pusa Prabhat.

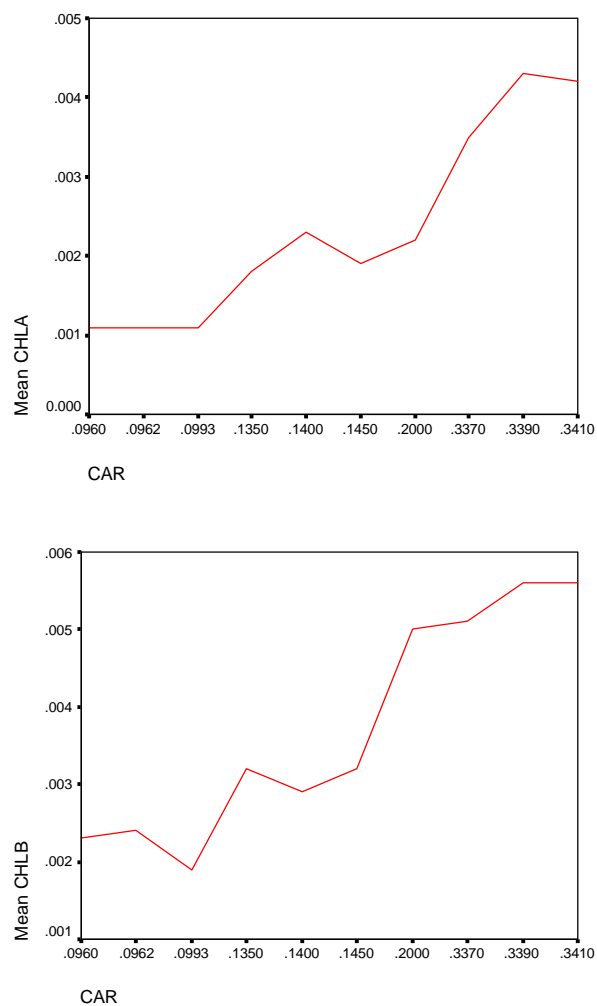


Fig.:4b. Relationship between varying level of carotinoids concentration with chlorophyll 'a' and 'b' concentration in leaf of Pusa Prabhat.

Correlation studies shows that the increase in concentration of solution is inversely related to the concentration of the all pigments while the correlations between carotinoids and chlorophyll 'a' and 'b' is positive as increase in concentration of chlorophyll increase in concentration of carotinoids.

#### 4. Discussion and Conclusions:

The present result showed that the germination of both varieties was significantly influenced by both chemical treatments. The results clearly indicates that increasing concentration of these two metals retrogrades the germination with its percentage increase. The result was consistent with Rout et. al. (2000). Jain et. al.(2000) Peralta (2001). The reduced germination of seeds under Cr stress could be a depressive effect of Cr on the activity of amylases and on subsequent transport of sugars to the embryo axes Zeid (2001).Heavy metal causes a significant adverse effect on seedling growth and pigment formation. The study showed reduction in root length, shoot length, no. of lateral roots and pigment formation. The effect of negative stress is max.in case of Cr then Cu. This could be due to the inhibition of the root cell division/root elongation or to extension of the cell cycle in roots Barcelo et. al. (1986).Result similar to Cu toxicity on root growth has been reported by Sheldon,A. and Neal W. Menzies (2004). Shoot growth reduction through the toxicity of Cr has been reported by Rout et. al. (1997). Barton (2000). The reduction in shoot growth is due to stunted root growth results lesser nutrients and water supplied to aerial parts, where Cr and Cu causes direct impact on the cellular metabolism causes shortning of shoot height. Hanus and Tomas(1993).At elevated level of Cu treatment of seedlings the Chl a, Chl b and carotinoids content decreases supported by Miller (1938), Patsikka (2002). Where author suggested that reduced chlorophyll contents observed in the plants grown in presence of very high copper content due to iron deficiency. Meanwhile the elevated level of chromium shows decreased level of Chl a,Chl b and carotinoids as supported by Ganesh Shankar,K. et. al. (2006).Where author have suggested that morphological germination study parameters were decreased with respect to increase of chromium concentrations. In concluding remark we can say that both metal treatments are harmful for the plant germination and seedling growth behaviors at elevated concentration level from its optimum. Where Cr is comparatively more toxic than Cu. The Pusa Prabhat is more sensitive to the metal pollution like Cr and Cu pollution than Malviya Matar-15.

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### Seed pretreatments enhance germination in *Occimum gratissimum* (lameaceae)

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**Abstract:** *Occimum gratissimum* (fever plant) is a crop with a wide range of use as a medicinal plant and as a spice. In spite of its huge economic importance, the plant is only grown in homestead. The study investigated the effects of light, temperature and water treatments on the germination of the seeds. The incubation of seeds at 25°C under light condition, coupled with leaching in running water for 12 hours was found to release the dormancy in the seeds of *Occimum gratissimum*. [The Journal of American Science. 2008;4(3):41-45]. (ISSN: 1545-1003).

**Keyword:** medicinal plants, endangered species, *occimum*, germination, dormancy,

#### Introduction

*Occimum gratissimum* (linn), the fever's plant belong to the group of labiates herbs with about 180 genera and 3500 species (Lancer and Gill, 1991; Masfield et al., 1973; Sofowora, 1982). The aromatic leaves add flavor to soups and sauces. *O. gratissimum* produces a volatile oil which has anti-microbial properties (Moundipa et al., 2005; Sofowora, 1982); as such it is being used as a vegetable spice for meat seasoning in Nigeria. Its efficacy in the control of food storage insects and as a fungicide has also been explored (Awuah, 1996; Eze et al., 2006). Its leaf extract is generally believed to cure respiratory infections (Agusiobo, 1984; Lasisi and Ajuwon, 2002). Additionally, it is also used in the treatment of epilepsy, high fever and mental illness (Abdulrahman, 1992; Oliver, 1980; Osifo, 1992). Furthermore, *O. gratissimum* is being used for the cure of diarrhoea, headache, ophthalmic (ocular) diseases, skin diseases, pneumonia, cough, fever, conjunctivitis, as well as digestive disorders (Obuekwe and Obuekwe, 2002; Onajobi, 1986; Sidhu et al., 2007; Sofowora, 1993). In India, it is believed to be used for aromatic baths of fumigations for the treatment of rheumatism and paralysis.

*O. gratissimum* is an erect perennial shrub, about 1.5m in height and propagates only by seeds. In spite of its enormous economic and industrial potentials, no deliberate attempt has been made to cultivate the crop as it is still grown as a garden plant. With development and its allied ecological problems such as bush burning moving to the rural areas, the compound farm concept is threatened, and as such under-utilized species like *O. gratissimum* are most endangered. Hence, there is need to engage in more research activities that would enhance its, conservation, improvement and processing. A preliminary report indicated frequent loss of viability in the seeds of *O. gratissimum* (Ojeifo and Denton, 1993). In this study, the effect of seed pretreatments on dormancy status of *O. gratissimum* was explored.

## Materials and methods

The seeds of *O. gratissimum* were collected from the parent stands. A white table spread of 1m<sup>2</sup> was spread at the base of the stand. Various terminal racemes on many branches on the stand were gathered by hand and shaken at the direction of the white table spread. The small seeds were then collected from this spread and then were sun-dried, de-husked and stored in the refrigerator.

Seeds of *O. gratissimum* were subjected to germination tests. De-husked seeds were surface-sterilized in 10% w/v chlorox for 30 seconds and rinsed in several changes of distilled water. Fifty seeds were planted in each of the 9 cm diameter petri dishes lined with moist sterile filter. The experiment was carried out under light and dark at 29±1°C. Light was supplied by four 40 watt white fluorescent lamps at a distance of 1 m (1200 lux). The petri dishes for the dark condition were wrapped completely with aluminum foil and kept in a dark cupboard. Fifty seeds were also planted for germination. The set up was subjected to combinations of light/dark and temperature requirements of 10°C, 15°C, 25°C, 30°C, 35°C and 40°C. Observations were made for up to day 10 of incubation. Radicle emergence of up to 1.5 mm was taken as a visible sign of germination.

Dormancy breaking by leaching in water was investigated. Seed lots were wrapped in clean sieve-cloth, placed in a beaker and put under running tap water for 12, 24, 36 and 48 hours. Control seeds were not leached. The seeds were then tested for germination at 25°C under light condition.

## Statistical analysis

All experiments were repeated 5 times. Data were statistically analyzed by the SAS software using a completely randomized design and means were compared at the  $p = 0.05$  level of significance using Duncan's multiple range test (SAS GLM,  $P < 0.05$ ; SAS Institute, 1989).

## Results and Discussion

Germination test showed that the seeds of *O. gratissimum* were in a state of dormancy (Table 1). Seeds germinated poorly especially in the dark with a maximum of 40% after 10 days (Table 1). Germination of seeds of *O. gratissimum* was enhanced under light conditions. 70% seed germination was observed as from the 6<sup>th</sup> up to the 10<sup>th</sup> day after sowing. The optimum temperature for seeds subjected to combination of light and temperature treatments was 25°C (Table 2). Light-induced germination was also observed in *Ocimum basilicum* seeds (Dawoud et al., 2003). It has long been established that light sensitivity of seeds operates through the phytochrome pigment systems (Black, 1972; Donohue, 2007). The dormancy challenge on the germination of *O. gratissimum* appears to be a combination of hard seed coat, the presence of germination inhibitors and a light requirement. Hence the release of dormancy from these seeds can only be achieved when the restriction to the entry of water and oxygen is eliminated and that the inhibiting substances are prevented from affecting the biochemistry of germination in these seeds. This would consequently trigger other processes that lead to embryo germination.

The leaching of seeds in running water for 12 hours, coupled with sowing at the optimum temperature and light conditions gave 100% germination (Table 3). It is plausible that germination inhibitors in the seeds, which act by blocking the enzymes sites, have been leached out by the running water. Çırak et al. (2007) also reported the release of exogenous dormancy in the seeds of a *Hypericum* species by presoaking the seeds in water for 24 hours. It was, however, surprising to note that further treatment for longer period under running water for 24-48 hours had deleterious effects on the seeds (Table 3).

On the whole, this study has indicated that *O. gratissimum* germination test should be conducted at 25°C preferably in light. Dormancy in this species can be released by 12 hour washing of the seeds under running tap water.

Table 1. Percentage germination of *O. gratissimum* seeds after 10 days incubation under light and dark at 29±1°C. Data are means of 5 replicates.

Period(days)	Percentage germination	
	Light	Dark
2	0.0±0.0 <sup>d</sup>	0.0±0.0 <sup>c</sup>
4	20±4.5 <sup>c</sup>	0.0±0.0 <sup>c</sup>
5	40±3.7 <sup>b</sup>	0.0±0.0 <sup>c</sup>
6	70±2.4 <sup>a</sup>	30±5.6 <sup>b</sup>
8	70±2.4 <sup>a</sup>	40±2.8 <sup>a</sup>
10	70±2.4 <sup>a</sup>	40±2.8 <sup>a</sup>

\*Mean ± SE. Means having the same letter are not significantly different (p=0.05) according to Duncan's multiple range test.

Table 2. Percentage germination of *O. gratissimum* seeds incubated under varying temperatures for 10 days. Data are means of 5 replicates.

Temperature (°C)	Percentage germination	
	Light*	Dark*
10	50±4.6 <sup>d</sup>	25±5.6 <sup>b</sup>
15	65±3.4 <sup>bc</sup>	20±2.6 <sup>b</sup>
25	80±4.6 <sup>a</sup>	45±4.8 <sup>a</sup>
30	70±4.7 <sup>b</sup>	50±3.6 <sup>a</sup>
35	60±4.2 <sup>cd</sup>	20±3.4 <sup>bc</sup>
40	35±4.6 <sup>e</sup>	15±2.1 <sup>c</sup>

\*Mean ± SE. Means having the same letter are not significantly different (p=0.05) according to Duncan's multiple range test.

Table 3. Effect of leaching on seed germination in *O. gratissimum*. Data are means of 5 replicates.

Treatment	Duration (hour)*	Percentage germination (10 <sup>th</sup> day)*
Leaching	12	100±0.0 <sup>a</sup>
	24	50±5.6 <sup>c</sup>
	36	30±3.4 <sup>d</sup>
	48	25±2.7 <sup>d</sup>
Control	-	77±6.5 <sup>b</sup>

\*Mean ± SE. Means having the same letter are not significantly different (p=0.05) according to Duncan's multiple range test.

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**ALTITUDINAL CHANGES IN DOMINANCE-DIVERSITY AND SPECIES RICHNESS OF TREE SPECIES IN A TEMPERATE FOREST OF GARHWAL HIMALAYA**

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**Abstract:** In this study, we examined the species composition, diversity and concentration of dominance of tree species along an altitudinal gradient of Garhwal Himalaya. Vegetational analysis of tree species was analysed identifying four forest stands along an altitude of (1200-1800m). *Quercus leucotrichophora* emerged as a dominant species on the (Stand III, 1600m and Stand IV, 1800m) with the highest IVI values 138.79 & 74.49 respectively. The total density ranged from 1166 to 1828 trees/ha. Species diversity ranged from 1.00 to 2.07 Maximum diversity reported for Stand IV, 1800m and minimum diversity (1.00) recorded for Stand I, 1200m. [The Journal of American Science. 2008;4(3):46-54]. (ISSN 1545-1003).

**Key words:** Altitude, Diversity, Species richness

### **Introduction**

The temperate forests of Western and Central Himalaya are usually distributed between (1200 and 3000 m asl) which are represented either by pure Oak or Oak- *Rhododendron* mixed forests. Puri (1960) considered that these forests represent climatic climax of one or other species of *Quercus* in upper altitudinal zones. The lower elevations of the temperate forest are occupied by Oak-Pine mixed forests and *Quercus semicarpifolia* with other coniferous at higher altitudes, normally form the climax vegetation. Other species of Oak are found above the Oak-Pine forests of Garhwal Himalaya (Osmoston, 1922). The oaks are the most preferred species in the entire region and used mainly for fuel, fodder and small timber. The forests also vary with altitude ranging from *Shorea robusta* in submontane zone to *Quercus semicarpifolia* near timberline and *Quercus leucotrichophora* and *Pinus roxburghii* in the montane zone (Bhandari *et al*, 1997). It is interesting that in the sub alpine zone to the north facing slopes bear timberline at lower altitude and on south facing slopes the *Quercus semicarpifolia* wood reaches to higher altitudes than on north facing slopes. The various changes in the Himalayan forests are appearing in their structure, density and composition due to global warming (Gaur, 1982), uncontrolled lopping and felling of trees for fuel wood, fodder and grazing (Bargali *et al*. 1998; Kumar *et al*. 2004 ;).

Species diversity and its distribution along the altitudinal gradient had been a subject of ecosystem. Earlier Rahbek (1997) viewed that approximately half of the studies detected a mid altitude peak in species richness, in a critical literature review on species richness patterns in relation to altitude. Grytnes and Vetaas (2002) have also reviewed these aspects in Nepalese Himalaya. Though the plant

community of a region is a function of time, nevertheless, altitude, slope, latitude, aspect, rainfall and humidity had a play a role in the formation of community composition.

A lot of work has been done by various workers on submontane and montane forests of Western and Central Himalaya. The analytical and synthetic behavior of high altitude forests of Kumaun Himalaya study by Ralhan *et al*, (1982) Saxena & Singh, (1982) Singh & Singh, (1987) Adhikari et al. (1991).

Though several studies have been done on the plant communities of the garhwal was done by (Tiwari et al, 1989; Joshi & Tiwari, 1990; Bisht & Kusumlata, 1993; Bhandari et al, 1995; & 1997; Kumar et al, 2004). In the present study we try to understand the variation in tree species along the altitudinal gradient in a temperate forest for Garhwal Himalaya.

## **Material and Methods**

### **Study site**

The present study was carried out along an altitudinal gradient in the temperate forest of Narayanbagar block of chamoli district. It lies in the Central Himalaya between the latitude  $29^{\circ}31'9''\text{N}$  and  $31^{\circ}26'5''\text{N}$  and longitude  $77^{\circ}33'5''\text{E}$  and  $80^{\circ}6'0''\text{E}$  with a total area of 29, 089 km<sup>2</sup>. A total of four forest stands were selected at different altitudes (1200m –1800m) to examine the changes on the tree vegetation (Table: 1).

### **Climate**

As the elevation of the district ranges from 800 m. to 8000 m above sea level the climate of the district very largely depends on altitude. The winter season is from about mid November to March. As most of the region is situated on the southern slopes of the outer Himalayas, monsoon currents can enter through the valley, the rainfall being heaviest in the monsoon from June to September.

### **Methods**

Vegetational analysis of the selected forest stands along an altitudinal gradient was carried out in the year 2004 by using 10 X 10 m quadrates. The quadrates were laid out randomly for tree species throughout the selected forest stands. The size and the number of quadrates were determined following (Misra, 1968 and Kershaw, 1973). In each quadrates, trees were recorded with  $>31.5$  cm cbh (Circumference at breast height i.e. 1.37m above the ground) individually measured. The vegetational data were quantitatively analysed for abundance, density and frequency according to the formulae given by Curtis and Mc Intosh (1950) and Mishra (1968). The relative values were summed up to represent Importance Value Index (IVI) as per Curtis (1959). The diversity index ( $H'$ ) was computed by using Shannon-Wiener information Index (Shannon and Wiener, 1963). The concentration of dominance (CD) was computed by Simpson's Index (Simpson, 1949).

## Results

The study showed that the total number of the tree species varied from (3 to 9) from (Stand I, 1200m Stand IV, 1800m). The highest number of tree species was recorded from (Stand IV 1800m) due to relatively open habitat which provided congenial environment for the growth of different species. Fig 1 shows the total density of tree species ranged between (1166 trees/ha Stand I to 1826 trees/ha Stand II, 1400m). Meanwhile total density (1311 trees/ha recorded for Stand III, 1600m and 1698 trees/ha for stand IV, 1800m) respectively.

Altitudinal changes in density Important value index and diversity are set in (Table 2, 3 and 4). On the basis of density *Quercus leucotrichophora* (1025 trees/ha and 687 trees/ha) was the dominated species on the (Stand I, 1200m) and (Stand IV, 1800m). Meanwhile Tree species *Pinus roxburghii* (783 trees/ha) dominated in (Stand I, 1200m) and *Rhododendron arboretum* (54 trees/ha) in (Stand III, 1600m) Table 2. Dominance of tree species were observed by calculating the Important value index and result are depicted in Table 3. Study revealed that *Quercus leucotricophora* is the most dominant species of (Stand III, 1600m and Stand IV, 1800m) with maximum IVI values (138.79 and 74.49) respectively.

Species diversity ( $H'$ ) ranged from (1.00-2.07). Species diversity and concentration of dominance are generally inversely related. The values of species richness (R), species diversity ( $H'$ ) and concentration of dominance (cd) are given in (Table 4). Species richness and diversity of tree species along an altitudinal gradient ranged between (3-9) & (1.00-2.07) respectively. Maximum diversity (2.07) reported for (Stand IV, 1800m) with the maximum number of species richness (9) whereas minimum diversity (1.00) recorded for (Stand I, 1200m) with minimum number of tree species (3). Concentration of dominance (cd) showed reverse trend as compared to species diversity. In the present study the value of concentration of dominance ranged between 0.13 (Stand IV 1800m) to 0.40 (Stand I, 1200 m).

**Table: 1 General characteristics of the study area**

Forest stand	Altitude (m)	Aspect	Dominant spp.
I	1200	South-West	<i>Pinus roxburghii</i> , <i>Machilus duthi</i>
II	1400	East	<i>Rhododendron arboreum</i> , <i>Lyonia ovalifolia</i>
III	1600	East	<i>Quercus leucotrichophora</i> , <i>Lyonia ovalifolia</i>
IV	1800	East	<i>Quercus leucotrichophora</i> , <i>Rhododendron arboreum</i>

**Table 2: Altitude wise variation in the values of Density (trees/ha)**

Tree species	Stand I 1200	Stand II 1400	Stand III 1600	Stand IV 1800
<i>Ficus semicordata</i> Buch-Ham.ex.J.E.Smith.,	183			
<i>Lyonia ovalifolia</i> (Wall)Drude		442	37	175
<i>Machilus duthi</i> King ex Hook.f.,	200			
<i>Myrica esculenta</i> Buch-Ham ex. D.Don		257		175
<i>Pinus roxburghii</i> Sargent	783	200	87	
<i>Pyrus pashia</i> Buch-Ham ex.D.Don			50	50
<i>Quercus floribunda</i> Lindley ex Rehder				200
<i>Quercus glauca</i> Thunb.				25
<i>Quercus. leucotrichophora</i> A.Camus		371	1025	687
<i>Rhododendron arboretum</i> Smith.		514	75	312
<i>Stranvaesia nussia</i> (Buch-Ham ex.D.Don)				12
<i>Symplocos paniculata</i> (Thunb.)Miq		42	37	62

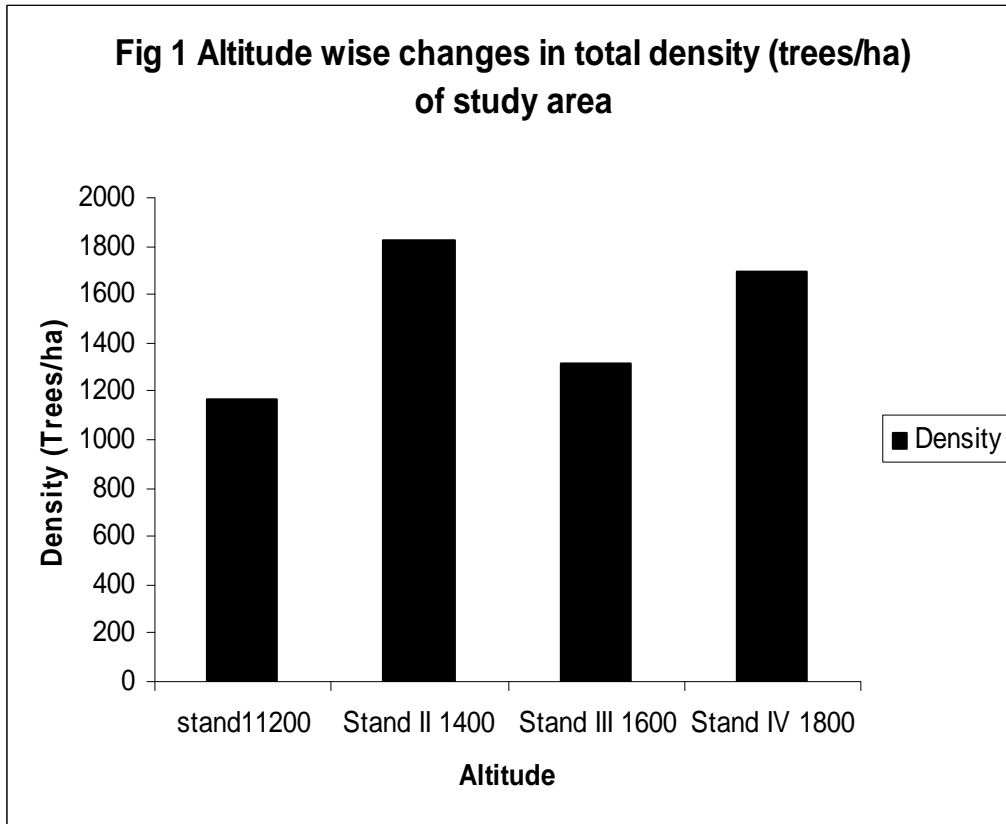
**Table 3. Altitude wise changes in values of IVI (Important value Index)**

Tree species	Stand I 1200	Stand II 1400	Stand III 1600	Stand IV 1800
<i>Ficus semicordata</i> Buch-Ham.ex.J.E.Smith.,	63.84			
<i>Lyonia ovalifolia</i> (Wall)Drude		60.19	44.39	34.17
<i>Machilus duthi</i> King ex Hook.f.,	72.12			
<i>Myrica esculenta</i> Buch-Ham ex. D.Don		51.02		32.36
<i>Pinus roxburghii</i> Sargent	164	54.45	39.23	
<i>Pyrus pashia</i> Buch-Ham ex.D.Don			17.42	15.5
<i>Quercus floribunda</i> Lindley ex Rehder.				33.63
<i>Quercus glauca</i> Thunb.				16.90
<i>Quercus. leucotrichophora</i> A.Camus		54.82	138.79	74.49
<i>Rhododendron arboretum</i> Smith.		64.01	29.73	47.2
<i>Stranvaesia nussia</i> (Buch-Ham ex.D.Don)				28.98

<i>Symplocos paniculata</i> (Thunb.)Miq		15.51	30.43	16.76
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**Table 34 Altitude wise Changes in Species Richness (R), Diversity (H') and Concentration of Dominance (Cd) for tree in selected forest stands**

Altitude	R	H'	Cd
Stand I (1200m)	3	1.00	0.40
Stand II (1400m)	6	1.72	0.18
Stand III (1600m)	6	1.53	0.27
Stand IV (1800m)	9	2.07	0.13



### Discussion

The present study was conducted along an altitudinal gradient in a temperate forest of Garhwal Himalaya. The highest number of tree species (9) was recorded from (Stand IV 1800m) due to relatively open habitat which provided congenial environment for the growth of different species. Fig 1 shows the total density of tree species ranged between (1166 trees/ha Stand I to 1826 trees/ha Stand II, 1400m). The

present values of density are higher as compared to those for submontane forest (Bhandari et al, 1997), suggesting that the present stands are much older than the submontane forests of Garhwal Himalaya. Study revealed that *Quercus leucotricophora* is the most dominant species of all stands. Oak (*Quercus* spp) forests are most extensively distributed between the altitudes 1000m to timberline and represent the climax stage, throughout the central Himalaya (Champion and Seth, 1968; Upreti *et al*; 1985). In these forests, one or other species of oak exhibits clear-cut dominance over other associated tree layer (Singh & Singh, 1986). Three oak species (*Quercus floribunda*, *Quercus glauca* and *Quercus leucotrichophora*) were recorded in present forest stands but none of these attains a clear-cut dominance.

Species richness and diversity of tree species along an altitudinal gradient ranged between (3-9) & (1.00-2.07) respectively. Concentration of dominance (cd) showed reverse trend as compared to species diversity. In the present study the value of concentration of dominance ranged between 0.13 (Stands IV 1800m) to 0.40 (Stand I, 1200m). These values are generally comparable with the values reported for temperate forests (Singh & Singh, 1987). Lower diversity in the temperate forests could be due to lower rate of evolution and diversification of community (Simpson, 1949) and severity in the environment (Connell and Oris, 1964). Species diversity and concentration of dominance are generally inversely related. The value of concentration of dominance ranged from (0.13 to 0.40). These findings support the range reported by Risser and Rice (1971) for temperate forests. The data in Table 4 indicates that increasing diversity and reduced concentration of dominance is associated with increased stability (Mc Naughton, 1967). Pandey and Singh (1985) have also reported increasing species diversity in disturbed ecosystem of Kumaon Himalaya.

It is a well known fact that the altitude represents a complex gradient along which many environmental variables change concomitantly. Rahbek (1997) explain the patterns in species richness decrease with altitude. Highly diverse compositional pattern of forests characteristic of central Himalaya, has been explored by (Singh & Singh 1987). Besides the ecosystem functions the distribution and occurrence of species had been affected by human interventions (Singh et al, 1987). Among human influence, commercial exploitation, agricultural requirements, forest fire, and grazing pressure are the important source of disturbance (Singh et al, 1992). The result of present study is pronounced that as well as the altitude is increase the tree diversity is also increase which is the result of above biotic disturbance and invasion by new species on these stands.

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## **Are Forests only temporary Carbon Absorbers in Nature? - The debate has added fuel to the fire on Global Warming.**

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**Abstract:** The world today, in which we are living is full of most serious dangers and threats. These perils and intimidation are not from any alien species, but from the most intelligent animal species of this world. Yes! We the Humans, who have deteriorated the natural ecosystems so much that in near future, we are going to see an end of all civilizations, and the mother planet earth will be *as lifeless as mars*. The most emerging anxiety these days is the “Global Warming”. It is so severe that it is precisely called, as “Global warming is the Global warning”. Due to heavy industrialization and ignoring the scientific parameters of safety, we have transformed our planet in a *fast furnace*, or simply a time bomb! We all know that due to heavy industrializations and burning of fossil fuels etc, the gaseous oxides of Carbon like Carbon-mono-oxide (CO), and Carbon-di-oxide (CO<sub>2</sub>) are emitted in the atmosphere. It is a proven study worldwide that green plants, with the help of their *chlorophyll* and accessory photosynthetic pigments take up the (CO<sub>2</sub>) and convert it to the glucose, with the help of sun light and water. This whole biochemical process of fixing Atmospheric Carbon-di-oxide by green plants is known as “Photosynthesis”. But now a new-fangled trepidation has taken birth within the scientific community. Some scientists believe that the forests, which play a major role in fixing the atmospheric CO<sub>2</sub>, are only the temporary source of carbon absorbers in nature. They argue that the current terrestrial carbon sinks (the forests) are themselves a result of change in land-use pattern. It is further argued that the forest will soon be vanished from the different areas of the world, due to the changes in local weather pattern. Then in the future for the full-fledged triumph against the global warming, the scientists should start searching for a more reliable and sustainable carbon sink and the best way to combat global warming is to cut the carbon emissions worldwide. The present paper is an endeavor to comprehend the legitimacy of such claims about the forests. [The Journal of American Science. 2008;4(3):55-61]. (ISSN: 1545-1003).

**Key Words:** Forests, Carbon absorbers, Global Warming, Sustainability.

**Introduction:** Carbon sequestration can be defined as the removal of CO<sub>2</sub> from **atmosphere (source)** into **green plants (sink)** where it can be stored indefinitely (**Watson et al. 2000**). These sinks can be above ground biomass (trees) or living biomass below the ground in soil (roots and micro organisms) or in the deeper sub surface environments. Sequestration, which is relatively a new term, can be described as storage of all forms of carbon, including storage in terrestrial, geological and oceanic ecosystem. Through practices and technologies sequestration seeks to quantify and enhance the storage ability of all potential sinks and expand the number and type of sinks in which carbon storage is possible. Enhancing the natural processes that remove CO<sub>2</sub> from the atmosphere is thought to be one of the most useful methods of mitigating the atmospheric levels of CO<sub>2</sub>. While the whole world is anxious of the adverse effects of *Global Warming* and taking this as a sign of *Global Warning*, a team of 30 scientists lead by Professor David Schimel, of the Max Planck Institute for Biogeochemistry in Jena, Germany, has published a paper in the renowned journal ‘*Nature*’, arising the question of the sustainability of forests. The researchers have revealed that the world should not expect the terrestrial ecosystems such as forests, grasslands and soils to soak up Carbon di oxide (CO<sub>2</sub>) far into the near future. The basis of their predictions is that these terrestrial “*carbon sinks*” **are themselves the product of temporary changes in land use**. They fear that the entire land-based carbon-sink could ultimately disappear. The study is also reviewed by distinguished environmentalist Alex Kirby

and is of utmost importance in the present scenario of global warming and climate change. This new revelation has evoked numerous queries in the minds of scientists worldwide. The supporters of the theory argue that forests are going to vanish from different landforms, since they are themselves the product of geological transformations and have become ardent on this issue. Moreover there are instances that certain boreal forest ecosystems in Canada and other northern countries are not sequestering the amount of atmospheric carbon, they were supposed to do. Because of anthropogenic emissions, atmospheric CO<sub>2</sub> has climbed to levels that are presently more than 30% higher than before the industrial revolution, (**Barnolla 1999; Keeling and Whorf, 2000**). Indeed, geochemical measurements made on ancient ocean sediments suggest that atmospheric CO<sub>2</sub> levels over the past 20 million years were never as high as they are today (**J. T. Houghton et al., eds, 2001**).

The terrestrial sink for atmospheric carbon is the theme of substantial disagreement at present, regarding not only its magnitude but also its cause. For many years, researchers have believed that the prevailing sink mechanism is the fertilizing effects of increased CO<sub>2</sub> concentrations in the atmosphere and the addition to soils of fixed nitrogen from fossil fuel burning and agricultural fertilizers. This fertilization mechanism has been incorporated into most existing models of the terrestrial biosphere that are used to predict future concentrations of atmospheric CO<sub>2</sub>. (**Sarmiento and Gruber, 2002**). However, a recent analysis of long-term observations of the change in biomass and growth rates, made by the **US Forest Service**, suggests that such fertilization effects are much too small to explain more than a small fraction of the observed sink in the United States of America (**Caspersen et al., 2000**). In addition, long-term experiments in which small forest patches and other land ecosystems have been exposed to elevated CO<sub>2</sub> levels for extended periods show a rapid decrease of the fertilization effect after an initial enhancement (**Schlesinger and Lichter 2001**).

**The Present Carbon sink:** When we talk about the biogeochemical cycles in nature, there are two important parts – one is the source and the other is the sink. The Source is the pool of that inorganic species, where it is found in free state. Whereas the sink is the region which absorbs that inorganic species. For example, if we talk about the biogeochemical cycle of Carbon di oxide (CO<sub>2</sub>) gas, then the source is the atmosphere and the sink is the forest and oceanic ecosystems, with the abundance of **green plants** or **algae** ("**Phykos**" or **sea weeds**). These **photosynthetic green plants**, with the help of *chlorophyll* and accessory photosynthetic pigments, **absorb the CO<sub>2</sub>** emitted into the atmosphere by human activities and **fix them in the form of carbohydrates**. This whole process of changing the atmospheric CO<sub>2</sub> into the solid glucose form is a very complex one and is known as "**photosynthesis**". Earlier some scientists believed that about 90% of the world's total photosynthesis is carried out by marine algae, but studies conducted later confirmed that only one-third of the total global photosynthesis could be attributed to oceanic algae. Almost all the climatologists believe that **CO<sub>2</sub>** and other **Green House Gases (GHGs)** are intensifying the climate's natural changeability. But precisely how much carbon they absorb is unknown. Scientists believe the land and the oceans together absorb about half the CO<sub>2</sub> given off by the burning of fossil fuels.

Forests are carbon stores, and they are carbon dioxide sinks when they are increasing in density or area. In Canada's boreal forests as much as 80% of the total carbon is stored in the soils as dead organic matter (**CFS Science Policy Note, 2007**).

Because CO<sub>2</sub> is noncreative in the atmosphere, it has a relatively long residence time there. However, its growth rate is presently less than half of what would be expected if all the CO<sub>2</sub> released by fossil fuel burning and land-use change remained in the atmosphere.

The growth rate is lower because the **terrestrial biosphere (plants and soils)** and the **ocean** are taking up a significant amount of anthropogenic CO<sub>2</sub>, that is, acting as "**sinks**." The scientific community has made much progress in establishing the relative role of these two major natural sinks on a global scale, and it appears that the missing carbon is about equally divided between them. However, scientists continue to debate aspects of the spatial distribution and mechanisms of these sinks. The future behavior of the sinks turns out to be highly sensitive to whatever mechanisms we assume. Thus, better understanding of their behaviors is key to predicting, and hopefully mitigating, the future impact of anthropogenic CO<sub>2</sub>. An important starting point for forecasting the future behavior is to understand its past.

The carbon balance is not fixed in time. As seen in, the atmospheric growth rate varies by a large amount from year to year. Most of the inter annual variability is correlated with the **El Niño southern oscillation** climate mode, with higher growth rates generally being related with **El Niño (warm climate)** episodes. The climate cooling caused by the **Mt. Pinatubo** eruption in the early 1990s appears to have contributed to reduce atmospheric growth rates. The primary cause of the variability remains controversial, but is probably due mostly to the response of terrestrial vegetation to climate variability, with a smaller contribution due to the oceanic response (**Quéré, et al., 2000**).

As CO<sub>2</sub> concentrations in the atmosphere continue to rise, increases in plant productivity and litter fall are likely. Results suggest that the balance of carbon stored in the soils (thought to be a long-term sink for carbon) can be changed with the addition of fresh leaf litter. The capacity of soils to store carbon might then reduce if global environmental changes such as CO<sub>2</sub> increases and nitrogen deposition boost plant productivity. The study has implications for policy makers considering new approaches to capping carbon emissions such as carbon sequestration. The results suggest unanticipated feedbacks to the carbon cycle that must be taken into account when estimating the potential for carbon sequestration in the soil (**Sayer EJ, Powers JS**, The 30 authors of the report in “Nature” found that the atmospheric CO<sub>2</sub> and oxygen data confirm, that the terrestrial biosphere was mainly neutral with respect to net carbon exchange during the 1980s, but became a net carbon sink in the 1990s. This recent sink can be largely credited to northern extra-tropical areas, and is roughly split between America and Eurasia. Tropical land areas, however, were approximately in equilibrium with respect to carbon exchange, implying a carbon sink that counterbalance emissions due to tropical deforestation. In North America, China and Europe, the authors say that the key reasons were most likely the regrowth of forests, often after farmland was abandoned in the 1980s and 1990s. (**Fig.1**). A decrease in the frequency of fires also contributed in this.



**Fig. 1. Abandoning Farming will help in increasing Carbon Sink.**

**Provincial differences:** Other aspects probably include changes in foliage, plant litter and soil microbes. These in turn are affected by changes in photosynthesis, respiration, fire and insect outbreaks, influenced by huge climate fluctuations such as **El Nino** and its **reverse La Nina effect** or more commonly **ENSO (El Nino Southern Oscillation)** in the **pacific ocean**. Growing trees soak up net quantities of CO<sub>2</sub>, and the higher levels of CO<sub>2</sub> and nitrogen in the atmosphere are themselves stimulating tree and plant growth.

But the scientists anticipate that *“these effects will reach up to a saturation point one day and cease to have an effect thereafter”*.

They found big regional variations in the effectiveness of sinks. **Much of Siberia, for example, has warmed by about 0.5 degrees Celsius a decade since the 1960s.**

*An increase in wildfires and insect damage appears to have changed it from a sink into a temporary source of CO<sub>2</sub>.*

In a possible pointer to future changes, Professor David Schimel articulated that, *“Globally, there appears to be a net release of carbon to the atmosphere during warm and dry years, and a net uptake during cooler years.”*

The most astonishing revelation of Professor David Schimel, is that, *“although carbon sinks have a role to play in absorbing excess CO<sub>2</sub>, it is possible that the net global terrestrial carbon sink may disappear altogether in the future.”*(Fig. 2).



**Fig. 2. Forests will not remain forever.**

**Actual Role of Forests as Carbon Sink:** There is certainly no doubt that forests do play a significant role in carbon fixation and are the sink for atmospheric carbon. But different studies worldwide also confirm the uneven effectiveness of the forests as atmospheric carbon sink. Japan appears most likely to rely most heavily on forest and biological sinks to meet its Kyoto targets. For Canada, sinks are likely to play a rather modest role. For the European Union (EU), the role of sinks is likely to be even smaller, with sinks playing no role for some EU countries (including Sweden)(Masahiro and Sedjo, 2003).

Carbon uptake via forest activities varies significantly depending on location (tropical, Great Plains, etc.), activity (forest conservation, tree planting, management, etc.), and the assumptions and methods upon which the cost estimates are based. (G Van Cornelis Kooten and Alison, J. Eagle, 2005). The new findings pointing towards the uncertainty of the sustainability of Forests also give an idea that the

best way to fight against the global warming is to cut carbon emissions worldwide. Some scientists also feel that in the future carbon sinks could become a source of CO<sub>2</sub> and other greenhouse gases, such as methane.

There are numerous examples of severe **forest fires** due to **EL NINO** and the reverse **LA-NINA** effect, which occur in a periodic cycle of about 5-7 years in the pacific countries like Australia, Costa-Rica etc. *Due to these horrible large-scale forest fires, the forest themselves become the sources of high CO<sub>2</sub> emissions in the atmosphere, thus adding to the problem of global warming.* So it is certainly not very much exciting to rely upon the terrestrial ecosystems for a future reliable source of Carbon Sink. Similar examples can also be taken from the northern boreal Canadian forests, which first of all do not sequester the atmospheric carbon in the desired amount and sometimes due to huge forest fires are transformed into big sources of atmospheric carbon (**Fig. 3**).



**Fig. 3. Crown Fire in Canadian Boreal Forest.**

The **world's forests** contain about **830 Pg C (10<sup>15</sup> g) Carbon** in their vegetation and soil, with about 1.5 times as much in soil as in vegetation. During the 1980s, analysis of Carbon budgets show that forest of the temperate and boreal countries were a net sink of atmospheric C of about 0.7 Pg yr<sup>-1</sup>, but the tropics were a net source of about 1.6 Pg yr<sup>-1</sup>. However, accounting for the imbalance in the global C cycle suggests that forest are not significantly contributing to the net increase in atmospheric CO<sub>2</sub> and thus not contributing to global climate change. However, this may not continue into the future as temperate and boreal forests reach maturity and become a smaller C sink, and if rates of tropical deforestation and degradation continue to accelerate (**Sandra Brown, 1997**).

The green house effect raising the global temperature may trigger a series of changes within the overall global climate system. For instance, **global sea levels have risen by 10-25 cm over the past 100 years**, and are expected to continue to rise due to increases in temperature. We are also seeing increases in severe weather events. Such impacts of climate change could have far-reaching and/or unpredictable environmental, social, and economic consequences. Indeed, the climate change problem and the related changes are among the most serious of the environmental issues that we face today (**Seth, 2005**).

In the U.S., trees and other growth expanded on abandoned agricultural land and a reduction in fires allowed forests to spread. Increased plant growth spurred by the increasing carbon dioxide and nitrogen deposits - a process more noticeable in Europe and Asia - also helped remove carbon dioxide.

But there is a limit to how much forests can fill in and spread. Eventually new trees and grasses reach maturity and soak up less carbon dioxide. In addition, global climate change may have impacts upon the well-being of ecosystems and cause them to decline in extent and vigor. Warm and dry weather was found to reduce the ability of terrestrial ecosystems to act as global sinks.

Under some conditions, forests and peat bogs may become sources of CO<sub>2</sub>, such as when a forest is flooded by the construction of a hydroelectric dam. Unless the forests and peat are harvested before flooding, the rotting vegetation is a source of CO<sub>2</sub> and methane comparable in magnitude to the amount of carbon released by a fossil-fuel powered plant of equivalent power. **Duncan Graham-Rowe (2005).**

Life expectancy of forests varies throughout the world, influenced by tree species, site conditions and natural disturbance patterns. In some forests carbon may be stored for centuries, while in other forests carbon is released with frequent stand replacing fires. Forests that are harvested prior to stand replacing events allow for the retention of carbon in manufactured forest products such as lumber. Only a portion of the carbon removed from logged forests ends up as durable goods and buildings - the remainder ends up as sawmill by-products such as pulp, paper and pallets. For instance, of the 1,692 teragrams of carbon harvested from forests in Oregon and Washington (U.S) from 1900 to 1992, only 23% is in long-term storage in forest products. **Harmon, Harmon, Ferrell and Brooks(1996).**

**Conclusion:** So, in the conclusion we can say that no doubt the forests are working as the terrestrial carbon sinks in nature presently, yet it appears that these carbon sinks are only temporary environmental entities being influenced by natural and anthropogenic activities. We have seen a lot of examples explaining about the temporary survivalship of forests in nature. The ever changing local weather and global wind patterns are also playing key roles in deciding the life expectancy of the forest lands. One thing is for sure that - the best way to fight against global warming is to cut the carbon emission and stop the use of fossil-fuels worldwide. To ensure this all the governments of world must work hand-in-hand and that is the only solution to stop the increasing concentration of the atmospheric carbon. New declarations and policies must be made considering the important revelation about the sustainability of the terrestrial carbon sinks in nature. Simultaneously the scientists should also seek for some new sources of carbon sink as well, which are not influenced by local weather pattern and might not become a source of carbon themselves in near future. We must start acting today to make a safe and better world for tomorrow.

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## HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-CoA reductase) (HMGR)

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**Abstract:** HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-CoA reductase, HMGCR) is the rate controlling enzyme (EC 1.1.1.88) of the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids. This enzyme is the target of the widely available cholesterol lowering drugs (statins). HMGCR is anchored in the membrane of the endoplasmic reticulum, and was long regarded as having seven transmembrane domains, with the active site located in a long carboxyl terminal domain in the cytosol. In humans, the gene for HMG-CoA reductase is located on the long arm of the fifth chromosome (5q13.3-14). Related enzymes having the same function are also present in other animals, plants and bacteria. [The Journal of American Science. 2008;4(3):62-64]. (ISSN: 1545-1003).

### Introduction

3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) catalyzes the formation of mevalonate - converts HMG-CoA to mevalonic acid (Zhang, Wan et al. 2007). In many classes of organisms, this is the committed step leading to the synthesis of essential compounds, such as cholesterol. However, a high level of cholesterol is an important risk factor for coronary heart disease, for which an effective clinical treatment is to block HMGR using inhibitors like statins. Recently the structures of catalytic portion of human HMGR complexed with six different statins have been determined by a delicate crystallography study (Zhang, Wan et al. 2007).

HMGR inhibitors have been shown to upregulate GTP cyclohydrolase I (GTPCH-I), the key enzyme for tetrahydrobiopterin de novo synthesis and to normalize tetrahydrobiopterin levels in hyperglycemic endothelial cells (Wenzel, Daiber et al. 2008).

The liver is responsible for controlling cholesterol homeostasis in the body. HMGR and the LDL receptor (LDL-r) are involved in this regulation and are also ubiquitously expressed in all major tissues (Mutungi, Torres-Gonzalez et al. 2007).

Statin reduces cerebrovascular events independent of its cholesterol lowering effect. Reduction of cerebrovascular events by statins may be brought by the direct inhibition of atherosclerotic change (Tsuchiya, Nagotani et al. 2007).

### Drugs inhibit HMGR

Drugs which inhibit HMGR, known collectively as HMGR inhibitors (or "statins"), are used to lower serum cholesterol as a means of reducing the risk for cardiovascular disease. These drugs include lovastatin (Mevacor), atorvastatin (Lipitor), pravastatin (Pravachol), and simvastatin (Zocor), etc.

Vytorin is drug that combines the use simvastatin and ezetimibe, which blocks the formation of cholesterol by the body, along with the absorption of cholesterol in the intestines. Pravastatin is a typical drug that inhibits HMGR.

### Hormones

HMGR is active when blood glucose is high. The basic functions of insulin and glucagon are to maintain glucose homeostasis. Thus, in controlling blood sugar levels they indirectly affect the activity of HMGR, but a decrease in activity of the enzyme is caused by an AMP-activated protein kinase which responds to an increase in AMP concentration, and also to leptin.

### Importance

HMGR is a polytopic, transmembrane protein that catalyzes a key step in the mevalonate pathway which is involved in the synthesis of sterols, isoprenoids and other lipids. In humans, HMG-CoA reductase

is the rate-limiting step in cholesterol synthesis and represents the sole major drug target for contemporary cholesterol-lowering drugs.

The medical significance of HMGR has continued to expand beyond its direct role in cholesterol synthesis following the discovery that it can offer cardiovascular health benefits independent of cholesterol reduction. Statins have been shown to have anti-inflammatory properties, most likely as a result of their ability to limit production of key downstream isoprenoids that are required for portions of the inflammatory response. Notably, blocking of isoprenoid synthesis by statins has shown promise in treating a mouse model of multiple sclerosis, an inflammatory autoimmune disease.

HMGR is also an important developmental enzyme. Inhibition of its activity and the concomitant lack of isoprenoids that yields can lead to morphological defects. HMGR is a key enzyme in the sterol biosynthesis pathway, but its subcellular distribution in the Trypanosomatidae family is somewhat controversial (Pena-Diaz, Montalvetti et al. 2004).

### **Regulation of HMGCR**

Regulation of HMGCR is achieved at several levels: transcription, translation, degradation and phosphorylation.

#### **Transcription of the reductase gene**

Transcription of the reductase gene is enhanced by the *sterol regulatory element binding protein* (SREBP). This protein binds to the *sterol regulatory element* (SRE), located on the 5' end of the reductase gene. When SREBP is inactive, it is bound to the ER or nuclear membrane. When cholesterol levels fall, SREBP is released from the membrane by proteolysis and migrates to the nucleus, where it binds to the SRE and transcription is enhanced. If cholesterol levels rise, proteolytic cleavage of SREBP from the membrane ceases and any proteins in the nucleus are quickly degraded.

#### **Translation of mRNA**

Translation of mRNA is inhibited by a mevalonate derivative which has been reported to be farnesol, although this role has been disputed.

#### **Degradation of reductase**

Rising levels of sterols increases the susceptibility of the reductase enzyme to proteolysis. Helices 2-6 (total of 8) of the HMG-CoA reductase transmembrane domain sense the higher levels of cholesterol and this leads to Lysine 248 being exposed. This lysine residue can become ubiquitinated, and this serves as a signal for proteolytic degradation. The protease (SCAP, SCREBP Cleavage Activating Protein) that activates SREBP is also sensitive to levels of sterols.

#### **Phosphorylation of reductase**

Short term regulation of HMG-CoA reductase is achieved by inhibition by phosphorylation (of Serine 872, in humans). Decades ago it was believed that a cascade of enzymes control the activity of HMG-CoA reductase: an HMG-CoA reductase kinase was thought to inactivate the enzyme, and the kinase in turn was held to be activated via phosphorylation by HMG-CoA reductase kinase kinase. An excellent review on regulation of the mevalonate pathway by Nobel Laureates Joseph Goldstein and Michael Brown adds specifics: HMG-CoA reductase is phosphorylated and inactivated by an AMP-activated protein kinase, which also phosphorylates and inactivates acetyl-CoA carboxylase, the rate limiting enzyme of fatty acid biosynthesis. Thus, both pathways utilizing acetyl-CoA for lipid synthesis are inactivated when energy charge is low in the cell, and concentrations of AMP rise. There has been a great deal of research on the identity of upstream kinases which phosphorylate and activate the AMP-activated protein kinase. Fairly recently LKB1 has been identified as a likely AMP kinase kinase which appears to involve calcium/calmodulin signaling. This pathway likely transduces signals from leptin, adiponectin, and other signaling molecules.

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## Does the Magical Himalayan Herb “Sanjeevani Booti” really exist in Nature?

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**Abstract:** For the past 1000 years or so in India, Asia and other parts of the world where the Hinduism is followed, the generations have been brought up listening and learning to the grand Hindu Epic “*The Ramayana*.” In the Ramayana, there is a description of a ‘magical herb’ called as “*Sanjeevani Booti*” or “*Life giving herb*” found in Indian Himalayas. It is believed that this magical herb has a unique property of ‘bioluminescence’ for its easy identification and is a “*panacea*” against all known and unknown diseases, which can give life even to a dying or dead person. I personally think that when the belief about a plant is so strong that it is worshiped for centuries in any part of the world then it becomes the duty of a botanist to find out the truth about the very existence of the herb itself and let people know the reality from scientific point of view. In this paper, I have tried to explain the botanical existence and the medicinal properties of this wonder herb from the Indian Himalayas. [The Journal of American Science. 2008;4(3):65-67]. (ISSN: 1545-1003).

**Key Words:** Sanjeevani Booti, Heterosporous Fern, Drought Resistant Plants, Himalayan Medicinal Herbs and Panacea.

**Introduction:** From time immemorial, generations in India and other countries have grown up hearing the grand Hindu Epic *Ramayana*. In this world famous epic of Hindi poet *Tulsidas*, there is also a description of a wonder herb called as *Sanjeevani booti (Life giving herb)*, which according to the popular belief had given life to the dying Laxmana, the younger brother of Lord Shree Rama.

Since then, the question of existence of such life-giving herb has been floating in the minds of people. According to the Ramayana, the *Sanjeevani booti* is found in the Indian Himalayas and has the unique property of **bioluminescence**, which helps in its easy identification. In Hindu mythology, Sanjeevani is a magical herb which has the power to cure every known and unknown malady. It is believed that medicines prepared from this herb can even revive a dead person also. This herb is mentioned in the Ramayana when, during the battle with Ravana (the King of Demons), Lakshmana was wounded and was nearly killed by Ravana's son Meghnaad. Hanuman was called upon to fetch this herb from the Dronagiri mountain in the Himalayas (Currently in Uttarakhand State of India). Upon reaching Mount Meru, Hanuman was unable to identify the herb and decided to lift the entire mountain and brought it to the battlefield.

**Botanical Analysis:** Now the real scientific question arises: **Is there really a wonder herb called *Sanjeevani booti* in the Indian Himalayas, with the property of bioluminescence?**

In fact according to the botanical science, this is not exactly the same plant described in the Ramayana, **but yes**- there is a plant from Indian Himalayas, with certain medicinal properties and high drought resistance, which has been given the name “*Sanjeevani booti*” by the local people and scientists. The *Sanjeevani booti* is actually a **heterosporous Indian Himalayan Pteridophyte**, which in botanical language known as *Selaginella bryopteris*. However it is a fern plant, a ‘Pteridophyte or vascular cryptogams’, and not a flower bearing ‘Angiosperm’ plant. Moreover it also does not have the power of bioluminescence, as described in the Ramayana.

**Table 1. Classification: Sanjeevani booti (*Selaginella bryopteris*) -a fern plant.**

	<b>Kingdom: Planatae</b>
	<b>Division: Lycopodiophyta</b>
<b>Class:</b>	<b>Lycopsida</b>
<b>Order:</b>	<b>Selaginallales</b>
<b>Family:</b>	<b>Selaginellaceae</b>
<b>Genus:</b>	<b><i>Selaginella</i></b>
<b>Species:</b>	<b><i>bryopteris</i></b>



**Fig 1. Sanjeevani Booti “*Selaginella bryopteris*”**

**Special Features/Future Prospects:** *Sanjeevani booti* (*Selaginella bryopteris*) has been reported to have the highest degrees of drought resistance. It is observed that this fern can withstand years of drought very effectively, and still there is no adverse effect in its vigor. The cause for this special power lies in its **drought resistant gene**. Amazed by this unique property of this fern, the scientists of NBRI (**National Botanical Research Institute**), Lucknow, India are working to isolate its special drought resistant *gene*, and they further plan to make **transgenic agricultural crops**, with the help of biotechnology by incorporating **its gene in the genomes of agricultural crops**, such as **wheat, paddy, legumes** etc. So that even in an event of monsoon failure, the agricultural crops do not suffer and can overcome the adverse condition due to **Sanjeevani’s gene**. As it is well known that India is an agricultural country, but lacks good irrigation system and the success of its agriculture depends upon the timely arrival of **monsoon** (monsoon is a local wind system in southern Asia and Africa, which produces copious amount of rain in this part of the world).

There are numerous examples of biotechnologists evolving “**plants of desire**” through **gene transfer techniques**. The examples of **Bt Cotton**, **Bt Tobacco** and other such transgenic crops can be taken, where the unique **cry gene** of the bacteria *Bacillus thuringiensis* (**Bt**) is incorporated in the genomes of normal **Cotton** (*Gossypium sp.* **Family- Malvaceae**) and **Tobacco** (*Nicotiana rustica*. **Family- Solanaceae**) to make them transgenic crops. These transgenic crops are now not affected by the attack of **Bollworm** (a insect harmful to these crops). Because the protein “**delta endotoxin**”, produced by the bacteria’s **cry gene** is now being produced by these transgenic crops and the attacking **bollworms are killed due to starvation** as the toxin dissolves the alimentary canal or gut lining of these harmful bollworms.

**Medicinal Properties:** Pharmacognosy is the branch of science, related to the study of medicinal plants and their use in curing of diseases. There are numerous drug-yielding plants distributed in all the possible habitats of plants. The Indian Himalaya also has a very rich species diversity of such medicinal plants, which are used to cure various diseases. The particular medicinal property of a plant is basically due to certain phytochemicals such as an alkaloid or its derivatives, which has a definite physiological action on the human body.

The Sanjeevani booti or *Selaginella bryopteris* also has many **therapeutic properties** and is in use by local people for centuries. Traditionally the plant has certain specific properties and is used for-

**(1) Relief from heat stroke and the burning sensation during urination-** *Selaginella bryopteris* is extensively used in **curing the heat strokes**, which is a very common complaint of humans working outside in the extremely hot hours in the month of May- June in the tropical part of the country.

**(2) Restoring menstrual irregularities to normal-**The cycle of reproductive activity shown by most sexually mature non-pregnant female mammals except most primates is called as the menstrual cycle/oestrous cycle/sexual cycle. The menstrual cycle is the periodic vaginal discharges in human and other mammals and the discharge is made up of blood and cells shed from the inner lining of uterus. *Selaginella bryopteris* is also used to cure the irregularities in the oestrous cycle or menstrual cycle.

**(3) Helping in easy delivery of pregnant women in minimizing the labor pain-**The female mammal hormone "**relaxin**" (released from ovaries) makes the delivery of the baby easy through the narrow passage of vaginal walls, by expanding its muscles. If this hormone is not produced in the adequate quantity by the body, then the labour pain (delivery pain) becomes unbearable. *Selaginella bryopteris* also has the power to help in easy delivery of pregnant women in minimizing the labor pain.

**(4) In the treatment of Jaundice-**Jaundice is the physiological disorder of human body in which the liver is affected by certain bacteria and becomes unable to perform its normal function i.e. to breakdown the harmful nitrogenous wastes and to make the internal environment clean and secure. Liver also secretes a greenish-yellow alkaline fluid called as '**Bile**', which help in the digestion and absorption of fats. Bile is stored in the gall bladder. Bile also contains the bile pigments- **Bilirubin and Biliverdin**, which is produced by the breakdown of blood pigment- haemoglobin.

In jaundice **excess bile pigments** –'bilirubin' starts gathering in the bloodstream and tissues giving a yellow to orange and even greenish colour to the skin, the white parts of the eyes and the mucous membrane. Jaundice may also be due to the blockage of bile flow start gathering in the blood, which ultimately impart yellow colour to the body and make the blood impure **Sanjeevani Booti or Selaginella bryopteris** is also used by traditional Indian people to cure the deadly disease i.e. Jaundice.

**Conclusion:** Well, we have seen the genesis of the world Sanjeevani (Life giving herb) from the Indian Himalayas, mentioned in the grand old Hindu Epic "The Ramayana." We have also explored the plant botanically and can conclude that this is exactly not the similar plant as described in the Ramayana but interestingly it's a fern plant that has been used by local peoples from centuries and has earned the name Sanjeevani for itself. It is above board that this particular plant locally known as Sanjeevani, finds a place in the group of Heterosporous Pteridophytes of Indian Himalayas, when studied from botanical point of view. Scientifically it is known as *Selaginella bryopteris*. There is no doubt that this plant has several important medicinal properties and has been worshipped among local people as a magical herb owing to its medicinal properties. But a more important aspect is the existence of a drought resistance gene inside the cells of this fern and it's sure that soon it will become a true Sanjeevani (Life giving herb) to the world agricultural crops, even in the event of absence of water!

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## **Are Forests only temporary Carbon Absorbers in Nature? - The debate has added fuel to the fire on Global Warming.**

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**Abstract:** The world today, in which we are living is full of most serious dangers and threats. These perils and intimidation are not from any alien species, but from the most intelligent animal species of this world. Yes! We the Humans, who have deteriorated the natural ecosystems so much that in near future, we are going to see an end of all civilizations, and the mother planet earth will be *as lifeless as mars*. The most emerging anxiety these days is the “*Global Warming*”. It is so severe that it is precisely called, as “*Global warming is the Global warning*”. Due to heavy industrialization and ignoring the scientific parameters of safety, we have transformed our planet in a *fast furnace*, or simply a time bomb! We all know that due to heavy industrializations and burning of fossil fuels etc, the gaseous oxides of Carbon like Carbon-mono-oxide (CO), and Carbon-di-oxide (CO<sub>2</sub>) are emitted in the atmosphere. It is a proven study worldwide that green plants, with the help of their *chlorophyll* and accessory photosynthetic pigments take up the (CO<sub>2</sub>) and convert it to the glucose, with the help of sun light and water. This whole biochemical process of fixing Atmospheric Carbon-di-oxide by green plants is known as “Photosynthesis”. But now a new-fangled trepidation has taken birth within the scientific community. Some scientists believe that the forests, which play a major role in fixing the atmospheric CO<sub>2</sub>, are only the temporary source of carbon absorbers in nature. They argue that the current terrestrial carbon sinks (the forests) are themselves a result of change in land-use pattern. It is further argued that the forest will soon be vanished from the different areas of the world, due to the changes in local weather pattern. Then in the future for the full-fledged triumph against the global warming, the scientists should start searching for a more reliable and sustainable carbon sink and the best way to combat global warming is to cut the carbon emissions worldwide. The present paper is an endeavor to comprehend the legitimacy of such claims about the forests. [The Journal of American Science. 2008;4(3):68-74]. (ISSN: 1545-1003).

**Key Words:** Forests, Carbon absorbers, Global Warming, Sustainability.

### **Introduction:**

Carbon sequestration can be defined as the removal of CO<sub>2</sub> from **atmosphere (source)** into **green plants (sink)** where it can be stored indefinitely (Watson *et al.* 2000). These sinks can be above ground biomass (trees) or living biomass below the ground in soil (roots and micro organisms) or in the deeper sub surface environments. Sequestration, which is relatively a new term, can be described as storage of all forms of carbon, including storage in terrestrial, geological and oceanic ecosystem. Through practices and technologies sequestration seeks to quantify and enhance the storage ability of all potential sinks and expand the number and type of sinks in which carbon storage is possible. Enhancing the natural processes that remove CO<sub>2</sub> from the atmosphere is thought to be one of the most useful methods of mitigating the atmospheric levels of CO<sub>2</sub>. While the whole world is anxious of the adverse effects of *Global Warming* and taking this as a sign of *Global Warning*, a team of 30 scientists lead by Professor David Schimel, of the Max Planck Institute for Biogeochemistry in Jena, Germany, has published a paper in the renowned journal ‘*Nature*’, arising the question of the sustainability of forests. The researchers have revealed that the world should not expect the terrestrial ecosystems such as forests, grasslands and soils to soak up Carbon di oxide (CO<sub>2</sub>) far into the near future. The basis of their predictions is that these terrestrial “*carbon sinks*” **are themselves the product of temporary changes in land use**. They fear that the entire land-based carbon-



sink could ultimately disappear. The study is also reviewed by distinguished environmentalist Alex Kirby and is of utmost importance in the present scenario of global warming and climate change. This new revelation has evoked numerous queries in the minds of scientists worldwide. The supporters of the theory argue that forests are going to vanish from different landforms, since they are themselves the product of geological transformations and have become ardent on this issue. Moreover there are instances that certain boreal forest ecosystems in Canada and other northern countries are not sequestering the amount of atmospheric carbon, they were supposed to do. Because of anthropogenic emissions, atmospheric CO<sub>2</sub> has climbed to levels that are presently more than 30% higher than before the industrial revolution, (**Barnolla 1999; Keeling and Whorf, 2000**). Indeed, geochemical measurements made on ancient ocean sediments suggest that atmospheric CO<sub>2</sub> levels over the past 20 million years were never as high as they are today (**J. T. Houghton et al., eds, 2001**).

The terrestrial sink for atmospheric carbon is the theme of substantial disagreement at present, regarding not only its magnitude but also its cause. For many years, researchers have believed that the prevailing sink mechanism is the fertilizing effects of increased CO<sub>2</sub> concentrations in the atmosphere and the addition to soils of fixed nitrogen from fossil fuel burning and agricultural fertilizers. This fertilization mechanism has been incorporated into most existing models of the terrestrial biosphere that are used to predict future concentrations of atmospheric CO<sub>2</sub>. (**Sarmiento and Gruber, 2002**). However, a recent analysis of long-term observations of the change in biomass and growth rates, made by the **US Forest Service**, suggests that such fertilization effects are much too small to explain more than a small fraction of the observed sink in the United States of America (**Caspersen et al., 2000**). In addition, long-term experiments in which small forest patches and other land ecosystems have been exposed to elevated CO<sub>2</sub> levels for extended periods show a rapid decrease of the fertilization effect after an initial enhancement (**Schlesinger and Lichter 2001**).

### **The Present Carbon sink:**

When we talk about the biogeochemical cycles in nature, there are two important parts – one is the source and the other is the sink. The Source is the pool of that inorganic species, where it is found in free state. Whereas the sink is the region which absorbs that inorganic species. For example, if we talk about the biogeochemical cycle of Carbon di oxide (CO<sub>2</sub>) gas, then the source is the atmosphere and the sink is the forest and oceanic ecosystems, with the abundance of **green plants or algae (“Phykos” or sea weeds)**. These **photosynthetic green plants**, with the help of **chlorophyll** and accessory photosynthetic pigments, **absorb the CO<sub>2</sub>** emitted into the atmosphere by human activities and **fix them in the form of carbohydrates**. This whole process of changing the atmospheric CO<sub>2</sub> into the solid glucose form is a very complex one and is known as **“photosynthesis”**. Earlier some scientists believed that about 90% of the world’s total photosynthesis is carried out by marine algae, but studies conducted later confirmed that only one-third of the total global photosynthesis could be attributed to oceanic algae. Almost all the climatologists believe that CO<sub>2</sub> and other **Green House Gases (GHGs)** are intensifying the climate's natural changeability. But precisely how much carbon they absorb is unknown. Scientists believe the land and the oceans together absorb about half the CO<sub>2</sub> given off by the burning of fossil fuels.

Forests are carbon stores, and they are carbon dioxide sinks when they are increasing in density or area. In Canada's boreal forests as much as 80% of the total carbon is stored in the soils as dead organic matter (**CFS Science Policy Note, 2007**).

Because CO<sub>2</sub> is noncreative in the atmosphere, it has a relatively long residence time there. However, its growth rate is presently less than half of what would be expected if all the CO<sub>2</sub> released by fossil fuel burning and land-use change remained in the atmosphere.

The growth rate is lower because the **terrestrial biosphere (plants and soils)** and the **ocean** are taking up a significant amount of anthropogenic CO<sub>2</sub>, that is, acting as **"sinks."** The scientific community has made much progress in establishing the relative role of these two major natural sinks on a global scale, and it appears that the missing carbon is about equally divided between them. However, scientists continue

to debate aspects of the spatial distribution and mechanisms of these sinks. The future behavior of the sinks turns out to be highly sensitive to whatever mechanisms we assume. Thus, better understanding of their behaviors is key to predicting, and hopefully mitigating, the future impact of anthropogenic CO<sub>2</sub>. An important starting point for forecasting the future behavior is to understand its past.

The carbon balance is not fixed in time. As seen in, the atmospheric growth rate varies by a large amount from year to year. Most of the inter annual variability is correlated with the **El Niño southern oscillation** climate mode, with higher growth rates generally being related with **El Niño (warm climate)** episodes. The climate cooling caused by the **Mt. Pinatubo** eruption in the early 1990s appears to have contributed to reduce atmospheric growth rates. The primary cause of the variability remains controversial, but is probably due mostly to the response of terrestrial vegetation to climate variability, with a smaller contribution due to the oceanic response (**Quéré, et al., 2000**).

As CO<sub>2</sub> concentrations in the atmosphere continue to rise, increases in plant productivity and litter fall are likely. Results suggest that the balance of carbon stored in the soils (thought to be a long-term sink for carbon) can be changed with the addition of fresh leaf litter. The capacity of soils to store carbon might then reduce if global environmental changes such as CO<sub>2</sub> increases and nitrogen deposition boost plant productivity. The study has implications for policy makers considering new approaches to capping carbon emissions such as carbon sequestration. The results suggest unanticipated feedbacks to the carbon cycle that must be taken into account when estimating the potential for carbon sequestration in the soil (**Sayer EJ, Powers JS**, The 30 authors of the report in “Nature” found that the atmospheric CO<sub>2</sub> and oxygen data confirm, that the terrestrial biosphere was mainly neutral with respect to net carbon exchange during the 1980s, but became a net carbon sink in the 1990s. This recent sink can be largely credited to northern extra-tropical areas, and is roughly split between America and Eurasia. Tropical land areas, however, were approximately in equilibrium with respect to carbon exchange, implying a carbon sink that counterbalance emissions due to tropical deforestation. In North America, China and Europe, the authors say that the key reasons were most likely the regrowth of forests, often after farmland was abandoned in the 1980s and 1990s. (**Fig.1**). A decrease in the frequency of fires also contributed in this.



**Fig. 1. Abandoning Farming will help in increasing Carbon Sink.**

**Provincial differences:**

Other aspects probably include changes in foliage, plant litter and soil microbes. These in turn are affected by changes in photosynthesis, respiration, fire and insect outbreaks, influenced by huge climate fluctuations such as **El Nino** and its **reverse La Nina effect** or more commonly **ENSO (El Nino Southern Oscillation)** in **the pacific ocean**. Growing trees soak up net quantities of CO<sub>2</sub>, and the higher levels of CO<sub>2</sub> and nitrogen in the atmosphere are themselves stimulating tree and plant growth.

But the scientists anticipate that *“these effects will reach up to a saturation point one day and cease to have an effect thereafter”*.

They found big regional variations in the effectiveness of sinks. **Much of Siberia, for example, has warmed by about 0.5 degrees Celsius a decade since the 1960s.**

*An increase in wildfires and insect damage appears to have changed it from a sink into a temporary source of CO<sub>2</sub>.*

In a possible pointer to future changes, Professor David Schimel articulated that, *“Globally, there appears to be a net release of carbon to the atmosphere during warm and dry years, and a net uptake during cooler years.”*

The most astonishing revelation of Professor David Schimel, is that, *“although carbon sinks have a role to play in absorbing excess CO<sub>2</sub>, it is possible that the net global terrestrial carbon sink may disappear altogether in the future.”*(Fig. 2).



**Fig. 2. Forests will not remain forever.**

**Actual Role of Forests as Carbon Sink:**

There is certainly no doubt that forests do play a significant role in carbon fixation and are the sink for atmospheric carbon. But different studies worldwide also confirm the uneven effectiveness of the forests as atmospheric carbon sink. Japan appears most likely to rely most heavily on forest and biological sinks to meet its Kyoto targets. For Canada, sinks are likely to play a rather modest role. For the European Union (EU), the role of sinks is likely to be even smaller, with sinks playing no role for some EU countries (including Sweden)(Masahiro and Sedjo, 2003).

Carbon uptake via forest activities varies significantly depending on location (tropical, Great Plains, etc.), activity (forest conservation, tree planting, management, etc.), and the assumptions and methods upon which the cost estimates are based. (G Van Cornelis Kooten and Alison, J. Eagle, 2005). The new findings pointing towards the uncertainty of the sustainability of Forests also give an idea that the best way to fight against the global warming is to cut carbon emissions worldwide. Some scientists also feel that in the future carbon sinks could become a source of CO<sub>2</sub> and other greenhouse gases, such as methane.

There are numerous examples of severe **forest fires** due to **EL NINO** and the reverse **LA-NINA** effect, which occur in a periodic cycle of about 5-7 years in the pacific countries like Australia, Costa-Rica etc. *Due to these horrible large-scale forest fires, the forest themselves become the sources of high CO<sub>2</sub> emissions in the atmosphere, thus adding to the problem of global warming.* So it is certainly not very much exciting to rely upon the terrestrial ecosystems for a future reliable source of Carbon Sink. Similar examples can also be taken from the northern boreal Canadian forests, which first of all do not sequester the atmospheric carbon in the desired amount and sometimes due to huge forest fires are transformed into big sources of atmospheric carbon (**Fig. 3**).



**Fig. 3. Crown Fire in Canadian Boreal Forest.**

The **world's forests** contain about **830 Pg C (10<sup>15</sup> g) Carbon** in their vegetation and soil, with about 1.5 times as much in soil as in vegetation. During the 1980s, analysis of Carbon budgets show that forest of the temperate and boreal countries were a net sink of atmospheric C of about 0.7 Pg yr<sup>-1</sup>, but the tropics were a net source of about 1.6 Pg yr<sup>-1</sup>. However, accounting for the imbalance in the global C cycle suggests that forest are not significantly contributing to the net increase in atmospheric CO<sub>2</sub> and thus not

contributing to global climate change. However, this may not continue into the future as temperate and boreal forests reach maturity and become a smaller C sink, and if rates of tropical deforestation and degradation continue to accelerate (**Sandra Brown, 1997**).

The green house effect raising the global temperature may trigger a series of changes within the overall global climate system. For instance, **global sea levels have risen by 10-25 cm over the past 100 years**, and are expected to continue to rise due to increases in temperature. We are also seeing increases in severe weather events. Such impacts of climate change could have far-reaching and/or unpredictable environmental, social, and economic consequences. Indeed, the climate change problem and the related changes are among the most serious of the environmental issues that we face today (**Seth, 2005**).

In the U.S., trees and other growth expanded on abandoned agricultural land and a reduction in fires allowed forests to spread. Increased plant growth spurred by the increasing carbon dioxide and nitrogen deposits - a process more noticeable in Europe and Asia - also helped remove carbon dioxide.

But there is a limit to how much forests can fill in and spread. Eventually new trees and grasses reach maturity and soak up less carbon dioxide. In addition, global climate change may have impacts upon the well-being of ecosystems and cause them to decline in extent and vigor. Warm and dry weather was found to reduce the ability of terrestrial ecosystems to act as global sinks.

Under some conditions, forests and peat bogs may become sources of CO<sub>2</sub>, such as when a forest is flooded by the construction of a hydroelectric dam. Unless the forests and peat are harvested before flooding, the rotting vegetation is a source of CO<sub>2</sub> and methane comparable in magnitude to the amount of carbon released by a fossil-fuel powered plant of equivalent power. **Duncan Graham-Rowe (2005)**.

Life expectancy of forests varies throughout the world, influenced by tree species, site conditions and natural disturbance patterns. In some forests carbon may be stored for centuries, while in other forests carbon is released with frequent stand replacing fires. Forests that are harvested prior to stand replacing events allow for the retention of carbon in manufactured forest products such as lumber. Only a portion of the carbon removed from logged forests ends up as durable goods and buildings - the remainder ends up as sawmill by-products such as pulp, paper and pallets. For instance, of the 1,692 teragrams of carbon harvested from forests in Oregon and Washington (U.S) from 1900 to 1992, only 23% is in long-term storage in forest products. **Harmon, Harmon, Ferrell and Brooks(1996)**.

### **Conclusion:**

So, in the conclusion we can say that no doubt the forests are working as the terrestrial carbon sinks in nature presently, yet it appears that these carbon sinks are only temporary environmental entities being influenced by natural and anthropogenic activities. We have seen a lot of examples explaining about the temporary survivalship of forests in nature. The ever changing local weather and global wind patterns are also playing key roles in deciding the life expectancy of the forest lands. One thing is for sure that - the best way to fight against global warming is to cut the carbon emission and stop the use of fossil-fuels worldwide. To ensure this all the governments of world must work hand-in-hand and that is the only solution to stop the increasing concentration of the atmospheric carbon. New declarations and policies must be made considering the important revelation about the sustainability of the terrestrial carbon sinks in nature. Simultaneously the scientists should also seek for some new sources of carbon sink as well, which are not influenced by local weather pattern and might not become a source of carbon themselves in near future. We must start acting today to make a safe and better world for tomorrow.

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**ANTHELMINTIC EFFECT OF *Solanum lycocarpum* IN MICE INFECTED WITH *Aspicularis tetraptera*.**

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**Abstract**

This approach intends to add new data on the helminthes parasites of laboratory mice. It has been investigated the anthelmintic activity of *Solanum lycocarpum* (*Solanaceae*) extracts against *Aspicularis tetraptera* in mice naturally infected. According to the analysis of the results it was observed and an extremely significant difference between TM and C (from  $5.64 \pm 3.33$  to  $1.56 \pm 3.16$ ). It was published that medicinal plants which were reported as useful in the treatment of diabetes the *S. lycocarpum* was the sixth most frequently mentioned. According to the results obtained in the present study, we can speculate that the anthelmintic effect of *Solanum lycocarpum* was noticed due to the concentration of steroidal alkaloid oligoglycosides and short-chain fatty acids. [The Journal of American Science. 2008;4(3):75-79]. (ISSN: 1545-1003).

Key words: *Solanum lycocarpum*, *Aspicularis tetraptera*, anthelmintic, mice, medicinal plants.

**INTRODUCTION**

*Solanum lycocarpum* was collected in the City of Três Marias, State of Minas Gerais and in the City of Seropédica, State of Rio de Janeiro. The botanical identification was carried through in the Department of Botany of the Rural Federal University of Rio de Janeiro, having been the exsiccates deposited under numbers RBR 28010 and RBR 14072.

*S. lycocarpum* is a plant which is shrubs ranging in height from 1.2 to 3 m. The fruit is yellow in color and resembles a medium sized tomato. Parts of the plant are poisonous if it gets in your system. When it is in bloom, it is medium blue. It blooms in the late winter, early spring, late fall, early winter, and mid winter. It is velvety or fuzzy. It needs water regularly. It is found in the Brazilian savannah but has been said to grow in San Antonio, Texas. *S. lycocarpum* is commonly used in Brazilian folk medicine. The Brazilian flora is one the world richest sources of bioactive material due to its biodiversity. Several plants are currently used in Brazilian traditional medicine to treat diabetes. *Solanum lycocarpum* St. Hill., *Solanaceae* has been widely used and commercialized as a hypoglycemic agent in Brazil. It was described by Vieira et al. (2003) the anti-inflammatory effects of the crude ethanol extract and it alkaloid fraction from *S. lycocarpum* fruit. Recently, it was carried out a chemical analysis of the starch and tried to correlate its supposed hypoglycemic activity with the polysaccharide content. However, these investigators did not conduct any experimental test to directly demonstrate the hypoglycemic effect attributed to the starch. *Solanaceae* or Lobeira is a plant used as a hypoglycemic agent. A study reported that the extract reduces glycemia in alloxan induced diabetic rats. It was reported that the potential of *S. lycocarpum* as antioxidant was capable reduce in 27% nitrate generation in diabetic animals. In literaturature has been demonstrated that *S. lycocarpum* is not ulcerogenic and restored haemoglobin and haematocrit to normal values in diabetic animals (Perez et al, 2006).

It this plant contains steroidal glycoalkaloids that can be transformed into an intermediate for steroidal drug production. In this way, it is very possible that these glycoalkaloids and its aglycone, once in the body by ingestion of *S. lycocarpum* fruits, may act by disrupting the endocrine system. Because its fruits may be consumed by pregnant animals in the fields, various studies determined the possible toxic effects of exposure to *S. lycocarpum* fruit from gestation. The unripe fruits contained 0.6% of solamargine and 0.9% of solasonine. It was related that *S. lycocarpum*, during gestation and the beginning of lactation reduces intrauterine growth. It is known that during adulthood, female offspring showed impaired sexual behavior and male offspring showed prominent degeneration of testis germinative cells, characterized by a reduced number of germ cells and vacuolation. It has been documented that the exposed offspring showed reduced hypothalamic norepinephrine (NOR), vanillylmandelic acid (VMA), 3-methoxy-4-hydrophenylglycol (MHPG) and homovanillic acid (HVA) levels, and reduced striatum NOR, HVA, VMA, MHPG, dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindolacetic acid (5-HIAA) levels. It is suggest that the fruit may act as an estrogen, with a long-term effect, impairing the receptive lordosis behavior of female offspring and promoting testis abnormalities in male offspring at adulthood. It appears to disrupt brain organization since important central monoamine level alterations were also related (Schwarz et al, 2005a).

Rodents, as mice and rats are the most common laboratory animals used in research and testing. They are seldom investigated for autochthonous ecto- and endoparasites prior their utilization in the experiments. Pinworms commonly infecting laboratory rodents include mainly the mice pinworms *Aspicularis tetraptera* (Gilioli et al, 2000; Perek-Matysiak et al, 2006).

Some plant extract may act differently due to its action against the parasite. In a study the anthelmintic activity of the extracts obtained from *Luxemburgia octandra* was evaluated naturally infected mice with *Aspicularis tetraptera* and *Vampirolepis nana*. The ethanolic and ethyl acetate extracts presented significant increase of the *V. nana* elimination, but did not present the nematicide effect against *A. tetraptera* (Silva et al, 2005).

In the present study we evaluated the anthelmintic activity of *Solanum lycocarpum* extracts in a concentration of 10% against *Aspicularis tetraptera* in mice in naturally infected.

#### MATERIALS AND METHODS.

Vegetal extracts: Dried leaves of units of had been used in the anthelmintic tests *Solanum lycocarpum* had been gotten by infusion (tea), submitted to the filtration in nylon and the express concentrations in g/100 ml (p/v).

Animals and anthelmintic tests: For anthelmintic test have been used lots of albinos mice, male and females weighted in media of 25g and naturally infecting for *Aspicularis tetraptera*, originated from Oswaldo Cruz Foundation – FIOCRUZ and held into the Institute of Biology from Rural Federal University from Rio de Janeiro. The animals have been held into bird cages individual of polypropylene (30x 20 x 13cm), it has at the bottom road of screen stark and stiff (network of 7x 7mm) upon a sheet of absorbent paper with the aim to facilitate the collection diary of excrement (Amorim et al., 1987 e Amorim e Borba, 1990).

The extracts were applied for oral saw (intragastric), into the volume of 0.04mL/g, with the employing of a dead and bend probe during three consecutive days. The excrement, collected 24 hours after each application, performing a total of four fecal collection, have been softened previously, transferred about to tames of network of 125 micrometers ( $\mu\text{m}$ ) and evaluated under microscope stereoscope, with the objective of behave the identification from the worm eliminated of the second to the fifth day of the experimental. Into the fifth and last days from the tests, the mice have been sacrificing for inhalation of vapors of ether ethyl, examining in the colon the number of the *A. tetraptera* remnants (Amorim et al., 1999). On the tests have been used the extracts of *Solanum lycocarpum* (leaves dried from Três Marias in the concentration of 10%) and (leaves dried from UFRRJ in the concentration of 10%). Additional lots of mice have been used with standard, they receiving doses of 20mg.kg<sup>-1</sup>day<sup>-1</sup> of mebendazol and 100mg.kg<sup>-1</sup>day<sup>-1</sup> of nitroscanato and they were submitted to the identical assessment anthelmintic description about to the animals treated with the plant extracts. A batch control, without a treatment served about to appraise the elimination spontaneous from the helminthes studied. The outcome antinematode also was denominated in terms percentile average of roundworm eliminated, considering the number of roundworm eliminated in the excrement in relation to the total number. Statistical analysis were performed and Tukey-Kramer Multiple Comparisons Test was applied to compare the results.



RESULTS AND DISCUSSION.

According to the analysis of the results it was observed that there were no differences ( $p > 0.05$ ) in the % of elimination between TM and UR (from  $5.64 \pm 3.33$  to  $3.15 \pm 3.16$ ), UR and C (from  $3.15 \pm 3.16$  to  $1.56 \pm 3.16$ ) and an extremely significant difference between TM and C (from  $5.64 \pm 3.16$  to  $1.56 \pm 3.16$ ) (Table 1).

Table 1. Anthelmintic activity of the extracts obtained of *Solanum lycocarpum* in the elimination of *Aspiculuris tetraptera* in mice naturally infected.

Used Parts	Administration form	Number of animals	Number of Helminthes		Elimination (%)
			Fecal Exam	Necropsy	
Leaves Dried from Três Marias (TM)	10%	10	61	1082	$5.64 \pm 3.33$
Leaves Dried from UFRRJ (UR)	10%	12	54	1717	$3.15 \pm 3.16$
Nitroscanato (NIT)		12	499	282	$64.0 \pm 0.00$
Mebendazol (MEB)		10	324	0.0	$100 \pm 0.00$
Control (C)		10	45	2836	$1.56 \pm 3.16$

The extracts were applied for oral saw (intra-gastric), into the volume of  $0.04\text{mL/g}$ , with the employing of a dead and bend probe during three consecutive days. The excrements, collected 24 hours after each application, performing a total of four fecal collection, have been softened previously, transferred about to tames of network of  $125\mu\text{m}$  and evaluated under microscope stereoscope, with the objective of behave the identification of the worm eliminated of the second to the fifth day of the experimental. Tukey-Kramer Multiple Comparisons Test was applied to compare the results.

Animal models have been exhaustively investigated regarding aspects related to their suitability for the development of experimental protocols under laboratory conditions. Nevertheless, in most of the adopted procedures, the prior detection of their ecto and endo parasites are generally overlooked related to the really effects of natural extracts in their biological cycle.

In the Brazilian cerate, a preparation obtained from the fruits of *Solanum lycocarpum* St.-Hill. (Solanaceae), popularly known as 'fruta-de-lobo' (wolf-fruit), have been widely employed for diabetes management, obesity and to decrease cholesterol levels. The medicinal preparation consists of the green fruits which are ground in aqueous solution and filtered. The white 'gum' deposited is decanted and slowly dried providing a powder which is commercialized in capsules with the name of 'polvilho-de-lobeira'. Through phytochemical analysis of this phytomedicine and the fruit of *S. lycocarpum* were found polysaccharides as the main component. Some polysaccharides slow gastric emptying and act on the endocrinous system affecting the liberation of gastrointestinal hormones, lowering blood glucose levels.

According to Schwarz et al (2005b) it is well known that this plant contain steroidal glycoalkaloids that can be transformed into an intermediate for steroidal drugs production, like oral contraceptives. In this way, it is very possible that these glycoalkaloids and its aglycone, once in the body by ingestion of *S. lycocarpum* fruits, may act disrupting to the endocrine system as well as it may probably affect the reproductive system of helminthes. The hypocholesterolemic activity could be due to the increased fecal bile acid excretion as well as to the action of the short-chain fatty acids, coming from fermentation, on the synthesis of delta-aminolevulinate and by the increase of the cholesterol 7-alpha-hydroxylase and 3-hydroxy-3-methylglutaryl CoA reductase synthesis (Dall'Agnol and Von Poser, 2000). Due to the effect related it may be possible that these fatty acids could act as an anthelmintic, although in the present study there was not observed differences between TM and UR extracts related to % of elimination in comparison one to another, although in comparison to the control group was evident a significative difference due to the TM group. Related to the obtained results due to the action of the TM extract it may be explained by their concentration as well as originated region which may explain the effect due to the biochemistry compounds in the equivalents proportions in spite of different conditions as soil composition, light and water availability.

The effect of TM extract may be support by possible modifications in ribosomal DNA spacer region suggesting that it could result in genetic and geographical variability as well as different bioactivity which may not be effective depend on the concentration of the extract (Arruda et al, 2003).

We can speculate that the other effect would be related to the low concentration of steroidal alkaloid oligoglycosides which in a optimal concentration may suppress the transfer of sucrose from the stomach to the small intestine which could diminish the support of glucose to helminthes together with its antioxidant effect which is capable of reducing the nitrate generation which can be used in the protein synthesis.

## CONCLUSION.

Based on the results we can suggested that the anthelmintic effect of *Solanum lycocarpum*, TM extract, is related to the possible concentration of steroidal alkaloid oligoglycosides as well as the short-chain fatty acids presents in the extract. The similar action of the extracts may be explained by adaptation mechanisms related to the genetic and geographical variability.

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**Mutagenesis and Selection of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for Potential use as Starter Culture.**

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**ABSTRACT:** Mutagenesis and Selection of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* isolated from “Kindirmo” a Nigerian local yogurt for potential use as starter culture was undertaken. The mutants isolated showed ability to grow on MRS agar, and were catalase negative. Their colony color varies from white to creamy grey. Most isolates were gram positive and were presumed to have high potentials of lactic acids bacteria and few were gram negative, and were presumed to have defective gene(s). The mutant isolates showed ability to produce acids in TSB and high degree of fructose utilization. The increased acids production with increase fructose utilization by some mutant cells may also suggest the use of fructose as a supplement to increase the rate of milk fermentation in “lazy-milks”. [The Journal of American Science. 2008;4(3):80-87]. (ISSN: 1545-1003).

**Key Words:** “Kindirmo”, “Lazy-Milk”, Mutagenesis and Selection.

## INTRODUCTION

In developing countries like Nigeria, simple biotechnological techniques like mutagenesis may be adopted in order to enhance food and goods productivity. Mutation has its harmful and beneficial effects (Allan and Greenwood, 2001; Kruz, 1995; Voet, *et al.*, 1999). There are numerous documented cases where beneficial mutations with survival advantages have arisen in a population. Such beneficial mutations occur frequently among viruses, bacteria and higher organisms as well (Brown, 1992). Some of the beneficial effects are; increase in enzyme activities of mutant strain of *Leuconostoc Messenteroides* was about 2.5 fold higher than normal (Kamal, *et al.*, 2003), evolution of a single clonal line of beer yeast cells with mutations in permease and phosphatase enzymes that results in increase beer production, when yeast cells are grown in a chemostat with limited phosphate (Francis and Hansche, 1972 & 1973 and Hansche, 1975), evolution of a new metabolic pathways of metabolism of fucose and lactose by *E. coli* ( Lin and Wu, 1984 and Kenneth, 1999) and Ethidium bromide-treated *Aspergillus nidulans* cell showed significantly greater homozygotization index (HI) than controls (Becker, *et al.*, 2003). Mutagenesis has been used in the selection and improvement of Lactic acid bacteria starter culture (Harlander, 1992).

The Fulani’s (cattle rearing tribe in Nigeria) have been using their starter culture for years in “kindirmo” (local yoghurt) production with little or no attempt to improve the starter culture strains for either, to enhance product flavors or texture.

Therefore, this study has undertaken a simple biotechnology technique, mutagenesis, to see if it can improve the local starter culture strains isolates used in the production of “kindirmo” (the local yoghurt).

## **MATERIAL AND METHODS**

### **Isolation of Lactic Acid Bacterial Strains**

Lactic acid bacteria strains (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) were isolated from “kindirmo” bought from Fulani women hawking “kindirmo” in Federal University of Technology, Yola, Nigeria. The culture was grown on MRS agar plates, (Oxoid, (2004))

### **Induction of Mutagenesis**

Mutagenesis with ethidium bromide and ultra violet (UV) light were done according to Gawel *et al.*, (2002) and Kamal *et al.*, (2003) respectively.

#### **(a). UV-Mutagenesis**

*Lactobacillus bulgaricus* and *Streptococcus thermophilus* were grown at 30°C and 42°C respectively in 100ml of tryptone soya broth (TSB) to cell optical density (O.D<sub>600</sub>) of 0.2 – 0.3. The cells were harvested by centrifugation at 5,000 X g for 15min and washed twice in 100ml cold, sterile, 0.9% NaCl solution. Portions of cell suspensions (8ml aliquots) was transferred to sterile petri dishes and radiated with UV-light (254nm) for four different periods (20, 25, 30 and 35 sec.). Each irradiated sample were centrifuged at 5,000 X g for 15min and re-suspended in 10ml TSB and incubated at 30°C and 42°C respectively for 18hr. The cultures were then diluted serially into sterile 0.9% NaCl solution and 0.1ml of serial dilution were plated on to MRS agar plates and incubated at 30°C and 42°C respectively. Then mutants were isolated

#### **(b). Ethidium Bromide Mutagenesis**

The two strains (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) were grown to late logarithmic phase of growth in TSB. The cells were harvested and washed twice with sterile 0.9% NaCl solution and 0.5, 0.1 and 1.5g/l of ethidium bromide were added to 2ml of each cell suspension. The mixture was aerated on a shaking incubator at 30°C and 42°C for 30min respectively. The treated cells were incubated in 10ml TSB washed twice and re-suspended in 0.9% NaCl solution and after serial dilution it was spread on MRS agar plates and incubated at 30°C and 42°C respectively for 48hr. Then mutants were isolated.

### **Characterization of Mutant Isolates**

Cell morphology was performed according to Ridge (1982), catalase test according to Schieri and Blazevic (1981), physiological test was as described by Oxoid (2004) and biochemical test according to methods of Harrigan and McCance (1993), Sambrook *et al.*, (1989) and Tserovska *et al.* (2002).

## **Results**

The results of mutants isolated from UV – light mutagenesis of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and their characterization is as shown in table 1.

All the mutants isolated showed; positive growth on MRS agar medium, negative catalase activity and all are gram positive. The mutants also showed creamy grey (CG) colony coloration with small colony size.

The cell morphology of most mutants showed rods, with only two (2) mutants (Stm 108 and Stm 109) showing cocci appearance. The mutant isolates (Lbm 102, 104, 105, 113 and 119) had scattered cell arrangement when examined with X100 oil immersion objective lens, while mutant isolate Lbm 106, had chains and scattered single cells; mutant isolate Stm 104 had single cells and scattered; and mutant isolates Stm 108 and 109 showed chains and single cells when examined with X100 oil immersion objective lens.

The mutant isolates showed three different levels of acid production in TSB. They are; positive, moderately positive and strongly positive were observed and only one mutant (Lbm 105) could not produce acid in TSB.

Positive acid production by isolates Stm 104, 108, 109; moderately positive acid production by isolates Lbm 104, 113, 119 and strongly positive acid production by isolates Lbm 102 and 106 were observed. And only one mutant (Lbm 105) could not produce acid in TSB.

The results for mutants isolated from ethidium bromide mutagenesis of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and their characterization is shown in Table 2. All the mutants isolated showed positive growth on MRS agar medium and negative catalase activity. The colony colour of most of the mutant isolates is white except for Stm 143 and 144, which were greyish white. Colony size varies: Isolates Stm 142, 143, 144 are very small; isolates Lbm 131, Lbm 134 are small and isolates Lbm 132, 133, 140 are bigger than others. Reaction to Grams test were positive for isolates Lbm 133, 134 and Stm 140; negative for Lbm 131 and Stm 142, 143 and the Grams reaction results for Lbm 132 and Stm 143 were not determined. Mutant cell morphology showed that Lbm 131 and Stm 140, 142, 144 were cocci like in shape; isolates Lbm 133, 134 were rod like in shape and cell morphology for isolates Lbm 132 and Stm 143 were not determined. The cell arrangement when observed in X100 immersion objective lens showed that isolates Lbm 131 and Stm 144 were singles; isolates Lbm 133, 134 are scattered; isolates Stm 140, 142 are singles and scattered and that of isolates Lbm 132 and Stm 143 were not determined.

The mutant isolates showed two (2) level of acid production in TSB. The mutant isolate Lbm 132 showed strongly positive level of acid production in TSB and the rest had moderately positive level of acid production in TSB.

The result for biochemical characterization (sugar utilization) of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* mutant isolates for UV – irradiated and ethidium bromide treated strains is as shown in Table 3. The mutant isolates showed three (3) levels of sugar utilization (positive, moderately positive and strongly positive). Glucose utilization was positive among isolates Lbm 102, 104, 105, 113, 119, 132, 133 and Stm 109, 142, 143. Moderately positive by isolates Lbm 106, 131 and strongly positive by isolates Lbm 134 and Stm 108, 140, 144. Galactose utilization was positive among most isolates except Lbm 106 with moderately positive galactose utilization and Lbm 105, which showed strongly positive galactose utilization. Fructose utilization among the mutant isolates varies: Positive by isolates Lbm 132 and Stm 142; moderately positive by isolates Lbm 113, 131 and Stm 104, 140, 144 and strongly positive among isolates Lbm 102, 104, 105, 106, 119, 134 and Stm 143.

**Table 1: Characterization of UV- Irradiated *Lactobacillus bulgaricus* Mutants (Lbm) and *Streptococcus thermophilus* Mutants (Stm) Isolates.**

Isolates	Growth on MRS agar production	Catalase activity	Colony colour	Colony size	Gram reaction	Cell morphology	Cell arrangement	Acid
Lbm102	+	-	CG	Small	+	Rods	Sc	+++
Lbm104	+	-	CG	Small	+	Rods	Sc	++
Lbm105	+	-	CG	Small	+	Rods	Sc	-
Lbm106	+	-	CG	Small	+	Rods	C/Sc	+++
Lbm113	+	-	CG	Small	+	Rods	Sc	++
Lbm119	+	-	W	Small	+	Rods	Sc	++
Stm104+	-	CG	Small		+	Rods	S/Sc	+
Stm108+	-	CG	Small		+	Cocci	C/S	+
Stm109+	-	CG	Small		+	Cocci	C/S	+

Key, CG- creamy grey, CW- creamy white, W- white, Sc- scattered, S- singly, C- chain, S/C- singles and chains,

C/Sc- chains and scattered, TSB- Tryptone Soya broth, - = negative, + = positive, ++ = moderately positive, +++ = strongly positive.

**Table 2: Characterization of Ethidium bromide treated *Lactobacillus bulgaricus* Mutants (Lbm) and *Streptococcus thermophilus* Mutants (Stm) Isolates.**

Isolates	Growth on	Catalase activity	Colony colour	Colony size	Gram reaction	Cell morphology	Cell arrangement	Acid
	MRS agar production							
Lbm131	+	-	W	Small	-	Cocci	S	++
Lbm132	+	-	W	Big	ND	ND	ND	+++
Lbm133	+	-	W	Big	+	Rods	Sc	++
Lbm134	+	-	W	Small	+	Rods	Sc	++
Stm140	+	-	W	Big	+	Cocci	S/Sc	++
Stm142	+	-	W	V. small	-	Cocci	S/Sc	++
Stm143	+	-	CG	V. small	ND	ND	ND	++
Stm144	+	-	CG	V. small	-	Cocci	S	++

**Key**, CG- creamy grey, W- white, S- singles, C- chains, Sc- scattered, S/Sc singles and scattered, V. small- very small,

TSB- Tryptone soya broth, ND- not detectable, + = positive, ++ = moderately positive, +++ = strongly positive, - = negative.

**Table 3: Biochemical Characterization of *Lactobacillus bulgaricus* Mutants (Lbm) and *Streptococcus thermophilus* Mutants (Stm) Isolates from UV – Irradiated and Ethidium Bromide-Treated Strains.**

Isolates	Treatment	Glucose	Galactose	Fructose
Lbm102	UV-25sec	+	+	+++
Lbm104	UV-35sec	+	+	+++
Lbm105	UV-35sec	+	+++	+++
Lbm106	UV-35sec	++	++	+++
Lbm113	UV-35sec	+	+	++
Lbm119	UV-35sec	+	+	+++
Lbm131	0.5g/dm <sup>3</sup>	++	+	++
Lbm132	0.5g/dm <sup>3</sup>	+	+	+
Lbm133	0.5g/dm <sup>3</sup>	+	+	++
Lbm134	1.0g/dm <sup>3</sup>	+++	+	+++



Stm104	UV-35sec	++	+	++
Stm108	UV-35sec	+++	+	+
Stm109	UV-35sec	+	+	+
Stm140	1.0g/dm <sup>3</sup>	+++	+	++
Stm142	1.0g/dm <sup>3</sup>	+	+	+
Stm143	1.5g/dm <sup>3</sup>	+	+	+++
Stm144	1.5g/dm <sup>3</sup>	+++	+	++

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**Key**, + = positive, ++ = moderately positive, +++ = strongly positive

## Discussion

The ability of mutant isolates to grow on MRS agar medium and are catalase negative is an indication that the mutant isolates still retain biological activities of wild type strain (Oxoid, 2004; Togo, 2002). The isolates that are Gram positive are presumed to have potentials of lactic acid bacteria and the rest could be mutants with defective gene. Lactic acid bacteria have been consistently demonstrated to be responsible for the lactic acid fermentation of milk (Brock and Madigan, 1991; Prescott *et al.*, 1999) and non-dairy materials (Steinkraus, 1996; Varnam, 2002). The mutant isolates showed ability to produce lactic acid in TSB and fructose utilization, which is in line with the work of Buchanan and Gibbons (1974). The increased fructose utilization observed may also explain the increased acid production in some mutant cells and this may likely suggest the use of fructose as supplement to increase the rate of milk fermentation in “lazy – milk” similar to research work of Igyor (2005) where he used yeast extract supplementation in milk fermentation.

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## **MAASTRO lab has a vacancy for a Senior scientist, Head of Laboratory Research in molecular oncology (M/F)**

**Vac.nr. 2007.009/KC**

MAASTRO, Maastricht Radiation Oncology, is a co-operation between MAASTRO clinic, the University of Maastricht (UM) and the University Hospital Maastricht (azM) (see [www.maastro.nl](http://www.maastro.nl)). MAASTRO consists of several division, including Maastricht Clinic, which offers state-of-the-art radiotherapy to more than 3500 cancer patients each year from the Mid and South Limburg area in the Netherlands. MAASTRO clinic is also world-wide reference centre for Siemens Medical. In addition, research and training at Maastricht is carried out in Maastricht Physics, Maastricht Trials, Maastricht School, and Maastricht Lab.

MAASTRO Lab is a basic and translational research laboratory embedded within the GROW research institute of the Faculty of Health, Medicine and Life Sciences at Maastricht University. Research carried out in the past has been focused on the tumour microenvironment and EGFR signalling pathways, both of relevance to radiation oncology. MAASTRO Lab has made several important discoveries in these fields, including demonstration that EGFR is up regulated by radiation and that hypoxia inhibits the initiation step of mRNA translation. In addition, we have initiated translational and clinical studies based on these results including both phase I novel treatment and molecular imaging trials as well as a Biobank project with more than 1500 patients included.

The lab has 4 permanent scientists, 5 technicians, more than 5 PhD students and is fully equipped for cell culture, molecular biology, flow cytometry, hypoxia, gene expression, proteomics and microscopy. Maastricht lab has set up the necessary infrastructure for controlled exposures to hypoxia and hypoxia/reoxygenation, including development of novel equipment that allows rapid and precise changes in oxygenation. Access to expertise, equipment and resources within the much larger GROW research institute and other facilities in the University are also readily available, including the genome centre, advanced microscopy, and the animal facility with its imaging facility (Optical imager, MRI 7Tesla and micro CTPET to come). MAASTRO has a structural collaboration with the VU in Amsterdam on molecular PET biomarkers, with the TU/Eindhoven on Systems Biology and is initiating a new collaboration with the University of Toronto on research related to the Unfolded Protein Response and tumour hypoxia.

**MAASTRO lab has a vacancy for a**

**Senior scientist, Head of Laboratory Research in molecular oncology  
(M/F)**

Vac.nr. 2007.009/KC

**In this position you will be responsible** for carrying out basic and translational research that is of relevance to radiation oncology in the broadest possible scope. You will initiate an independent research program based on demonstrated skills and expertise in fundamental aspects of biology. In addition, you will be chiefly responsible for the scientific research and training within the lab of experimental Radiation Oncology (MAASTRO lab). As head of research you will manage the laboratory scientific research, direct the research policy, and participate actively in ongoing and newly initiated research lines and projects. Successful grant applications to prestigious (inter)national organizations to support expansion of research activities will constitute an important part of your work.

Depending on experience, the process to appoint you as professor or associate professor at the faculty of Health, Medicine and Life Sciences from Maastricht University will be started. You will participate in research and educational activities within the faculty. The emphasis in this faculty appointment is on *microenvironment of solid tumours and cell signalling (EGFR)* but there is room for your specific area of expertise.

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**Your application letter, Curriculum Vitae and listing of publications** can be sent before to the department of Personell to the attention of mrs. M.T.V. Vaessens, pbox 5800, 6202 AZ Maastricht, the Netherlands.

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