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Review

Application of Real-time Polymerase Chain Reaction (RT-PCR)

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Abstract: The real-time polymerase chain reaction (RT-PCR), also called quantitative real-time polymerase chain reaction (QRT-PCR) or kinetic polymerase chain reaction (kPCR), is a technique used to simultaneously quantify and amplify a DNA molecule. It is used to determine whether a specific DNA sequence is present in the sample; and if it is present, the number of copies in the sample. It is the real-time version of quantitative polymerase chain reaction (qPCR), itself a modification of polymerase chain reaction (PCR). The procedure of RT-PCR follows the regular PCR procedure, but the DNA is quantified after each round of amplification. Two common methods of quantification are the use of fluorescent dyes that intercalate with double-strand DNA, and modified DNA oligonucleotide probes that fluoresce when hybridized with a complementary DNA. RT-PCR could be combined with reverse transcription polymerase chain reaction to quantify messenger RNA (mRNA) at a particular time for in a particular cell or tissue type. [The Journal of American Science. 2006;2(3):1-15].

Keywords: DNA; polymease chain reaction (PCR); real-time (RT); RNA

1. Introduction

Polymease chain reaction (PCR) is a technique that allows logarithmic amplification of short DNA sequences (100 to 600 bases) within a longer double stranded DNA molecule. This technique was invented in 1980's by Dr. Kary Banks Mullis in California. Kary Banks Mullis awarded Norbel Prize of chemistry for the invention of PCR (Greer, 2006; Mullis, 2006). The purpose to put the three pictures of Dr. Mullis in this article is to see the characteriztion of the PCR inventor. From the photos we can see that Mullis is a thoughtful man. Let's thank and remember Dr. Mullis for the invetion of PCR when we do PCR.

Higuchi et al. pioneered the analysis of PCR kinetics by constructing a system that detects PCR products as they accumulate. This "real-time" system includes the intercalator ethidium bromide in each amplification reaction, an adapted thermal cycler to irradiate the samples with ultraviolet light, and detection of the resulting fluorescence with a computer-controlled cooled CCD camera. Amplification produces increasing amounts of double- stranded DNA, which binds ethidium bromide, resulting in an increase in fluorescence. By plotting the increase in fluorescence versus cycle number, the system produces amplification plots that provide a more complete picture of the PCR process than assaying product accumulation after a fixed number of cycles. This technique to measure the accumulation of PCR products in a real time is called real-time PCR (RT-PCR), where the real-time is abbreviated as RT and PCR is the abbreviation of polymease chain reaction. As a milestone of the RT-PCR, Higuchi et al. wrote in the journal Biotechnology in 1993 as the following: "We describe a simple, quantitative assay for any amplifiable DNA sequence that uses a video camera to monitor multiple polymerase chain reactions (PCRs) simultaneously over the course of thermocycling. The video camera detects the accumulation of double-stranded DNA (dsDNA) in each PCR using the increase in the fluorescence of ethidium bromide (EtBr) that results from its binding duplex DNA. The kinetics of fluorescence accumulation during thermocycling are directly related to the starting number of DNA copies. The fewer cycles necessary to produce a detectable fluorescence, the greater the number of target sequences. Results obtained with this approach indicate that a kinetic approach to PCR analysis can quantitate DNA sensitively, selectively and over a large dynamic range. This approach also provides a means of determining the effect of different reaction conditions on the efficacy of the amplification and so can provide

insight into fundamental PCR processes" (Higuchi, et al., 1993).

PCR uses a pair of primers (about 20 bp each), that are complementary to a specific sequence on each of the two strands of the target DNA. These primers are extended by a DNA polymerase and the sequence of the new DNA pieces matches the sequence followed the primer. After the new DNA synthesis, the same primers will be released and used again. This gives the DNA a logarithmic amplification. Since the DNA amplification is processed under the single strand condition, it needs high temperature to separate the double strand DNA in each round of the amplification process. The milestone of the life science research is the discovery of a thermo-stable DNA polymerase (Taq polymerase) that is isolated from *Thermus aquaticus,* a bacterium that grows in hot pools near volcanic vent. For PCR, it is not necessary to add new polymerase in every round of amplification. After some rounds of amplification (about 40), the PCR product is analyzed on an agarose gel and is abundant enough to be detected with an ethidium bromide (EB) stain. In order to measure messenger RNA (mRNA), the method is extended using reverse transcriptase to convert mRNA into complementary DNA (cDNA) which is amplified by PCR and, again analyzed by agarose gel electrophoresis. In many cases this method has been used to measure the levels of a particular mRNA under different conditions but the method is actually even less quantitative than PCR of DNA because of the extra reverse transcriptase step. Reverse transcriptase PCR analysis of mRNA is often referred to as "RT-PCR" also which is unfortunate as it can be confused with "real-time PCR" that also abbreviated as RT-PCR (Abdul-Careem, 2006**)**. In this article anywhere the RT-PCR appears that represents real-time PCR.

There are two type quantifications from RT-PCR. One is absolute quantification which requires an input standard curve with series diluted template. Another one is relative quantification which used to determine fold different in input target that do not need a standard curve and is very commonly used for gene expression analysis.

For living cells in a specific time some genes are expressed and some are not. When a particular protein is required by a cell, the gene coding for that protein is activated. The first step to synthesize a protein is to transcribe an mRNA from the gene's DNA sequence. The amount of mRNA produced correlates with the amount of protein eventually synthesised and measuring the amount of a particular mRNA produced by a given cell or tissue is often easier and more important than measuring the amount of the final protein, as the protein could be in a dynamic status in the cell's living cycle.

Traditionally, mRNA amount can be measured by Northern blot and it is still used to measure mRNA by many laboratories with different proposes. Northern blot needs larger of mRNA sample, and RT-PCR was developed to measure small amount of mRNA. As the sensitivity if higher for RT-PCR method, the contamination sould be pay attention. For RT-PCR, it does not need to measure the concentrations of mRNA or cDNA in a sample before the detection. The other method for RNA measurement is RNase protection assay.

Normal reverse transcriptase PCR is only semi-quantitative at best because of the insensitivity of EB. PCR is the most sensitive method and can discriminate closely related mRNAs. Northern blot and ribonuclease protection assays are the standard methods, since no amplification is involved, whereas in situ hybridization is qualitative rather than quantitative. Techniques such as Northern blot and ribonuclease protection assays work very well, but require more RNA than is sometimes available. PCR methods are therefore particularly valuable when amounts of RNA are low, since the fact that PCR involves an amplification step means that it is more sensitive. In contrast to regular reverse transcriptase-PCR and analysis by agarose gels, RT-PCR gives quantitative results. An additional advantage of RT-PCR is the relative ease and convenience of use compared to some older methods. RT-PCR offers scientists a powerful tool for the quantitation of target nucleic acids.

In U'Ren, et al's study, A TaqMan allelic-discrimination assay designed around a synonymous single-nucleotide polymorphism was used to genotype Burkholderia pseudomallei and Burkholderia mallei isolates. The assay rapidly identifies and discriminates between these two highly pathogenic bacteria and does not cross-react with genetic near neighbors, such as Burkholderia thailandensis and Burkholderia cepacia (U'Ren, 2005).

In the traditional PCR technique, a PCR uses a peltier heat pump to quickly heat and cool the DNA and uses the Taq polymerase. Thermophilus aquaticus (Taq) is a bacterium that lives by volcanic sulfer jets at the bottom of the ocean. They can withstand extreme temperatures, and that is why they are so valuable in PCR. Primers are short strands of RNA that bind to specific known sites on the DNA molecule. DNA polymerases need to have RNA primers in order to begin replication. Four dNTPs (deoxyribonucleotide triphosphates) (dGTP, dCTP, dATP and dTTP) are letters of the DNA alphabet and the taq polymerase uses the sNTPs to build the new molecular chains.

Brief Steps of Traditional PCR:

1) The DNA strands are denatured at high temperature, breaking the weak hydrogen bonds that bind one side of the helix to the other and separating the rails of DNA.

2) The temperature is lowered and primers (short bits of DNA) are added. The primers bond to their specific sites.
3) The temperature is brought back up to body temperature and taq polymerase is added.
4) Repeat step one for n cycles, amplifying the DNA.
5) The product of PCR is 2^n copies of the selected DNA strand, where n is the number of cycles run.

PCR makes a revolution for the life science. As Dr. Kary Banks Mullis wrote in *Scientific American*, "Beginning with a single molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in an afternoon. The reaction is easy to execute. It requires no more than a test tube, a few simple reagents and a source of heat. The DNA sample that one wishes to copy can be pure, or it can be a minute part of an extremely complex mixture of biological materials. The DNA may come from a hospital tissue specimen, from a single human hair, from a drop of dried blood at the scene of a crime, from the tissues of a mummified brain or from a 40,000-year-old wooly mammoth frozen in a glacier" (Mullis, 1990).

RT-PCR offers the ability to monitor the real-time progress of the PCR product via fluorescent detection. The point characterizes this in time during cycling when amplification of a PCR product is first detected rather than the amount of PCR product accumulated after a fixed number of cycles. These PCR based fluorescent homogenous assays can be monitored using either labeled hybridization probe(s) (Taq Man, Molecular Beacons) or labeled PCR primer (Amplifluor) and SYBR Green (Applied Biosystems).

2. Principle of Methodology of RT-PCR

Currently, there are three techniques for RNA measurement: Reverse transcription PCR, Northern blot analysis and RNase protection assay. Reverse transcription PCR is the most sensitive technique for mRNA detection and quantitation. Compared to the other two techniques for quantifying mRNA levels (Northern blot analysis and RNase protection assay) Reverse transcription PCR can be used to quantify mRNA levels from much smaller samples. In fact, this technique is sensitive enough to enable quantitation of RNA from a single cell.

RT-PCR principle is based on the properties of the PCR reaction kinetics. A quantification of the PCR products synthesized during the PCR is obtained at each cycle. From the PCR cycle number curves obtained for each sample, a threshold is defined. The cycle threshold (C_T) corresponds to the intersection of the threshold and the PCR amplification curve. The threshold is chosen to intersect with all the PCR amplification curves during their exponential phases.

RT-PCR can detect sequence-specific PCR products as they accumulate in real-time during the PCR amplification process. As the PCR product is produced, RT-PCR can detect their accumulation and quantify the number of substrates exist in the initial PCR mixture before amplification start.

RT-PCR was developed from the PCR technique that measures the amplification of small DNA amount. For RT-PCR, mRNA or total RNA is isolated from a particular sample before producing a DNA copy of complementary DNA (cDNA) of each mRNA molecule. The gene expression levels are then further amplified from the cDNA mixture together with a housekeeping gene (internal control). Housekeeping genes are those whose expression levels remain roughly constant in all samples and include such genes as actin, hypoxanthine-guanine phosphoribosyltransferase (HGP) and glyceraldehyde phospho-dehydrogenase (GAPDH), the endogenous contal to correct for potential variation in RNA loading, cDNA synthesis or efficiency of the amplification reaction. For the RT-PCR principle, more mRNA is in a sample, the earlier it will be detected during repeated cycles of amplification. Many systems produced that amplify DNA with a fluorescent dye. RT-PCR machines can detect the amount of fluorescent DNA and thus the amplification progress. Amplification of a given cDNA over time follows a curve, with an initial flat-phase, followed by an exponential phase. As the experiment reagents are used up, DNA synthesis slows and the exponential curve flattens into a plateau.

Threshold is a level of normalized reporter signal that is usde for C_T determination in real-time assays. The level is set to be above the baseline but sufficiently low to be within the exponential growth region of an amplifivation curve. The cycle number at which the fluorescence signal associated with a particular amplicon accumulation crosses the thrshold is referred to as the C_T. C_T is threshold cycle, the cycle number at whitch the fluorescence generated within a reaction crosses the threshold line. C_T values are logarithmic and are used either directly or indirectly for the quantitative analyses. As an example, suppose that we want to measure the expression level of "Gene-M" in two cell samples. After RT-PCR amplification we finds that in sample 1, Gene-M reaches a pre-determined threshold of detection after 18 cycles, known as the C_T value, where as in sample 2 it does not reach the threshold until 22 cycles. If the housekeeping gene has a C_T value of 17 in both cases then the difference between C_T values, or ΔC_T, will be 1 for sample 1 and 5 for sample 2. In this case Gene-M is more highly expressed in sample 1 than in sample 2.

Normally a housekeeping gene will not have the same C_T value over all samples analysed. Many

softwares and spreadsheets have been produced with that the user can input C_T values and produce a numerical output showing gene expression levels compared between different cell samples, expressed as a fold difference between samples. Such programs also allow statistical analysis of data, such as calculation of standard error and standard deviation.

According to chemistries, currently four different chemical principles of methodology are available for RT-PCR: (1) TaqMan® (Applied Biosystems, Foster City, CA, USA); (2) Molecular Beacons; (3) Scorpions®; (4) SYBR® Green (Molecular Probes). All the four methods do the detection of PCR products via the generation of a fluorescent signal. TaqMan probes, Molecular Beacons and Scorpions depend on Förster Resonance Energy Transfer (FRET) to generate the fluorescence signal through the coupling of a fluorogenic dye molecule (5' end) and a quencher moeity (3' end) to the same or different oligonucleotide substrates. SYBR Green is a fluorogenic dye that exhibits little fluorescence when in solution, but emits a strong fluorescent signal upon binding to double-stranded DNA (Dharmaraj, 2006). The old method for RT-PCR is **e**nd-point RT-PCR (relative RT-PCR, competitive RT-PCR and comparative RT-PCR). In spite of the rapid advances made in the area of real-time PCR detection chemistries and instrumentation, the end-point RT-PCR still remains a very commonly used technique for measuring changes in gene-expression in small sample numbers.

1) TaqMan Probes

TaqMan probes depend on the 5'- nuclease activity of the DNA polymerase used for PCR to hydrolyze an oligonucleotide that is hybridized to the target amplicon. TaqMan probes are oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher moeity coupled to the 3' end. These probes hybridize to an internal region of a PCR product. In the unhybridized state (5' end with fluorogenic dye binds 3' end with quencher), the proximity of the fluor and the quench molecules prevents the detection of fluorescent signal from the probe. During PCR, when the polymerase replicates a template on which a TaqMan probe is bound, the 5'- nuclease activity of the polymerase cleaves the probe. This decouples the fluorescent and quenching dyes, and FRET no longer occurs. So that fluorescence increases in each cycle and the fluorescence increasing has a linear relationship with the amount of probe cleavage. Well-designed TaqMan probes require very little optimization. In addition, they can be used for multiplex assays by designing each probe with a unique fluor/quench pair. However, TaqMan probes can be expensive to synthesize, with a separate probe needed for each mRNA target being analyzed (a primer costs about US$20, but a probes costs about US$250).

2) Molecular Beacons

Like TaqMan probes, Molecular Beacons also use FRET to detect and quantitate the synthesized PCR product through a fluor coupled to the 5' end and a quench attached to the 3' end of an oligonucleotide substrate. Unlike TaqMan probes, Molecular Beacons are designed to remain intact during the amplification reaction, and must rebind to target in every cycle for signal measurement. Molecular Beacons form a stem-loop structure when free in solution (a hairpin, 5' end with fluorogenic dye binds 3' end with quencher). Thus, the close proximity of the fluor and quench molecules prevents the probe from fluorescing. When a Molecular Beacon hybridizes to a target, the fluorescent dye and quencher are separated, and the fluorescent dye emits light upon irradiation. Like TaqMan, Molecular Beacons can be used for multiplex assays by using separated fluor/quench moieties on each probe. As with TaqMan probes, Molecular Beacons can be expensive to synthesize, with a separate probe required for each target.

3) Scorpions

With Scorpion probes, sequence-specific priming and PCR product detection is achieved using a single oligonucleotide. The Scorpion probe maintains a stem-loop configuration in the unhybridized state. The fluorophore is attached to the 5' end and is quenched by a moiety coupled to the 3' end. The 3' portion of the stem also contains sequence that is complementary to the extension product of the primer. This sequence is linked to the 5' end of a specific primer via a non-amplifiable monomer. After extension of the Scorpion primer, the specific probe sequence is able to bind to its complement within the extended amplicon thus opening up the hairpin loop. This prevents the fluorescence from being quenched and a signal is observed.

4) SYBR Green

SYBR Green provides the simplest and most economical format for detecting and quantitating PCR products in real-time reactions. SYBR Green binds double-stranded DNA, and upon excitation emits light. Thus, as a PCR product accumulates, fluorescence increases. The advantages of SYBR Green are that it is inexpensive, easy to use, and sensitive. The disadvantage is that SYBR Green will bind to any double-stranded DNA in the reaction, including primer-dimers and other non-specific reaction products, which results in an overestimation of the target concentration. For single PCR product reactions with well designed primers, SYBR Green can work extremely well, with spurious non-specific background only showing up in

very late cycles. SYBR Green is the most economical choice for real-time PCR product detection. Since the dye binds to double-stranded DNA, there is no need to design a probe for any particular target being analyzed. However, detection by SYBR Green requires extensive optimization. Since the dye cannot distinguish between specific and non-specific product accumulated during PCR, follow up assays are needed to validate results.

5) Real-time Reporters for Multiplex PCR

TaqMan probes, Molecular Beacons and Scorpions allow multiple DNA species to be measured in the same sample (multiplex PCR), since fluorescent dyes with different emission spectra may be attached to the different probes. Multiplex PCR allows internal controls to be co-amplified and permits allele discrimination in single-tube, homogeneous assays. These hybridization probes afford a level of discrimination impossible to obtain with SYBR Green, since they will only hybridize to true targets in a PCR and not to primer-dimers or other spurious products.

6) End-Point RT-PCR (Relative RT-PCR, Competitive RT-PCR and Comparative RT-PCR)

End-point RT-PCR can be used to measure changes in expression levels using three different methods: relative, competitive and comparative. The most commonly used procedures for quantitating end-point RT-PCR results rely on detecting a fluorescent dye such as ethidium bromide, or quantitation of P^{32}-labeled PCR product by a phosphorimager or, to a lesser extent, by scintillation counting.

Relative quantitation compares transcript abundance across multiple samples, using a co-amplified internal control for sample normalization. Results are expressed as ratios of the gene-specific signal to the internal control signal. This yields a corrected relative value for the gene-specific product in each sample. These values may be compared between samples for an estimate of the relative expression of target RNA in the samples.

Absolute quantitation, using competitive RT-PCR, measures the absolute amount (copies) of a specific mRNA sequence in a sample. Dilutions of a synthetic RNA (identical in sequence, but slightly shorter than the endogenous target) are added to sample RNA replicates and are co-amplified with the endogenous target. The PCR product from the endogenous transcript is then compared to the concentration curve created by the synthetic competitor RNA.

Comparative RT-PCR mimics competitive RT-PCR in that target message from each RNA sample competes for amplification reagents within a single reaction, making the technique reliably quantitative. Because the cDNA from both samples have the same PCR primer binding site, one sample acts as a competitor for the other, making it unnecessary to synthesize a competitor RNA sequence.

Both relative and competitive RT-PCR quantitation techniques require pilot experiments. In the case of relative RT-PCR, pilot experiments include selection of a quantitation method and determination of the exponential range of amplification for each mRNA under study. For competitive RT-PCR, a synthetic RNA competitor transcript must be synthesized and used in pilot experiments to determine the appropriate range for the standard curve. Comparative RT-PCR yields similar sensitivity as relative and competitive RT-PCR, but requires significantly less optimization and does not require synthesis of a competitor.

(1) Relative RT-PCR

Relative RT-PCR uses primers for an internal control that are multiplexed in the same RT-PCR reaction with the gene specific primers. Internal control and gene-specific primers must be compatible — that is, they must not produce additional bands or hybridize to each other. The expression of the internal control should be constant across all samples being analyzed. Then the signal from the internal control can be used to normalize sample data to account for tube-to-tube differences caused by variable RNA quality or RT efficiency, inaccurate quantitation or pipetting. Common internal controls include ß-actin, GAPDH mRNAs and 18S rRNA, etc. Unlike Northern blot and nuclease protection assays, where an internal control probe is simply added to the experiment, the use of internal controls in relative RT-PCR requires substantial optimization.

For relative RT-PCR data to be meaningful, the PCR reaction must be terminated when the products from both the internal control and the gene of interest are detectable and are being amplified within exponential phase. Because internal control RNAs are typically constitutively expressed housekeeping genes of high abundance, their amplification surpasses exponential phase with very few PCR cycles. It is therefore difficult to identify compatible exponential phase conditions where the PCR product from a rare message is detectable. Detecting a rare message while staying in exponential range with an abundant message can be achieved several ways: (A) by increasing the sensitivity of product detection; (B) by decreasing the amount of input template in the RT or PCR reactions; (C) by decreasing the number of PCR cycles.

As an internal control 18S rRNA shows less variance in expression across treatment conditions than ß-actin and GAPDH. However, because of the abundance of 18S rRNA in cells, it is difficult to detect the PCR product for rare messages in the exponential phase of amplification of 18S rRNA.

The biochemical company Ambion's patented Competimer™ Technology solves this problem by attenuating the 18S rRNA signal even to the level of rare messages. Attenuation results from the use of competimers — primers identical in sequence to the functional 18S rRNA primers but that are blocked at their 3' end and cannot be extended by PCR. Competimers and primers are mixed at various ratios to reduce the amount of PCR product generated from 18S rRNA. Ambion's QuantumRNA 18S Internal Standards contain 18S rRNA primers and competimers designed to amplify 18S rRNA in all eukaryotes. The Universal 18S Internal Standards function across the broadest range of organisms including plants, animals and many protozoa. The Classic I and Classic II 18S Internal Standards by Ambion can be used with any vertebrate RNA sample. All 18S Internal Standards work well in multiplex RT-PCR. These kits also include control RNA and an Instruction Manual detailing the series of experiments needed to make relative RT-PCR data significant. For those researchers who have validated ß-actin as an appropriate internal control for their system, the QuantumRNA ß-actin Internal Standards are available.

(2) Competitive RT-PCR

Competitive RT-PCR precisely quantitates a message by comparing RT-PCR product signal intensity to a concentration curve generated by a synthetic competitor RNA sequence. The competitor RNA transcript is designed for amplification by the same primers and with the same efficiency as the endogenous target. The competitor produces a different-sized product so that it can be distinguished from the endogenous target product by gel analysis. The competitor is carefully quantitated and titrated into replicate RNA samples. Pilot experiments are used to find the range of competitor concentration where the experimental signal is most similar. Finally, the mass of product in the experimental samples is compared to the curve to determine the amount of a specific RNA present in the sample. Some protocols use DNA competitors or random sequences for competitive RT-PCR. These competitors do not effectively control for variations in the RT reaction or for the amplification efficiency of the specific experimental sequence, as do RNA competitors.

(3) Comparative RT-PCR

While exquisitely sensitive, both relative and competitive methods of qRT-PCR have drawbacks. Relative RT-PCR requires extensive optimization to ensure that the PCR is terminated when both the gene of interest and an internal control are in the exponential phase of amplification. Competitive RT-PCR requires that an exogenous competitor be synthesized for each target to be analyzed. However, comparative RT-PCR achieves the same level of sensitivity as these standard methods of qRT-PCR, with significantly less optimization. Target mRNAs from 2 samples are assayed simultaneously, each serving as a competitor for the other, making it possible to compare the relative abundance of target between samples. Comparative RT-PCR is ideal for analyzing target genes discovered by screening methods such as array analysis and differential display.

3. Brief Description for the RT-PCR Procedure (Protocol online, 2006)

1) mRNA or total RNA is copied to cDNA by reverse transcriptase using an oligo dT primer (random oligomers may also be used). In RT-PCR, it usually uses a reverse transcriptase that has an endo H activity. This removes the mRNA allowing the second strand of DNA to be formed. A PCR mix is then set up which includes a heat-stable polymerase (such as Taq polymerase), specific primers for the gene of interest, deoxynucleotides and a suitable buffer.
2) cDNA is denatured at more than 90^{o}C (~94^{o}C) so that the two strands separate. The sample is cooled to 50^{o}C to 60^{o}C and specific primers are annealed that are complementary to a site on each strand. The primers sites may be up to 600 bases apart but are often about 100 bases apart, especially when RT-PCR is used.
3) The temperature is raised to 72^{o}C and the heat-stable Taq DNA polymerase extends the DNA from the primers. Now we have four cDNA strands (from the original two). These are denatured again at approximately 94^{o}C.
4) Again, the primers are annealed at a suitable temperature (normally between 50^{o}C and 60^{o}C).
5) Taq DNA polymerase binds and extends from the primer to the end of the cDNA strand. There are now eight cDNA strands
6) Again, the strands are denatured by raising the temperature to 94^{o}C and then the primers are annealed at 60^{o}C.
7) The temperature is raised and the polymerase copies the eight strands to sixteen strands.
8) The strands are denatured and primers are annealed.
9) The fourth cycle results in 32 strands.
10) Another round doubles the number of single stands to 64. Of the 32 double stranded cDNA molecules at this stage, 75% are the same size, that is the size of the distance between the two primers. The number of cDNA molecules of this size doubles at each round of synthesis (logarithmically) while the strands of larger size only increase arithmetically and are soon a

small proportion of the total number of molecules.

After 30 to 40 rounds of synthesis of cDNA, the reaction products are usually analyzed by agarose gel electrophoresis. The gel is stained with EB. This type of agarose gel-based analysis of cDNA products of reverse transcriptase-PCR does not allow accurate quantitation since EB is rather insensitive and when a band is detectable, the logarithmic stage of amplification is over. EB is a dye that binds to double stranded DNA by interpolation (intercalation) between the base pairs. Here it fluoresces when irradiated in the UV part of the spectrum. However, the fluorescence is not very bright. Other dyes such as SYBR green and TaqMan Gene Expression Assays that are much more fluorescent than EB are used in RT-PCR.

SYBR green is a dye that binds to double stranded DNA but not to single-stranded DNA and is frequently used in RT-PCR reactions. When it is bound to double stranded DNA it fluoresces more brightly than EB. Other methods such as TaqMan Gene Expression Assays can be used to detect the product during RT-PCR.

A gene that is to be used as a loading control (or internal standard) should have various features:

1) The standard gene should have the same copy number in all cells
2) It should be expressed in all cells
3) A medium copy number is advantageous since the correction should be more accurate

However, the perfect standard does not exist; therefore whatever we decide to use as a standard or standards should be validated for your tissue. If possible, we should be able to show that it does not change significantly in expression when your cells or tissues are subjected to the experimental variables you plan to use.

Commonly used standards are:

1) Glyceraldehyde-3-phosphate dehydrogenase mRNA
2) Beta actin mRNA
3) MHC I (major histocompatibility complex I) mRNA
4) Cyclophilin mRNA
5) mRNAs for certain ribosomal proteins e.g. RPLP0 (ribosomal protein, large, P0). This is also known as 36B4, P0, L10E, RPPO, PRLP0, 60S acidic ribosomal protein P0, ribosomal protein L10, Arbp or acidic ribosomal phosphoprotein P0.
6) 28S or 18S rRNAs (ribosomal RNAs)

4. Time Required for RT-PCR

Normally the sample preparation (cell/tissue obtained and RNA isolation, etc) is the most time cost for the research and the time cost is different for the different experiments. The following table gives the minimum time required for RT-PCR experiment (Table 1).

Table 1. Time required for RT-PCR

1	cDNA synthesis: 2 hours
2	RT-PCR: 2 hours
3	Dissociation curve analysis: 0.5 hour
Total Time	4.5 hours

5. Quantitation of RT-PCR Results

Normally, either of the two methods can be used to quantify RT-PCR results: the standard curve method and the comparative threshold method.

1) Standard Curve Method

In this method, a standard curve is constructed from an RNA of known concentration. This curve is then used as a reference standard for extrapolating quantitative information for target mRNA. Though RNA standards can be used, their stability can be a source of variability in the final analyses. In addition, using RNA standards would involve the construction of cDNA plasmids that have to be in vitro transcribed into the RNA standards and accurately quantitated, a time-consuming process. However, the use of absolutely quantitated RNA standards will help generate absolute copy number data.

In addition to RNA, other nucleic acid samples can be used to construct the standard curve, including purified plasmid dsDNA, in vitro generated ssDNA or any cDNA sample expressing the target gene. Spectrophotometric measurements at 260 nm can be used to assess the concentration of these DNAs, which can be converted to a copy number value based on the molecular weight of the sample used. cDNA plasmids are the preferred standards for standard curve quantitation. However, since cDNA plasmids will not control for variations in the efficiency of the reverse transcription step, this method will only yield information on relative changes in mRNA expression. However, this can be corrected by normalization to a housekeeping gene.

2) Comparative C_T Method

As described earlier, C_T is the cycle threshold. Another quantitation method for RT-PCR described

here is the comparative C_T method. The comparative C_T method involves comparing the C_T values of the samples with a control (or calibrator) such as a non-treated sample or RNA from normal tissue. The comparative C_T values of both the calibrator and the samples are normalized to an appropriate endogenous housekeeping gene.

Comparative C_T method is also known as the $2^{-\Delta\Delta CT}$ method, where $\Delta\Delta C_T=\Delta C_{T,sample}-\Delta C_{T,reference}$ Here, $\Delta C_{T,sample}$ is the C_T value for any sample normalized to the endogenous housekeeping gene and $\Delta C_{T,\ reference}$ is the C_T value for the calibrator also normalized to the endogenous housekeeping gene.

For the $\Delta\Delta C_T$ calculation to be valid, the amplification efficiencies of the target and the endogenous reference must be approximately equal. This can be established by looking at how ΔC_T varies with template dilution. If the plot of cDNA dilution versus ΔC_T is close to zero, it implies that the efficiences of the target and housekeeping genes are similar. If a housekeeping gene cannot be found whose amplification efficiency is similar to the target, then the standard curve method should be used.

6. RT-PCR Equipments and Reagents

1) Instruments for RT-PCR

RT-PCR requires an instrumentation platform that consists of a thermal cycler, a computer, optics for fluorescence excitation and emission collection, and software for data acquisition and analysis. These machines, available from several manufacturers, differ in sample capacity (some are 96-well standard format, some process fewer samples or require specialized glass capillary tubes), method of excitation (some use lasers, some broad spectrum light sources with tunable filters), and overall sensitivity. There are also platform-specific differences in how the software processes data. An RT-PCR machine cost about US$20,000 to US$150,000.

Now there are many RT-PCR equipments available and the parts do not match each other. As an example, the company of Applied Biosystems (Foster City, California, USA) has the following RT-PCR instruments:

(1) 96-Well & 0.2 ml Tube Instruments:

A. 7000 Real-Time PCR System: The ABI Prism® 7000 Sequence Detection System effective March 31, 2006. After this date, the ABI Prism® 7000 Sequence Detection System will no longer be available for sale. We have decided to discontinue the sale of this product because of the introduction of newer and more affordable technology.

B. 7300 Real-Time PCR System: The Applied Biosystems 7300 Real-Time PCR System is an integrated platform for the detection and quantification of nucleic acid sequences. Applied Biosystems supplies a Dell™ Notebook with the 7300 System. The current price is US$34,900.00.

(2) 7500 Real-Time PCR System: The Applied Biosystems 7500 Real-Time PCR System is a leading edge system with features to support labs requiring more capabilities from their real time PCR platform. The current price is US$42,500.00.

(3) 96-Well Instruments

7500 Fast Real-Time PCR System: The Applied Biosystems 7500 Fast Real-Time PCR System enables high speed thermal cycling in a 96-well format, reducing the run times to less than 40 minutes and accelerating your research by providing access to results more quickly than ever before. The current price is US$49,900.00.

(4) 96- & 384-Well Instruments

7900HT Fast Real-Time PCR System: The Applied Biosystems 7900HT Fast Real-Time PCR System is the only real-time quantitative PCR system that combines 96- and 384-well plate compatibility and the TaqMan® Low Density Array with fully automated robotic loading-and now also offers optional Fast real-time PCR capability. The current price is US$131,500.00.

Summarily, the current prices of the RT-PCR instruments by Applied Biosystems are as the following:

The Applied Biosystems 7300 Real-Time PCR System	US$ 34,900.00
The Applied Biosystems 7500 Real-Time PCR System	US$ 42,500.00
The Applied Biosystems 7500 Fast Real-Time PCR System	US$ 49,900.00
7900HT Fast Real-Time PCR System	US$ 131,500.00

(Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404, USA. Telephones: 650-638-5800, 1800-327-3002;

Fax: 650-638-5884, Email: NA_ActSrvInfo@appliedbiosystems.com, Website: http://www.appliedbiosystems.com).

2) Reagents and Tools for RT-PCR

Table 2 gives the basic reagents and equipments needed for RT-PCR of SYBR method (Table 2).

Table 2. Reagents and equipments needed for RT-PCR (SYBR method)

1	Oligonucleotide Primers.
2	Mouse total liver RNA (Stratagene).
3	Mouse total RNA master panel (BD Biosciences / Clontech).
4	SYBR Green PCR master mix, 200 reactions (Applied Biosystems).
5	Optical tube and cap strips (Applied Biosystems).
6	SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen).
7	25 bp DNA ladder (Invitrogen).
8	ABI Prism 7000 Sequence Detection System (Applied Biosystems).
9	ABI Prism 7000 SDS software (Applied Biosystems).
10	3% ReadyAgarose Precast Gel (Bio-Rad).
11	Agarose gel electrophoresis apparatus (Bio-Rad).

Table 3 gives the basic reagents and equipments needed for RT-PCR of TaqMan method (Table 3).

Table 3. Reagents and equipments needed for RT-PCR (TaqMan method)

1	Isolated RNA (RTIzol can be used for the RNA isolation)
2	Primer 1 (sense primer)
3	Primer 2 (antisense primer)
4	Probe with dye
5	RT-PCR enzymatic kit (one step or two step)
6	Reaction plates
7	RT-PCT instrument (e.g. ABI Prism 7000 Sequence Detection System, Applied Biosystems)

Ambion's MessageSensor™ RT Kit includes an RNase H+ MMLV RT that clearly outperforms MMLV RT enzymes that have abolished RNase H activity in real-time RT-PCR experiments. Unlike many other qRT-PCR kits, MessageSensor includes a total RNA control, a control human GAPDH primer set, RNase inhibitor, and nucleotides, as well as a buffer additive that enables detection with SYBR® Green dye.

The Cells-to-cDNA™ II Kit produces cDNA from cultured mammalian cells in less than 2 hours. No RNA isolation is required. This kit is ideal for those who want to perform reverse transcription reactions on small numbers of cells, numerous cell samples, or for scientists who are unfamiliar with RNA isolation. Ambion's Cells-to-cDNA II Kit contains a novel Cell Lysis Buffer that inactivates endogenous RNases without compromising downstream enzymatic reactions. After inactivation of RNases, the cell lysate can be directly added to a cDNA synthesis reaction. Cells-to-cDNA II is compatible with both one-step and two-step real-time RT-PCR protocols.

Genomic DNA contamination can lead to false positive RT-PCR results. Ambion offers a variety of tools for eliminating genomic DNA contamination from RNA samples prior to RT-PCR. Ambion's DNA-free™ DNase Treatment and Removal Reagents are designed for removing contaminating DNA from RNA samples and for the removal of DNase after treatment without Proteinase K treatment and organic extraction. In addition, Ambion has also developed TURBO™ DNase, a hyperactive enzyme engineered from wild-type bovine DNase. The proficiency of TURBO DNase in binding very low concentrations of DNA means that the enzyme is particularly effective in removing trace quantities of DNA contamination.

Ambion now also offers an economical alternative to the high cost of PCR reagents for the ABI 7700 and other 0.2 ml tube-based real-time instruments. SuperTaq™ Real-Time performs as well or better than the more expensive alternatives, and includes dNTPs and a Reaction Buffer optimized for SYBR Green, TaqMan, and Molecular

7. Detailed Procedure of RT-PCR

In a RT-PCR reaction, a fluorescent reporter molecule is used to monitor the PCR as it progresses.

The fluorescence emitted by the reporter molecule manifolds as the PCR product accumulates with each cycle of amplification. Based on the molecule used for the detection, RT-PCR techniques can be categorically placed under two heads: Non- Specific detection using DNA binding dyes and specific detection target specific probes (PREMIER Biosoft International., 2006).

1) Non-specific detection using DNA binding dyes

In RT-PCR, DNA binding dyes are used as fluorescent reporters to monitor the PCR reaction. The fluorescence of the reporter dye increases as the product accumulates with each successive cycle of amplification. By recording the amount of fluorescence emission at each cycle, it is possible to monitor the PCR reaction during exponential phase. If a graph is drawn between the log of the starting amount of template and the corresponding increase the fluorescence of the reporter dye fluorescence during real-time PCR, a linear relationship is observed.

SYBR® Green is the most widely used double-strand DNA-specific dye reported for RT-PCR. SYBR® Green binds to the minor groove of the DNA double helix. In the solution, the unbound dye exhibits very little fluorescence. This fluorescence is substantially enhanced when the dye is bound to double strand DNA. SYBR® Green remains stable under PCR conditions and the optical filter of the thermocycler can be affixed to harmonize the excitation and emission wavelengths. Ethidium bromide can also be used for detection but its carcinogenic nature renders its use restrictive.

Although these double-stranded DNA-binding dyes provide the simplest and cheapest option for RT-PCR, the principal drawback to intercalation based detection of PCR product accumulation is that both specific and nonspecific products generate signal.

2) Specific detection using Target specific probes

Specific detection of RT-PCR is done with some oligonucleotide probes labeled with both a reporter fluorescent dye and a quencher dye. Probes based on different chemistries are available for real-time detection, these include:

A. Molecular Beacons
B. TaqMan® Probes
C. FRET Hybridization Probes
D. Scorpion® Primers

3) Reverse Transcription Procedure

Reverse transcription is the process by which RNA is used as a template to synthesize cDNA. Among the first options to consider when selecting a method to perform the reverse transcription is whether to use a one-step real-time or a two-step real-time method. Now, many companies are offering reverse transcription reagent kit. The kit is classified into two types: one-step and two-step real–time methods. As an example, Applied Biosystems offers the one-step reagent and two-step reagent of reverse transcription reagent kit.

A. **One-step reagent:** Requires single reaction mix because real-time and PCR occur in the same tube. It may get better limit of detection with rare transcripts and requires sequence-specific primer for cDNA synthesis. 2 µl of RNA solution was analyzed with a random reverse-transcriptase (RT)–PCR assay.

   a. The Superscript II platinum Taq polymerase one-step RT-PCR kit by Invitrogen contains 10 µl of buffer concentrate, 2 mM of magnesium sulphate, 0.8 µl of enzyme mixture, and 1.9 µM of each of two primers (20 µl total volume).
   b. TaqMan® One-Step RT-PCR Master Mix Reagents of Applied Biosystems (AB) is another easy-to-use kit that contains all components needed for one-step RT-PCR applications and the kit employs AmpliTaq Gold® DNA polymerase for enhanced performance (Table 4).
   c. TaqMan® EZ RT-PCR Core Reagents combine rT*th* DNA polymerase with the fluorogenic 5' nuclease assay in a one-step reverse transcription-PCR (RT-PCR) format. This format is ideally suited to high sample throughput and provides the additional benefit of high temperature reverse transcription, which can prove beneficial when amplifying targets in regions of RNA with abundant secondary structure (Table 5).
   d. The TaqMan® Gold RT-PCR kit provides the individual components needed to perform reverse transcription-PCR (RT-PCR) of RNA to cDNA using the 5' nuclease assay. You can use this kit to perform both one-step and two-step RT-PCR (Table 6).

B. **Two-step reagent:** Requires two reaction mixes (real-time reaction and PCR reaction) and cDNA can be store for later use. Using random primer, it can simultaneously reversely stranscribe all mRNA as well as 18s rRNA (targets+endogenous controls), and it can use

sequence-specific primer, random primer or oligo $d(T)_{16}$ for cDNA synthesis.

Table 4. TaqMan® One-Step RT-PCR Master Mix Reagents of Applied Biosystems

Product Name	Part Number	Quantity / Package	Price (US$)
TaqMan® One-Step RT-PCR Master Mix Reagents Kit	4309169	200 reactions	590.00
10-Pack, TaqMan® One-Step RT-PCR Master Mix Reagents Kit	4313803	2000 reactions	5420.00

Table 5. TaqMan® EZ RT-PCR Core Reagents of Applied Biosystems

Product Name	Part Number	Quantity / Package	Price (US$)
TaqMan® EZ RT-PCR Core Reagents without Controls	N8080236	200 reactions	755.00
10-Pack, TaqMan® EZ RT-PCR Core Reagents	403028	2000 reactions	6900.00

Table 6. TaqMan® Gold RT-PCR kit of Applied Biosystems

Product Name	Part Number	Quantity / Package	Price (US$)
TaqMan® Gold RT-PCR Reagents without Controls	N8080232	200 reactions	755.00
10-Pack, TaqMan® Gold RT-PCR Reagents without controls	4304133	2000 reactions	6900.00

Reverse transcription is carried out with the SuperScript First-Strand Synthesis System for RT-PCR. The following procedure is based on Invitrogen's protocol. In addition, Bio-Rad (USA) and other companies also have complete kit for RT-PCR.

A. Prepare RNA/primer mixture in each tube (Table 7).

B. Incubate the samples at 65°C for 5 min and then on ice for at least 1 min.

C. Prepare reaction master mixture for each reaction (Table 8)

D. Add the reaction mixture to the RNA/primer mixture, mix briefly, and then place at room temperature for 2 min.

E. Add 1 μl (50 units) of SuperScript II RT to each tube, mix and incubate at 25°C for 10 min.

F. Incubate the tubes at 42°C for 50 min, heat inactivate at 70°C for 15 min, and then chill on ice.

G. Add 1 μl RNase H and incubate at 37°C for 20 min.

H. Store the 1st strand cDNA at -20°C until use for RT-PCR.

Table 7. RNA/primer mixture

Total RNA	5 μg
Random hexamers (50 ng/μl)	3 μl
10 mM dNTP mix	1 μl
DEPC H_2O	to 10 μl

Table 8. Reaction master mixture

10x RT buffer	2 μl
25 mM $MgCl_2$	4 μl
0.1 M DTT	2 μl
RNAaseOUT	1 μl

4) RT-PCR Procedure

A. Normalize the primer concentrations and mix gene-specific forward and reverse primer pair. Each primer (forward or reverse) concentration in the mixture is 5 pmol/μl.

B. Set up the experiment and the following PCR program on ABI Prism SDS 7000. Do not click on the dissociation protocol if it needs to check the PCR result by agarose gel. Save a copy of the setup file and delete all PCR cycles (used for later dissociation curve analysis).

   a. 50°C 2 min, 1 cycle
   b. 95°C 10 min, 1 cycle
   c. 95°C 15 s -> 60 °C 30 s -> 72 °C 30 s, 40 cycles
   d. 72°C 10 min, 1 cycle

C. An RT-PCR reaction mixture can be either 50 μl or 25 μl (Table 9).
D. After PCR is finished, remove the tubes from the machine. The PCR specificity is examined by 3% agarose gel using 5 μl from each reaction.
E. Put the tubes back in SDS 7000 and perform dissociation curve analysis with the saved copy of the setup file.
F. Analyze the RT-PCR result with the SDS 7000 software. Check to see if there is any bimodal dissociation curve or abnormal amplification plot.

Table 9. RT-PCR reaction mixture of 50 μl or 25 μl

25 μl SYBR Green Mix (2x) 0.5 μl tissue cDNA 2 μl primer pair mix (5 pmol/μl each primer) 22.5 μl H_2O	Or	12.5 μl SYBR Green Mix (2x) 0.25 μl tissue cDNA 1 μl primer pair mix (5 pmol/μl each primer) 11.3 μl H_2O

7. Applications of RT-PCR

Fro the name we can see that RT-PCR is a technique to detect the progress of a PCR reaction in real time. At the same time, a relatively small amount of PCR product (DNA, cDNA or RNA) can be quantified. RT-PCR is based on the detection of the fluorescence produced by a reporter molecule which increases, as the reaction proceeds. This occurs due to the accumulation of the PCR product with each cycle of amplification. These fluorescent reporter molecules include dyes that bind to the double-stranded DNA (i.e. SYBR® Green) or sequence specific probes (i.e. Molecular Beacons or TaqMan® Probes). RT-PCR facilitates the monitoring of the reaction as it progresses. It can start with minimal amounts of nucleic acid and quantify the end product accurately. Moreover, there is no need for the post PCR processing which saves the resources and the time. These advantages of the fluorescence based QPCR technique have completely revolutionized the approach to PCR-based quantification of DNA and RNA. Real-time assays are now easy to perform, have high sensitivity, more specificity, and provide scope for automation.

There are numerous potential applications for RT-PCR. Normally we want to know how the genetic expression of a particular gene changes over time, such as during germination, or in response to changes in environmental conditions. RT-PCR has been used to detect changes in gene expression in a tissue in response to an administered pharmacological agent and is thus an important technique in drug discovery and testing. In recent years, RT-PCR has been slightly superseded by DNA microarray technology, which allows the expression of many genes to be quantified in a cell sample instead of just one. However, a standard RT-PCR experiment is still cheaper and easier to set up than an average microarray, and so remains an important tool in molecular biology labs (Ma, 2005).

One reason that makes reverse transcriptase-PCR non-quantitative is that EB is a rather insensitive stain. Methods such as competitive PCR are developed to make the method more quantitative but they are very cumbersome and time-consuming to perform. Thus, RT-PCR (or reverse transcriptase RT-PCR) was developed.

RT-PCR has simplified and accelerated PCR laboratory procedures and has increased information obtained from specimens including routine quantification and differentiation of amplification products. Clinical diagnostic applications and uses of real-time PCR are growing exponentially, real-time PCR is rapidly replacing traditional PCR, and new diagnostic uses likely will emerge (Kaltenboeck, 2005).

RT-PCR can be used for quantitative or qualitative evaluation of PCR products and is ideally suited for analysis of nucleotide sequence variations (point mutations) and gene dosage changes (locus deletions or insertions/duplications) that cause human monogenic diseases. Real-time PCR offers a means for more rapid and potentially higher throughput assays, without compromising accuracy and has several advantages over end-point PCR analysis, including the elimination of post-PCR processing steps and a wide dynamic range of detection with a high degree of sensitivity. This review will focus on real-time PCR protocols that are suitable for genotyping monogenic diseases with particular emphasis on applications to prenatal diagnosis, non-invasive prenatal diagnosis and preimplantation genetic diagnosis (Traeger-Synodinos, 2006). According to Wortmann et al study, real-time PCR offers rapid (within hours) identification of Leishmania to the

complex level and provides a useful molecular tool to assist both epidemiologists and clinicians **(**Wortmann, 2005**).**

Reverse transcription PCR is also a basic technique for molecular biological research and it ahs a widely usage in the life sciences. The exponential amplification of complementary sequence of mRNA or RNA sequences via reverse transcription PCR allow for a high sensitivity detection technique, where low copy number or less abundant RNA molecules can be detected. It is also used to clone mRNA sequences in the form of complementary DNA, allowing libraries of cDNA (cDNA libraries) to be created which contain all the mRNA sequences of genes expressed in a cell. Furthermore, it allows the creation of cDNA constructs which were cloned by reverse transcription PCR and allow the expression of genes at the RNA and protein levels for further study.

Combined with Western blot, ELISA and microarray methods, RT-PCT will be very benefit on the gene expression studies (Ma, 2006).

Summarily, the applications of RT-PCR include **(**PREMIER Biosoft International, 2006**):**

1. Quantitative gene expression studies (mRNA synthesis) (qPCR).
2. DNA copy number measurements in genomic or viral DNAs.
3. Allelic discrimination assays or SNP genotyping.
4. Microarray result verifications. .
5. Drug designs.
5. Drug therapy efficacy exploring.
6. DNA damage measurements.

8. Troubleshooting

1) **Little or no PCR product**. Poor quality of PCR templates, primers, or reagents may lead to PCR failures. First, please include appropriate PCR controls to eliminate these possibilities. Some genes are expressed transiently or only in certain tissues. In our experience, this is the most likely cause for negative PCR results. Please read literature for the gene expression patterns. One caveat is that microarrays are not always reliable at measuring gene expressions. After switching to the appropriate templates, we obtained positive PCR results in contrast to the otherwise negative PCRs.

2) **Poor PCR amplification efficiency.** The accuracy of real-time PCR is highly dependent on PCR efficiency. A reasonable efficiency should be at least 80%. Poor primer quality is the leading cause for poor PCR efficiency. In this case, the PCR amplification curve usually reaches plateau early and the final fluorescence intensity is significantly lower than that of most other PCRs. This problem may be solved with re-synthesized primers.

3) **Primer dimer.** Primer dimer may be occasionally observed if the gene expression level is very low. If this is the case, increasing the template amount may help eliminate the primer dimer formation. Carefully designed primers will help to limited this problem that requires: limited length of primer in 18 to 24 bps; 50 to 60 % overall GC content; limit structures of G or G's longer than 3 bases; no Gs on the 5' end; limited self binding structure and self pine formation.

4) **Multiple bands on gel or multiple peaks in the melting curve.** Agarose gel electrophoresis or melting curve analysis may not always reliably measure PCR specificity. From our experience, bimodal melting curves are sometimes observed for long amplicons (> 200 bp) even when the PCRs are specific. The observed heterogeneity in melting temperature is due to internal sequence inhomogeneity (e.g. independently melting blocks of high and low GC content) rather than non-specific amplicon. On the other hand, for short amplicons (< 150 bp) very weak (and fussy) bands migrating ahead of the major specific bands are sometimes observed on agarose gel. These weak bands are super-structured or single-stranded version of the specific amplicons in equilibrium state and therefore should be considered specific. Although gel electrophoresis or melting curve analysis alone may not be 100% reliable, the combination of both can always reveal PCR specificity in our experience.

5) **Non-specific amplicons**. Non-specific amplicons, identified by both gel electrophoresis and melting curve analysis, give misleading real-time PCR result. To avoid this problem, please make sure to perform hot-start PCR and use at least 60°C annealing temperature. We noticed not all hot-start Taq polymerases are equally efficient at suppressing polymerase activity during sample setup. The SYBR Green PCR master mix described here always gives us satisfactory results. If the non-specific amplicon is

persistent, you have to choose a different primer pair for the gene of interest (PrimerBank, 2006).

9. Biography of Kary Banks Mullis

Lastly, in the following it gives the PCR inventor Kary Banks Mullis' biography:

1) 1944, December 28: Kary Banks Mullis (male) was born on in Lenoir North, North Carolina, USA.
2) 1966: received a bachelor degree in chemistry from the Georgia Institute of Technology, USA.
3) 1972: obtained his Ph.D. in biochemistry from the Berkeley of University of California, USA.
4) 1972 - 1973: Taught biochemistry at Berkeley Berkeley of University of California, USA.
5) 1973 – 1977: postdoctoral fellow in pediatric cardiology (in the areas of angiotensin and pulmonary vascular physiology) at the University of Kansas Medical School, USA.
6) 1977 - 1979: postdoctoral fellow in pharmaceutical chemistry at the University of California, San Francisco, USA. **1979 - 1985, Cetus Corp. in Emeryville, California, USA, on the DNA chemistry study. During this period he conducted research on oligonucleotide synthesis and invented PCR.**
7) 1986: director of molecular biology at Xytronyx, Inc. in San Diego of California of USA, on DNA technology and photochemistry.
8) 1987: began consulting on nucleic acid chemistry for more than a dozen corporations, including Angenics, Cytometrics, Eastman Kodak, Abbott Labs, Milligen/Biosearch and Specialty Laboratories.
9) 1990: awarded the William Allan Memorial Award of the American Society of Human Genetics, USA.
10) 1990: awarded the Award of Preis Biochemische Analytik of the German Society of Clinical Chemistry and Boehringer Mannheim, German.
11) 1991: awarded the National Biotechnology Award, USA.
12) 1991: awarded the Gairdner Award, Toronto, Canada.
13) 1991: awarded the R&D Scientist of the Year, USA.
14) 1992: awarded California Scientist of the Year Award, USA.
15) **1993: obtained a Nobel Prize in chemistry for his invention of PCR.**
16) 1993: obtained the Japan Prize for the PCR invention, Japan. It is one of international science's most prestigious awards.
17) 1993: obtained Thomas A. Edison Award, USA.
18) 1994: obtained the honorary degree of Doctor of Science from the University of South Carolina, USA.
19) 1998: inducted into the National Inventors Hall of Fame, USA.
20) Mullis has several major patents, such as the PCR technology and UV-sensitive plastic that changes color in response to light. His most recent patent application covers a revolutionary approach for instantly mobilizing the immune system to neutralize invading pathogens and toxins, leading to the formation of his latest venture, Altermune LLC.
21) His many publications include "The Cosmological Significance of Time Reversal" (Nature), "The Unusual Origin of the Polymerase Chain Reaction" (Scientific American), "Primer-directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase" (Science), and "Specific Synthesis of DNA In Vitro via a Polymerase Catalyzed Chain Reaction" (Methods in Enzymology). His autobiography, "Dancing Naked in the Mind Field," was published by Pantheon Books in 1998.
22) He is currently a Distinguished Researcher at Children's Hospital and Research Institute in Oakland, California. He serves on the board of scientific advisors of several companies, provides expert advice in legal matters involving DNA, and is a frequent lecturer at college campuses, corporations and academic meetings around the world. He is living with his wife, Nancy Cosgrove Mullis, in Newport Beach, California, USA and in Anderson Valley, California, USA.
23) Besides the invention of PCR and his biochemistry studies, Dr. Mullis also did a lot of thought in the subjects that cross the scientific principles to parapsychology and global social problems, which we can read from his book "Dancing Naked in the Mind Field" (Figure 6) (Mullis, 1998).
24) Dr. Kary Banks Mullis' website is: http://www.karymullis.com.

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References

1. Abdul-Careem MF, Hunter BD, Nagy E, Read LR, Sanei B, Spencer JL, Sharif S. Development of a real-time PCR assay using SYBR Green chemistry for monitoring Marek's disease virus genome load in feather tips. J Virol Methods. 2006;133(1):34-40.
2. Amazon. Dancing Naked in the Mind Field. http://www.amazon.com/gp/product/0679774009/qid=1018646044/sr=1-1/ref=sr_1_1/002-1565632-1875245?n=283155. 2006.
3. Dharmaraj S. RT-PCR: the basics. Ambion, Inc. http://www.ambion.com/techlib/basics/rtpcr/index.html. 2006.
4. Fergus Greer. http://www.karymullis.com/. 2006.
5. Higuchi R, Fockler C, Dollinger G, Watson R. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Biotechnology (NY). 1993;11(9):1026-30.
6. Kaltenboeck B, Wang C. Advances in real-time PCR: application to clinical laboratory diagnostics. Adv Clin Chem 2005;40:219-59.
7. Ma H, Chen G. Stem cell. J Am Sci 2005;1(2):90-2.
8. Ma H. ELISA Technique. Nat Sci. 2006;4(2):36-7.
9. Ma H. Western Blotting Method. J Am Sci 2006a;2(2):23-7.
10. Mullis KB. Autobiography of Kary B. Mullis. Pantheon Books, New York, 1998. http://nobelprize.org/nobel_prizes/chemistry/laureates/1993/mullis-autobio.html.
11. Mullis KB. Dancing Naked in the Mind Field. ISBN: 0679774009. Pantheon Books, New York, USA. 1998:1-240.
12. Mullis KB. The unusual origin of the polymerase chain reaction. Sci Am 1990;262(4):56-61, 64-5.
13. PREMIER Biosoft International. http://www.premierbiosoft.com/tech_notes/real_time_PCR.html. 2006.
14. PrimerBank. http://pga.mgh.harvard.edu/primerbank/PCR_protocol.html. 2006.
15. Protocol online. http://www.protocol-online.org/prot/Molecular_Biology/PCR/Real-Time_PCR/. 2006.
16. Traeger-Synodinos J. Real-time PCR for prenatal and preimplantation genetic diagnosis of monogenic diseases. Mol Aspects Med 2006;27(2-3):176-91.
17. U'Ren JM, Van Ert MN, Schupp JM, Easterday WR, Simonson TS, Okinaka RT, Pearson T, Keim P. Use of a real-time PCR TaqMan assay for rapid identification and differentiation of Burkholderia pseudomallei and Burkholderia mallei. J Clin Microbiol 2005;43(11):5771-4.
18. Wang X. http://biowww.net/browse-56.html. 2003.
19. Wortmann G, Hochberg L, Houng HH, Sweeney C, Zapor M, Aronson N, Weina P, Ockenhouse CF. Rapid identification of Leishmania complexes by a real-time PCR assay. Am J Trop Med Hyg 2005;73(6):999-1004.

Multiple Antibiotic Resistant Index and Plasmid of *Escherichia coli* in Beef in Ekpoma

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ABSTRACT: *Escherichia coli (E. coli),* a member of the family Enterobacteriacease, has been known to cause infection in man and animals. Sixty samples of beef were collected randomly from Ekpoma market, in Edo State. *E. coli* were isolated from 40/60 (66.7%) of samples. All isolates were sensitive to ciprofloxacin, 78% were resistant to tetracycline, 73% resistant to cefuroxime, 43% resistant to cotrimoxazole, 35% to nalidixic acid. All isolates were 100% resistant to chloramphenicol and ampicillin. Nine different resistant patterns were observed. Multiple antibiotics resistance was observed among isolates. Seven of the nine resistant patterns observed were screened for plasmid, it was observed that they haboured one or more plasmid that was of sizes 23.13 kb and 4.361 kb. This study point to the fact that farmers should exercise caution in the use of antibiotics in farms. [The Journal of American Science. 2006;2(3):16-18].

Keywords: Multiple Antibiotic Resistant Index; infection; plasmid

INTRODUCTION

Meat is defined as the flesh of animals, which are suitable for use as food (Forest *et al*., 1975). Meat also include the parts of livestock muscles that is skeletal or is found in the tongue diaphragm, heart, oesophagus with or without the accompanying of over laying fat and the portion of the bone, skin sinew, nerves and blood tissue that normally accompany the muscle tissue and are not separated from it in the process of dressing (Billy, 1996).

The quality of meat is affected by the physiology of the animal and handling of animal before slaughtering. The environmental conditions also play a vital role in determining the appearance of fresh meat (Ikeme, 1990). The main source of spoilage bacteria with which fresh meat become contaminated is the hide of cattle or the skin of hogs. It has been shown that cattle hide and hogs, skin may contain millions of bacteria (anaerobes and aerobes) per square centimeter of surface in the area where the stick knife is inserted.

Contamination caused by hides, skin and intestinal tract of animals get into meat during bleeding, handling, and processing of animal meat (like slaughtering, scalding, eviscerating and washing). Meat is an ideal culture medium for many organisms because it is high in moisture and rich in vitamin and nitrogen compound. Some organisms involved in meat contamination are *Acetobacter species, Acinetobacter species , Citrobacter species, Proteus species, Salmonella species, Escherichia species, Shigella species, Staphylococcus species,* and *Streptococcus species* (Alonge, 1982).

The wide spread of antibiotic by farmers have resulted in increasing antibiotic resistance. The aims of this paper are to determine the prevalence of multi-drug resistant *Escherichia coli (E. coli)* isolates from raw beef and to determine the plasmid profile of the isolates.

MATERIALS AND METHODS

Sixty beef samples were randomly purchased from different sites in Ekpoma market, Ekpoma, Edo State. Specimen comprises of stomach, liver, heart muscles and lungs. They were transported in sterile cellophane to the laboratory.

One gram of the meat were weighed and suspended in 9 ml of sterile nutrient broth. 10-fold dilution was made. 0.1 ml of each dilution was plated on Nutrient agar and MacConkey agar and incubated aerobically at 37^0C for 24 h. *E. coli* was identified with conventional biochemical tests (Forbes *et al*, 1998).

Susceptibility of the organisms to antibiotics were tested by the disk diffusion method on Brain Heart Infusion medium according to Bauer *et al* (1966). Antibiotics used include Nitrofurantion, Cefuroxime, Norfloxacin, Cotrimozaxole, Gentamycin, Tetracycline,

Ciprofloxacin, Nalidxic acid, Chloramphenicol and Ampicillin.

The method of Birboin and Doly (1979) was used to screen for plasmid. The DNA was electrophoresed on 0.8% agarose gel, stained with ethidium bromide, visualized by UV trans-illumination. Molecular weights were calculated based on molecular weight standard.

RESULTS

Out of the sixty samples from different parts of beef, *E coli* were isolated from 40 samples (66.7%). Fifteen from stomach, eight from muscle and liver, five from heart and four from lungs (Table 1).

All isolates were resistant to ampicillin and chloramphenicol, 90% resistance to nitrofurantion, 78% resistance to tetracycline, 73% to cefuroxime, 43% resistance to cotrimozole, 35% resistance to nalidixic acid, 33% resistance to norfloxacin, 28% resistance to gentamycin, while all isolates were sensitive to ciprofloxacin (Table 2).

Multiple resistances were observed all through the study. Nine different resistance patterns were observed (Table 3).

These different resistance patterns isolated were screened for plasmid, they were found to harbour one or more plasmid of sizes 23.1 and 4.36 kb (Table 4).

Table 1. Isolation of *E. coli* from various beef samples

Meat	Number examined	Number positive
Muscles	12	8+ve
Liver	14	8+ve
Heart	10	5+ve
Lungs	07	4+ve
Stomach	17	15+ve

Table 2. Percentage Antibiotics Resistance of *E. coli* isolates from beef

Antibiotics	No. of Resistant Strain	Percentage Resistance
Nitrofurantion	36	90
Cefuroxime	29	73
Norfloxacin	13	33
Cotrimozaxole	17	43
Gentamycin	11	28
Tetracycline	31	78
Ciprofloxacin	0	0
Nalidixic	14	35
Chloramphenicol	40	100
Ampicillin	40	100

Table 3. Antibiotic Resistance Patterns of *E. coli* from beef

Pattern of Resistance	Frequency of Occurrence	Percentage
Cf,NB,Gn,Te,Na,C,Am	4	10
N,Cf,Co,Gn,Te,C,Am	4	10
N,Cf,Na,C,Am	4	10
N,Te,C,Am	2	5
N,Cf,Te,C,Am	8	20
N,Cf,NB,Co,Gn,Te,C,Am	3	7.5
N,Cf,C,Am	5	12.5
N,Cf,NB,Co,Te,Na,C,Am	6	15
N,Co,Te,C,Am	4	10

Key: Cf = Cefuroxime, NB = Norfloxacin, Gn = Gentamycin, Te = Tetracycline, Na = Nalidixic acid, C = Chloramphenicol, Am = Ampicillin, N = Nitrofurantion

Table 4. Plasmid containing *E.coli* isolates and their Molecular Weight in Kilobase

Resistance Pattern	Number of Plasmid	Plasmid size (kb)
N,Cf,NB,Co,Gn,Te,C,Am	1	23.1
Cf,NB,Gn,Te,Na,C,Am	1	23.1
N,Cf,C,Am	2	23.1, 4.36
N,Co,Te,C,Am	1	4.36
N,Cf,NB,Co,Te,N,C,Am	2	23.1, 4.36
N,Cf,Co,Gn,Te,C,Am	1	23.1
N,Cf,Te,C,Am	1	23.1

DISCUSSION

The use of antibiotic in food animal product has been controversial from the standpoint of health workers and food consumers. Although the drugs afford the food animal industry the ability to generate affordable products, the resulting increases in antibiotics resistance bacteria associated with their use is both well documented and problematic (Langlios and Dawson, 1999).

From this study, it was observed that *E. coli* isolates were resistant to commonly used antibiotics in clinical medicine. Multiple antibiotic resistance was observed in this study. There has been increasing concern of the possible development of resistance to antimicrobial agents in the *Enterbacteriaceae*, especially *E. coli*, as a result of the use of such agent in animal feed (Willis, 2000). This resistance is quite high and it could be as a result of the widespread use of such agent in animal feeds in Nigeria. It could also be from drinking water. Calomiris *et al*. (1984) isolated multi resistance bacteria from drinking water. Another possible source of the resistance isolates may be from the hands, clothing of butcher/sellers and buyers; and knives or water used in washing the beef.

Plasmid profile analysis of the isolates revealed that most of the strains that were resistant to 4 antibiotics harboured plasmid.

The plasmid isolated were 23.1 kb and 4.36 kb. This was similar to what was observed by Smith *et al*. (2003). They isolated 3 plasmids from cow, which were 23.13, 4.361 and 0.564 kb.

Beef is commonly consumed in Nigeria and from this study; it would be observed that eating beef that is not properly cooked could be a source of multi drug resistant bacteria.

The isolates were highly resistant to commonly used antibiotic. Farmers should be advice on the dangers of using antibiotic in feeds. Since multi drug resistance can be transferred from animals to human and also taking into account the limited choice of antimicrobial agents for treatment and the possibility of transfer of resistance to other enteric organism.

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REFERENCES

1. Alonge, O.O. (1982). Bacteria causing beef spoilage in a meat shop in Ibadan. *Nig. J. Microbiol. 22*:168-172.
2. Bauer, A.W.; Kirby, W.M.; Sherris, J.C. and Turck, M. (1966). Antibiotic susceptility testing by a standard single disc method. *Am. J. Clin. Pathol. 45*:493-496.
3. Billy TJ (1996). What is meat?
3. http/www.fsis.usda.gov/OPPDE/FSISDirective/FSISDir7/60-2.
4. Birnboim, H.C. and Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acid Res. 7:* 1513- 1523.
5. Calomiris, J.; Armstrong, L. and Seidler, J. (1994). Association of metal tolerance with multiple antibiotics resistance of bacteria isolated from drinking water. *Appl. Environ. Microbiol. 47:*1238- 1242.
6. Forbes. B.A. (1998). Enterobacteriaceae. Bail and Scott's Diagnostic Microbiology, Baltimore, Mosby.Pp. 509-526.
7. Forest, J.C.; Aberle, E.D.; Hedrick, B.B.; Judge, M.D. and Markel, R.A. (1995). *Principle of Meat Science*. W.H. Freeman and Co. San Francisco. Pp.500.
8. Ikeme, M. (1990). *Meat Science and Technology.* African Feb Publishers ltd. Pp. 370.
9. Langlois B.E. and Dawson, K.A. (1999). Antimicrobial resistance of Gram-negative enteric bacteria from pigs in a non-antimicrobial exposed herb before and after transportation. *J. Food Protect. 62*: 797- 799.
10. Smith, S.; Aboaba, O.O.; Odeigha, P.; Shodipo, K.; Adeyeye, T.A.; Ibrahim, A.; Adebiyi, T.; Onibokun, H. and Odunukwe, N.N. (2003). Plasmid profile of *Escherichia coli* 0157:H7 from apparently healthy animals. *Afr. J. Biotechnol. 2 (9):* 322- 324.
11. Willis, C. (2000). Antibiotics in the food chain: their impact on the consumer. *Rev. Med. Microbiol. 11:* 153-160.

New Mycotic Infection Associated with Mortalities in Small Mouthed Salamander (*Ambystoma texanum*)

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Abstract: The recent global decline in amphibian population is a mysterious environmental puzzle. While some declines are undoubtedly because of habitat obliteration, others are clearly linked to diseases. Two emerging diseases are blamed to the increasing reports of mortalities among wild amphibians, Chytridiomycosis and Ranavirus infection. Since first report of both diseases during the last decades, there have been an increasing number of observations of the two diseases in amphibian populations, due partly the increased worldwide interest in amphibians as indicators of declining ecosystem health. Nonetheless, the decline due to mortalities associated with additional and as yet unreported pathogens of amphibian have not been considered. Here, we report a new cutaneous Zygomycosis infection caused by *Mucor* sp. associated with mortalities in an endangered salamander species in Michigan. The infection was observed in terrestrial individuals at the start of the breeding season. Affected salamanders showed sluggish escape reflex which allowed easily catching of the infected individuals. Clinical examination revealed scaly and nodular appearance of the skin of the posterior half, especially in the tail region. Mycotic examination resulted in isolation Mucor sp from deep layer of the epidermis of at the affected site. Histopathological examination is currently performed to detect the pathological changes associated with fungus infection. This is considered the first report of zygomycosis in a salamander in the world. [The Journal of American Science. 2006;2(3):19-22].

Keywords: mycotic infection; mortalities; salamander (*Ambystoma texanum*)

1. Introduction

The continuing worldwide decline in amphibian population is an enigmatic environmental puzzle (Blaustein *et al*., 1990; Daszak *et al*., 1999). While some declines are undoubtedly the result of habitat obliteration, others are not clearly linked to environmental changes. A number of etiological factors may contribute individually or in synergy with these declines. The declines include disappearance and presumed extinction of some amphibian species in certain parts of the world (Richards *et al*., 1993; Mahony, 1996; Pounds *et al*., 1997; Lips, 1998; Williams and Hero, 1998; Lips, 1999). Some of population declines occurred in ecologically pristine areas, such as forest reservations, where anthropogenic impact is thought to be negligible. These declines along with recent findings of amphibian species mass mortalities in these areas suggest that the extinctions are not normal population fluctuations. Recently, there are increasing reports of mortalities caused by newly emerging infectious diseases among wild animals, including amphibians, (Daszak *et al.*, 2000; Pounds *et al.*, 2006). Studies during the last decade have found two emerging infectious diseases accountable for amphibian mass mortalities in different geographical areas. Chytridiomycosis caused by zoospore forming fungal pathogens has been found in more than 90 amphibian species, including Salamanders, in North America, and worldwide, since its first report in 1998, (Berger *et al.*, 1998; Daszak *et al.*, 2003; Hopkins and Channing, 2003; Berger *et al.*, 2004). Ranavirus infection is another emerging disease associated with amphibian mortalities. This disease has been occasionally reported to cause up to 100% mortality and affect both amphibian adults and larval stages (Green *et al.*, 2002; Jancovich *et al.*, 1997; Berger *et al.*, 2000; Greer *et al.*, 2005). Since the first report of both diseases, an increasing number of observations of the two diseases in amphibian populations, due partly the increased worldwide interest in amphibians as indicators of declining ecosystem health. Nonetheless, the population decline caused by unreported pathogens of amphibian should be also considered.

In the present study, cutaneous Zygomycosis infection caused by *Mucor* sp. associated with mortalities is reported for the first time in a threatened species of salamander in Michigan, the small mouthed salamander (*Ambystoma texanum*, Ambystomatidae:

Caudata). The fungus was isolated from salamanders mortality during a mortality event during the breeding season in spring 2005. Results she light on a new infection associated with mortalities among a threatened species of salamander in the wild.

2. Materials and Methods

2.1 Animals

Four *A. texanum* salamanders were collected during a species' breeding period visual survey in southern Michigan. These surveys were part of a comprehensive program to locate breeding populations of the species. Four salamanders with small skin lesions were found under rotting wood, and leaves in terrestrial habitat, within approximately 50 m of a breeding pond. All animals were 100 m or less apart and were located at N41^{o}42'19.5", W84^{o}40'18.7". No other *A. texanum* were found at this site. The search was conducted during the early afternoon and the temperature was 2^{o}C. Species identification was made visually and thus consideration is needed of the potential for confusion with hybrid members of the *Ambystoma jeffersonianum gynogenetic* complex. The single infected male is highly likely *A. texanum* because triploid hybrid males are infrequent (0.3% - 0.03%, Clanton, 1934; Uzzell, 1964; Morris and Brandon, 1984; Lowcock et al., 1991; J. Ball, pers. comm.). Infected females may have been *A. texanum* hybrids (potentially with *Ambystoma laterale*) and electrophoretic species determination was not attempted.

2.2 Clinical examination

The animals were placed into ventilated plastic containers and brought alive to the lab. The salamanders were euthanized with a large dose of MS222 (tricaine methane sulfonate, Finquel- Argent Chemical Laboratories, Washington), followed by clinical examination and recording of all internal and external abnormalities.

2.3 Parasitic examination

Skin scrapings and wet mount preparation were performed from anaesthetized salamanders to examine external parasites. Intestinal scrapping, compression smears from liver and smear were done to examined internal parasites.

2.4 Bacterial examination

Skin scrapings and 1mm pieces of skin tissues were collected from anaesthetized salamanders using sterile, disposable scalpel blades and examined unstained with a light microscope or preserved in sterile whirl Pak bags in the freezer. Deep skin samples for bacteriology were collected after disinfection of the skin lesions using 70% ethanol and streaked onto Trypticase soy agar (TSA) and Hsu agar media for primary isolation. Bacterial isolates were further investigated by Gram stain and the use of API 20E system (BioMerieux, Charbonnier les Bains, France).

2.5 Mycotic examination

The scrapings were collected for mycology where placed on 10% KOH for microscopic analysis. Biopsies from the affected skin were taken from deep layer of the epidermis and cultured onto 2% Sabouraud dextrose (SDA) agar plates. Inoculated plates were then incubated at room temperature for 5 days.

2.6 Viral examination

Presence of viruses was investigated in the collected samples. Frozen tissue samples of liver, kidney, muscle, skin and gonad, were stored at -20°C until the processing. The stored samples were investigated by isolation of viral particles from the homogenate using a Biomaster Stomacher-80 (Wolf Laboratories Limited) at the high speed setting for 120 seconds. The homogenate tissues were then allowed to settled on ice for 15 minutes, passed through a 0.45-mm filter, followed by dilution to produce 10^{-2} and 10^{-3} samples. The diluted supernatant were then used to inoculate two cell lines: FHM (fathead minnow: Gravell and Malsburger, 1965) and CHSE-214 (chinook salmon embryo: Lannan *et al.*, 1984). The inoculated cells were then incubated at 20°C and 16°C for FHM and CHSE respectively and examined for the appearance of cytopathic effect (CPE) for two passages of at least 14 days each.

3. Results

3.1 Clinical examination

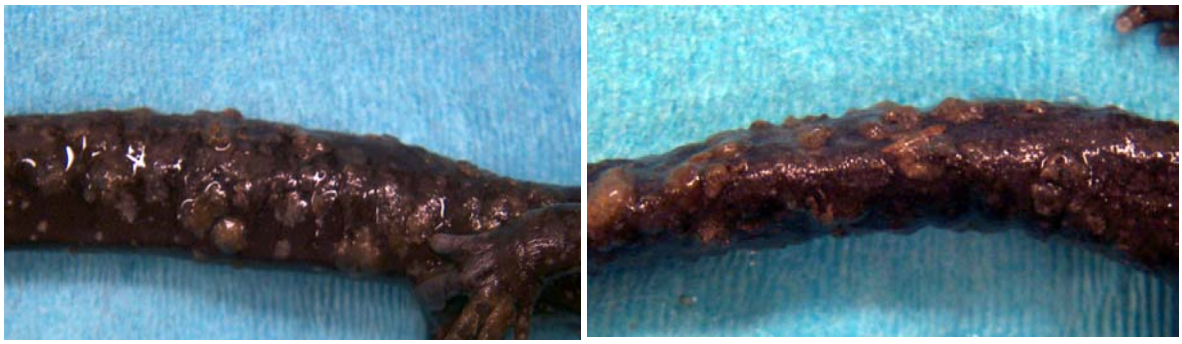
Clinical examination of the four animals with skin lesions revealed decrease response to stimuli, lethargic and sluggish movements. The animals were easily caught and showed minimal escape reflex. Consistent gross lesions were observed on the skin of all four sick salamanders. The lesions appeared as areas of abnormal thickening and scaly-looking epidermis with lost normal pigmentation limited to posterior half of the animal and the tail region (Figure 1). While the rest of salamander body appeared normal with normal pigmentation.

3.2 Parasitic examination

No external parasite was detected in wet mount preparation of skin scrapping. Examining intestinal scrapping, and smears from liver and spleen failed to identify any internal parasites of cysts.

3.3 Bacterial examination

Bacterial isolation from skin lesion on TSA yielded two dominant colony types. Gram stain of the bacteria indicated the resulted bacteria are Gram negative; however biochemical and identification are underway to identify the species of bacteria isolated.



A. Lateral view of salamander's tail affected by roughness and scale like appearance.

B. Dorsal view of salamander's tail affected by roughness and scale like appearance.

Figure 1. Tail of salamander affected with mucor infection. Note the rough and scaly appearance of the tail in both lateral and dorsal appearance

3.4. Mycotic examination

Skin scrapping failed to show fungal hyphae. However, culture on SDA consistently showed the isolation of the same fungal organism from all four salamander skin samples. The colonies were characterized by the relatively rapid growth of aerial cottony-like mycelia that covered the whole plate in about five days. At maturation, the colonies showed the presence of small dark structures, later identified as sporangia containing sporangiospores. Microscopically, hyaline coenocytic ribbon-type hyphae developing globose sporangia were the main characteristics of the isolate. Some of the observed globose sporangia containing sporangiospores possessed spherical columella, but lacked apophysis (a support-like structure below the collarette at the base of the sporangia). Rhizoids or zygospores were not produced by this strain. On the basis of the macroscopic and microscopic features this isolate was identified as *Mucor* sp.

3.5. Viral examination

No CPE were detected on FHM or CHSE cell lines after two passages of at least 14 days each.

4. Discussion:

Clinical examination of the four animals with skin lesions revealed reduced escape reflexes, lethargic and sluggish movements. It is well documented that the ability of amphibian to survive in wild and their fitness are greatly affected by infection and surrounded environmental stressors. For example; infection with iridovirus associated with environmental stress was reported to associated with fitness reduction by altering life-history traits in the long-toed salamander *macrodactylum*) (Forson and Storfer, 2006). Likewise, infection with chytrid fungus *Batrachochytrium dendrobatidis* influenced the proportion of frogs that were recaptured (Retallick *et al.*, 2004).

The inability to detect fungus hyphae in wet mount preparation most probably due to the fact that the hyphae were confined within pockets or enclosures scattered within the epidermis of affected animals. The colonies were characterized by the relatively rapid growth of aerial cottony-like mycelia that covered the whole plate in about five days. At maturation, the colonies showed the presence of small dark structures, later identified as sporangia containing sporangiospores. Microscopically, hyaline coenocytic ribbon-type hyphae developing globose sporangia were the main characteristics of the isolate. Some of the observed globose sporangia containing sporangiospores possessed spherical columella, but lacked apophysis (a support-like structure below the collarette at the base of the sporangia). Rhizoids or zygospores were not produced by this strain. On the basis of the macroscopic and microscopic features this isolate was identified as *Mucor* sp. The primarily role of this fungal pathogen in these salamanders is not clear. *Mucor* spp. is well known for its low virulence and for causing disease only in severely immunocompromised humans and animals (Ribes, 2000). This could suggest that the investigated salamanders might have an underlying condition.

Bacteriological examination for the lesions isolated a two species of bacteria from affected salamanders, yet to be identified. These bacterial infections most likely are secondary invaders under conditions associated with breeding season of salamander and post hibernation stresses. It is well documented that some secondary invaders as *A. hydrophila* infection increased during the breeding season in amphibians due to reduced immune ability after hibernation and high stress associated with breeding (Forbes et al 2004). Also, other bacteria as *Flavobacterium* spp. are known to be common aquatic flora and associated with stress-related infection in aquatic animals (Starliper et al 1998; Delaney et al 2001; Madsen et al 2005).

Although this study provides the first report of a new fungus infection in salamander associated with mortalities, the results did not provide conclusive evidence for the origin of fungal infection. Moreover, the associated infection with bacteria and/or sequences of infections with fungus and bacteria need further elucidation. Further studies are needed to investigate the prevalence of infection among salamander populations in Michigan, source of infection, and fungus pathogenesis in infected animals.

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5. References

[1] Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragani M, Hyatt A, McDonald K, Hines H, Lipsi K, Marantelli G, Parkes H. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. Proceedings of the National Academy of Sciences USA 1998; 95:903-906.

[2] Berger L, Speare R, Hines H, Marantelli G, Hyatt AD, McDonald KR, Skerratt LF, Olsen V, Clarke JM, Gillespie G, Mahony M, Sheppard N, Williams C, Tyler M. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Australian Veterinary Journal 2004; 82:31-36.

[3] Berger L, Speare R, Hyatt AD. Chytrid fungi and amphibian declines: Overview, implications and future directions. In: Campbell A, ed. Declines and disappearances of Australian frogs. Environmental Australia, Canberra, Australia: Environmental Australia 2000:21-31.

[4] Blaustein, AR, Wake DB. Declining amphibian populations: A global phenomenon. Trends in Ecology and Evolutions 1990;5:203-204.

[5] Clanton, W. An unusual situation in the salamander *Ambystoma jeffersonianum* (Green). Occasional Papers of the Museum of Zoology, University of Michigan 1934; 290:1-15.

[6] Daszak P, Cunningham AA, Hyatt AD. Emerging infectious diseases of wildlife- threats to biodiversity and human health. Science 2000; 287:443-449.

[7] Daszak P, Cunningham AA. Extinction by infection. Trends in Ecology and Evolution 1999; 14:279.

[8] Daszak P, Cunningham AA, Hyatt AD. Infectious disease and amphibian population declines. Diversity and Distribution 2003;9:141-150.

[9] Delaney MA, Brady YJ, Beam DR, Arana E, Worley SD. A N-halamine disinfectant for the treatment of columnaris *Flavobacterium columnare* on channel catfish *Ictalurus punctatus*. Aquaculture 2001; Book of Abstracts. 176 p.

[10] Forbes MR, McRuer DL, Rutherford PL. Prevalence of *Aeromonas hydrophila* in relation to timing and duration of breeding in three species of ranid frogs. Ecoscience 2004;11:282-285.

[11] Forson D, Storfer A. Effects of Atrazine and iridovirus infection on life history traits of the long-toes salamander (*Ambystoma macrodactylum*) Environmental Toxicology and Chemistry 2006; 25:168–173.

[12] Gravell M, Malsburger RG. (1965) A permanent cell line from the fathead minnow (*Pimephales promelas*). Annals of the New York Academy of Sciences 1965; 126:555–565.

[13] Green DE, Converse KA, Schrader AK. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996-2001. Annals of the New York Academy of Sciences 2002; 969:323-339.

[14] Greer AL, Berrill M, Paul J. Wilson PJ. Five amphibian mortality events associated with ranavirus infection in south central Ontario, Canada Diseases of Aquatic Organisms 2005;67:9-14.

[15] Hopkins S, Channing A. Chytrid fungus in northern and western cape frog populations, South Africa. Herpetology Review 2003; 34:334-336.

[16] Jancovich JK, Davidson EW, Morado JF, Jacobs BL, Collins JP. Isolation of lethal viruse from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. Diseases of Aquatic Organisms 1997; 31:161-167.

[17] Lannan CN, Winton JR, Fryer JL. Fish cell lines: establishment and characterization of nine cell lines from salmonids. *In Vitro* 1984; 20:671–676.

[18] Lips KR. Decline of a tropical montane amphibian fauna. Conservation Biology 1998; 12:106-117.

[19] Lips KR. Mass mortality and population declines of anurans at an upland site in western Panama. Conservation Biology 1999; 13:117- 125.

[20] Lowcock LA, Griffith H, Murphy RW. The *Ambystoma laterale-jeffersonianum* complex in central Ontario: ploidy structure, sexration. and breeding dynamics in a bisexual-unisexual community. Copeia 1991; 1991:87-105.

[21] Madsen L, Moeller JD, Dalsgaard I. *Flavobacterium psychrophilum* in rainbow trout, *Oncorhynchus mykiss* (Walbaum), hatcheries: studies on broodstock, eggs, fry and environment. Journal of Fish Diseases 2005; 28:39-47.

[22] Mahony M. The decline of the green and golden bell frog *Litoria aurea* viewed in the context of declines and disappearances of other Australian frogs. Australian Zoologist 1996;30:237-247.

[23] Morris MA, Brandon, RA. Gynogenesis and hybridization between Ambystoma platineum and *Ambystoma texanum* in Illinois. Copeia 1984:324-337.

[24] Pounds JA, Bustamante MR, Coloma LA, Consuegra JA, Fogden MPL, Foster PN, La Marca E, Masters KL, Merino-Viteri A, Puschendorf R, Ron SR, Sa´nchez-Azofeifa GA, Still CJ, YounG BE. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 2006; 439:161-167.

[25] Pounds JA, Fogden MP, Savage JM, Gorman GC. Tests of null models for amphibian declines on a tropical mountain. Conservation Biology 1997; 11:1307-1322.

[26] Retallick RW, McCallum RH, Speare R. Endemic infection of the amphibian chytrid fungus in a frog community postdecline. PLoS Biology 2004; 2:e351

[27] Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. Clinical Microbiology Reviews. 2000; 13:236-301.

[28] Richards SJ, Mcdonald KR, Alford RA. Declines in population of Australia's endemic tropical forest frogs. Pacific Conservation Biology 1993;1:66-77.

[29] Starliper CE, Villella R, Morrison P, Mathias J. Studies on the bacterial *flora* of native freshwater bivalves from the Ohio river. Biomedical Letters 1998; 58:85-95.

[30] Uzzell TM. Relations of the diploid and triploid species of the *Ambystoma jeffersonianum* complex (Amphibia, Caudata) Copeia,1964:257-299.

[31] William SE, Hero JM. Rainforest frogs of the Australian Wet Trpoica; Guild classification and the ecological similarity of declining species. Proceedings of the Royal Society of London. Series B 1998;265:597-602.

Analysis of Actinic Effect after Radiotherapy in the Uterine Cervix Carcinomas

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Abstract: The boarding more effective for the control of the cancer of the uterine cervix is the precocious diagnosis. However a great number of malignant tumors are identified in very advanced periods of training, reflecting the lack of access to the preventive examination. The control of the effectiveness of the treatment for radiation if makes with the colpocytological analysis, where it is possible to follow the patients, detecting residual or recurrent tumors, allowing in skillful time, the administration of therapies you add. This work had as objective: the identification of the frequency of examinations, with presence of epithelial cells presenting radiotherapy effect and the evaluation the evolution, through the colpocytological, of cases with uterine cervix cancer treated with radiation. For the election of the group of study of the present work, a survey of the cytopathological examinations of control was made radiotherapy, of the 270 patients, through the archive of the Division of Pathology/DIPAT of the National Institute of the Cancer, 112 patients had been excluded, for diverse reasons. The total of colpocytological carried through by the 158 patients, of this retrospective study, was of 307, varying of 2 the 6 examinations for each patient, in a space of 18 months. The diagnostic had been separate in five Groups. Group A: Cells with effect of actinic nature, negative for malignity. Group B: Negative for malignity, normal cytological examination. Group C: Atypical cells with radiotherapy effect, displasia after-irradiation?. Group D: Positive for malignity. Group E: Unsatisfactory material and/or no conclusive. Results: Group A - 68.73%, followed of Group C – 15.30%; Group B – 11.73%; Group E- 2.61% and finally Group D with 1.63%, which represents the positive cases. The evolution of the cases of control, after radiotherapy, showed that 81.25% had kept the diagnostic. It was observed that 12.50% had passed of suspicious to negatives; 6.25% had been passed by negative to suspected for and did not have none case that evolved of negatives/suspicious for positives. The last diagnostic had been considered as well as first too. On the basis of the analysis of the control diagnostic cytological after radiotherapy, was possible to conclude that the great majority (81.25%) had kept the result. In 86.07% of the cases, the result was of the Group A and the B (negative for malignity, with presence and absence of cells actinic effect, respectively). Our treatment got an excellent index of tumor reply, exactly being the prognostic directed only to the patients of the study. [The Journal of American Science. 2005;1(1):23-28].

Keywords: cytological; actinic; radiotherapy; uterine; cervix; carcinomas

Introduction

According to Health Ministry, in Brazil, enters the death causes, the cancer appears in according to place, representing a 15% tax. Analyzing the projection of the National Institute of the Cancer in 2003 it was observed that the uterine cervix cancer comes reaching as the place in the feminine population (graphic 01), however the Registers of Cancer of Population Base (RCPB), with consolidated information sample that the uterine cervix cancer was the tumor most incident in the cities of Belém and Goiânia and the second more incident tumor in Fortaleza, Campinas and Porto Alegre. 1,2,3,4,5

In the world-wide scale, malignant cervix tumors correspond to the second more frequent feminine neoplasia and the index of deaths it shows decreasing, due to precocious detention and the institutions that take care of adequately of the therapeutic processes. 6,7,8

Despite the knowledge each acquired bigger time through innumerable research in the whole world, the boarding more effective for the control of the uterine cervix cancer, is the precocious, possible diagnosis through the cytopathological examination - known popularly as test of Papanicolaou. This examination is painless, cheap and efficient, being perfectly possible to detect intra-epithelial injuries and to cure them before if becoming cancer. 5,9,10,11,12

The treatment for a cervical intra-epithelial lesions depends on some factors that include the fact, of the patient to desire to have children, age and general

health. A patient with lesions of high degree (HSIL, High grade squamous intra epithelial lesion/ LSIL, low grade squamous intra epithelial lesion) can not need additional treatment, especially if the abnormal area completely was removed during the biopsy, but it must carry through a test of regularly carried through Papanicolaou and a pelvic examination.

When the injury requires a treatment, the doctor will be able to use destructive therapies as the cryosurgery (freezing), cauterization (burning), or laser (surgery to destroy the abnormal area without damaging the fabric healthy adjacent). However, a great number of diagnosis is made when the tumors are in periods of training very advanced, reflecting the lack of access to the preventive examination, for difficulties in long-distance relation, ignorance or even though misinformation. The supervened average of the women diagnosed with uterine cervix tumors, classified as stadiums IIB (classification according to FIGO/*Fédération Internationale de Gynécologie et d'Obstétrique*) in ahead, for example, that they make the conventional treatment for pelvic radiotherapy, turns around 40%.

The control of the effectiveness of the treatment for radiation if makes with the colpocytological analysis, where it is possible to follow the patients, detecting residual or recurrent tumors, allowing in skillful time, the administration of therapies you add. the 8,13,14,15,16 Even so cellular alterations provoked by the action of the ionizing radiation well are told in literature and are of great relevance, in some countries of the world, our Country still have great necessity of more studies. The cytological evaluation of the actinic effect, deserves great attention and training, for account

This work had as objective: the identification of the frequency of examinations, with presence of epithelial cells presenting radiotherapy effects and the evaluation the evolution, through the colpocytology, of cases with uterine cervix cancer treated with radiation. of the importance in the therapeutic control of the patients after radiotherapy. 17,18,19,20,21,22,23

Materials and Methods

Characteristic of the Sample

In July of 2003, through the folder of archive, in the Service of Gynecology of the Hospital of Oncology (HC II/INCA), the patients with scaly uterine cervix carcinoma had been catalogued, directed to the radiotherapy, in the period of January the July of 2000, representing a total of 270 patients. All the cases had cytological examination confirmed by the biopsy, before the treatment.

In accordance with the registers, in the great majority of the directed patients, the tumors were local advanced; the clinical staging was carried through on the basis of the rules of the FIG (*Fédération Internationale de Gynécologie et d'Obstétrique*) is demonstrated (picture 01).

Election of the Patients

For the election of the group of study of the present work, a survey of the cytopathological examinations of control was made after radiotherapy, of the 270 patients, through the archive of the Division of Pathology/DIPAT of the National Institute of the Cancer. Later, it had the analysis of handbooks of the cases that had colpocytology of control, for attainment of excellent data.

By not possessing no cytological finding of control after radiotherapy 90 patients had been excluded, in the archives of INCA, 06 for not returning and 16 for arriving at the death. The total of colpocytology carried through for the 158 patients, of this retrospective study, was of 307, varying of 2 the 6 examinations for each patient, in a space of 18 months. The first colpocytology of control after radiotherapy, of each patient, after had an interval of 3 to 6 months the ending of the treatment.

Considering that the frequency of examinations, carried through after radiotherapy, varied of patient the patient; the concrete analysis was made through the result of last examination of control, what it reflects the situation of the evolution of the patients until November of 2003. The diagnostic had been separate in five Groups. Group A: Cells with effect of actinic nature, absence of malignity. Group B: Negative for malignity, normal cytological examination. Group C: Atypical cells with radiotherapy effect, displasia after-irradiation? (suggestive of return). Group D: Positive for malignity. Group E: Unsatisfactory material and/or inconclusive.

Results

The ages of the patients had varied of 35 to 88 years, with average of 59.5 years. Related to the colpocytology examinations which has been carried through, were observed that more of the half of the diagnostic they were of the Group A - 68.73%, followed of Group C – 15.30%; Group B – 11.73%; Group E - 2.61% and finally Group D with 1.63%, which represents the positive cases, as indicated in the Table 1.

In the Table 2 is demonstrated the result of the last colpocytology examination of control after radiotherapy of each group.

The evolution of the cases of control, after radiotherapy is represented in Table 3, where the percentage of each diagnosis is demonstrated, considering only first and the last diagnosis.

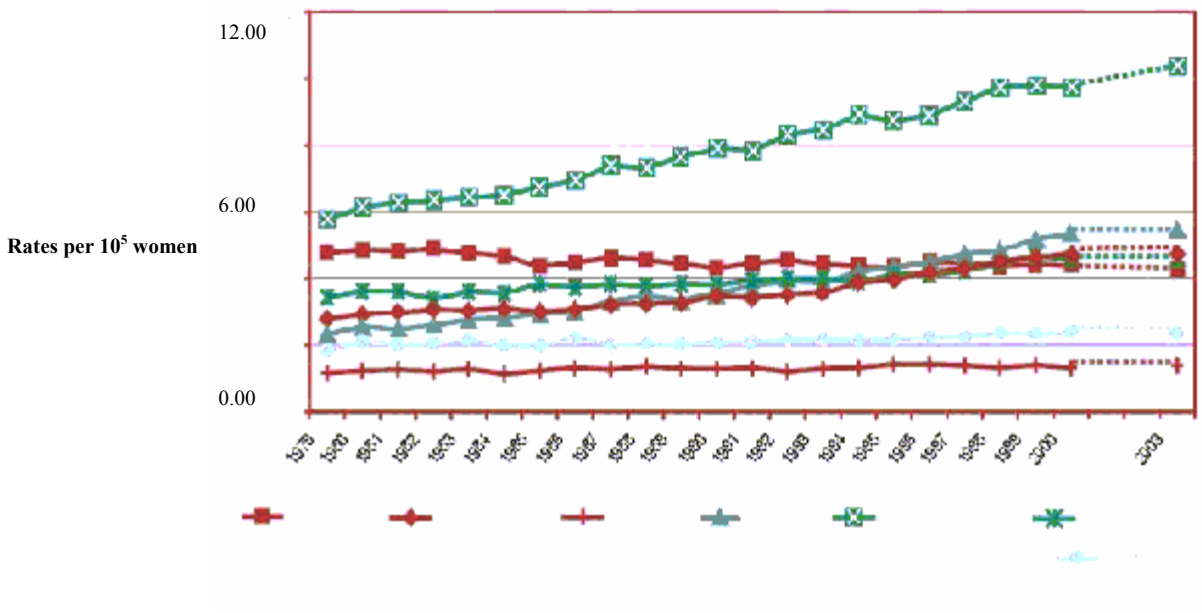

Figure 1. Rude taxes of mortality for 100,000 women for the tumor most frequent, Brazil, 1979-2000 and projection 2003. Source: Brazil, Health Ministry, National Institute of the Cancer. Estimate of the incidence and mortality by Cancer in Brazil.

Square 1. Clinical staging, according to FIGO by each patient

STAGES OF FIGO	NUMBER OF CASES	% OF THE CASES BY EACH CLINICAL STAGE
Stage I	---	---
IA	---	---
IA1	---	---
IA2	---	---
IB	19	7.04
IB1	---	---
IB2	03	1.11
Stage II	03	1.11
IIA	02	0.74
IIB	109	40.37
Stage III	01	0.37
IIIA	01	0,37
IIIB	124	45.93
Stage IV	01	0.37
IVA	06	2.22
IVB	01	0.37

Table 1. Diagnostics of each group of the whole colpocytological exams realized after radiotherapy.

Groups of the diagnostics	*Findings with each diagnostic*	*Frequency of the cases (%)*
Group A	211	68.73
Group B	36	11.73
Group C	47	15.30
Group D	05	1.63
Group E	08	2.61

Table 2 has shown the results of the last colpocytological exam of control after radiotherapy of each group.

Table 2. Diagnostics results of the last colpocytological exam of control after radiotherapy of each group.

Groups of the diagnostics	*Findings with each diagnostic*	*Frequency of the cases (%)*
Group A	118	74.68
Group B	16	11.39
Group C	18	10.13
Group D	03	1.90
Group E	03	1.90

The evolution of the cases of the control after radiotherapy is represented in the table 03 where is demonstrated the percentage of each group only considering the first and the last diagnostic.

Table 3. Evolution of the cases of the control after radiotherapy.

Evolution	*% of cases*
Keep the diagnostics	81.25
Suspects for negatives	12.50
Negatives for suspects	6.25
Negatives / suspects for positives	00

Discussion

The radiated cells show some cytochemistry alterations that can be due to enzyme release for the cytoplasmic destruction of organelles. The increase of the RNA synthesis occurs to nucleolus followed for the reduction of the synthesis of the DNA. Small doses of radiation can intermittently intervene with the production of messenger RNA e, therefore, to diminish the protein synthesis. Great doses inhibit the DNA synthesis and can take the irreversible damages in the nuclear DNA. The mitotic activity can be suppressed temporarily. Chromosomal and genetic abnormalities can be observed and occasionally resulting in the loss of the ability of dividing while the nuclear and cytoplasmic metabolism continues. 20,24,25

According to Shuheko et al, the cytology showed to be good method of control of uterine cervix cancer treated by radiotherapy, therefore in accordance with its studies, had not had cases false-positives and nor false negatives. 21,22

The pursuing through the colpocytology is important, mainly in the two first immediate years to therapeutic, due to the fact that 50% of the recurrences are detected in the first year of pursuing as well as 85% with two years. In five years of pursuing, 95% of the returns will have been detected. Some groups of patients can after benefit with a systematic pursuing the treatment. 9,10,21,22

Campos has observed that the identification of malignant cells to the end of the treatment indicates that the tumor was not sterilized, while that its absence always does not mean activity lack, therefore can be had carried through a local sterilization of the tumor while persists in lymphatic or parametric ganglia. More than 80% of the patients were into menopause, however 60% of the smears were observed little more than were atrophics. 26

In accordance with some studies, the return occurs precociously during the pursuing and about 85% of these patient ones they go the death in two years. Our findings show that before the pursuing 06% of the patients had arrived at the death. 27,28,29,30

According to Teixeira et al, only 24% of the patients who had had complete reply to the radiotherapy had come back to have tumor activity, mainly in the two first years of pursuing, evidencing that the therapeutic reply it could be used as parameter for the prognostic definition in advanced cases. 16

Conclusion

On the basis of the analysis of the control cytological-diagnostic after radiotherapy, was possible to conclude that the great majority (81.25%) had kept the result. In 86.07% of the cases, the result was of the Group A and the B (negative for malignity, with presence and absence of cells c/actinic effect, respectively).

The treatment got an excellent index of tumor reply, exactly being the prognostic directed only to the patients of the study. The analysis of the evolution, through the colpocytology disclosed a small index of cases with actinic effect (negative for malignity) that the return cases had evolved suspicious - displasia after-irradiation (6.25%), and accurately the double of this value (12.50%), they were suspected and they had become negative.

The result of the last examination of control disclosed an increase in relation to the total of examinations of the diagnostic of the Group A (5.95%), and a discrete reduction in group B (0.34%), which are negative, the first ones, with effect, actinic and as without evident actinic effect. It was a reduction of 5.17% in the diagnostic number of the Group C. The group D has shown a discrete reduction-0,27% and the diagnostic of the group E has presented an increase of 0.71%.

The accompaniment of the patients must be made through the colpocytology examination, approximately 6 weeks after the treatment for radiation, with semester harvests in the 3 first years and after that annual, in case that it does not have return suspicion, thus preventing, any possibility of unsatisfactory materials for the reading, beyond negative false results, since the cytological criteria, continue, for times, subjective.

It is of great relevance that the study continues, using others diagnostic techniques, as immunohistochemistry and cytogenetic, in the search of new markers that could differentiate the recidivates injuries of the actinic effect. Therefore the morphologic aspects are only insufficient, in some cases, to affirm a positive or negative diagnosis, without the necessity of the evolutionary accompaniment of the alterations, through annual the semester colpocytology.

This comparative study it would search the evaluation of the values predictive-positives and predictive-negative, being able to make possible the reduction of the cases false-positives and false-negative, therefore method in study is, for times, limited.

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REFERENCES

1. AMERICAN College of Obstetricians and Gynecologists. **Cervical cytology**: evaluation and management of

abnormalities. ACOG Techinical Bulletin. Washington: American College of Obstetricians and Gynecologists, 1984.
2. BERRINO, F., et al. Efficacy of screenig in preveting invasive cervical cancer: caso-control study in Milan, Italy. **IARC Sci Publ**, v. 76, p. 111-123, 1986.
3. BIBBO, M. **Comprehensive citopathology**. Philadelphia: W.B. Saunders Company, 1991.
4. BRASIL. Ministério da Saúde. DATASUS. **Sistema de informações sobre a mortalidade**: 1999. Disponível em:<http://www.datasus.gov.br>. A cesso em: no. 2003.
5. BRASIL. Ministério da Saúde. Instituto Nacional de Câncer. Disponível em: <http://www.inca.gov.br/estimativas/2003/index.asp?link=serieh istorica.asp.>. Acesso: jul. 2004.
6. BRASIL. Ministério da Saúde. Instituto Nacional de Câncer. **Controle do câncer**: uma proposta de integração ensino serviço. 3. ed. 1999.
7. BRASIL. Ministério da Saúde. Secretaria de Assistência a Saúde. Instituto Nacional de Câncer. **Viva mulher**: programa nacional de controle do câncer de colo de útero e mama. 2002.
8. BRINTON, L. A., et al. Epidemiology of cervical cancer by cell type. **Cancer Res**, v. 47, p. 1706-1911, 1982.
9. CAMPOS, J. R. C. Persistent tumor cells in the vaginal smears and prognosis of cancer of the radiated cervix. **Acta Cytol**, v. 14, p. 519, 1970.
10. CASTRO, S. V. **Anatomia Fundamental**. 3. ed. São Paulo: Makron books. 1985 .
11. DALALOYE, J. F., et al. Effect of total treatment time on event-free survival in carcinoma of the cervix. **Gynecol Oncol**, v. 60, p. 42-48, 1996.
12. DANGELO, J. G.; FATTINI, C. A. **Anatomia humana básica**. São Paulo: Ed. Atheneu, 1990.
13. FERLAY, J.; BRAY, F.; PISANI, P.; PARKIN, D. M. **Globocan 2000**: cancer incidence, mortality and prevalence worldwide [computer program]. Verssion 1.0. Lyon: Iarcpress, 2001. Disponível em: <http://www.dep.iarc.fr/globocan/globocan.htm>. Acesso em: nov. 2003.
14. GARDNER, E.; GRAY, D. J.; O'RAHILLY, R. **Anatomia**: estudo regional do corpo humano. 4. ed. Rio de Janeiro: Guanabara Koogan. 1985.
15. GIMENO, M. H. Quimioterapia experimental II: estudo de los efectos citomorfológicos de la radiacion y de la quimioterapia antitumoral sobre tejidos neoplasicos. **Rev. Esp. Oncologia**, v. 27, p. 393-403, 1980.
16. GOMPEL, C.; KOSS, L. G. **Citologia ginecológica e suas bases anatomoclínicas**. [s. l.]: Ed. Manole, 1997.
17. GRIGSBY, P. W.; PEREZ, C. A. Radiotherapy alone for medically inoperable carcinoma of the cervix: stage IA and carcinoma *in situ*. **Int J RadiatOncol Biol Phys**, v. 21, p. 375-378, 1991.
18. HARIMA, Y.; SHIRAHAMA, S.; HARIMA, K.; AOKI, S.; OHNISHI, T.; TANAKA, T.. Genetic alterations on chomossome 17p associated with response to radiotherapy in bulky cervical cancer. **British Journal of cancer,** v. 81, n. 1, p. 108-113, 1999.
19. LA VECCHIA, C., et al. Pap smears and the risk of cervical neoplasia: quantitative estimates from a case-control study. **Lancet**, v. 2, p. 779-782, 1984.
20. LUDGATE, S. M., et al. Synchrounus 5-fluorouracil, mitomycin C and radiation therapy in the tratament of locally advanced carcinoma of the cervix. **Int J Radiat Oncol Biol Phys**, v. 15, p. 893-899, 1988.
21. PERRZ, C. A.; KURMA, R. J.; STEHMAN, F. B.; THIGPEN, J. T. Uterine cervix. In: HOSKINS, W. J.; PEREZ, C. A.; YOUNG, R. C. (Eds). **Principles and practice of gynecologic oncology**. Philadelphia: JB Lippincott, 1992. p. 591-662.
22. VIÉGAS, Célia Maria Pais. **Químio e radioterapia em carcinoma localmente avançado de colo do útero**. 2002. Tese (Doutorado em Medicina)-Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2002.
23. JACYNTHO, C.; ALMEIDA FILHO, G.; MALDONADO, P. **HPV infecção genital feminina e masculina**. Rio de Janeiro: Revinter, 1994.
24. JIMENEZ AYALA, Matias; NAGALES ORTIZ, Francisco. **Citopatologia ginecológica**. Tradução: Alfredo Moraes, Silva Filho. [Madri]: Ed. Artes Médicas, 1978.
25. MARCIAL, V.A.; MARCIAL, L. V. Radiation therapy of cervical cancer: new develops. **Cance**r, v. 71, p. 1438-1445, 1993.
26. McKEE, G. T. **Citopatologia**. São Paulo: Artes Médicas, São Paulo, 1997.
27. MONTENEGRO, M. R; FRANCO, M. **Patologia processos gerais**. 4. ed. Rio de Janeiro: Atheneu, 1999.
28. SCHIFF, M. H., et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neolplasia. **J Natl Cancer Inst**, v. 85, p. 958-964, 1993.
29. SCHINEIDER, M. L.; SCHINEIDER, V. S. **Atlas de diagnóstico diferencial em citologia ginecológica**. Germany: Revinter, 1998.
30. SHUHEKO, Marina; LONGATTO FILHO, Adhemar; MAEDA, Marina Sakamoto; SHIH, Lai Wun Song; BORTOLAN, Jeni. Importância do "follow-up" de carcinomas do colo uterino irradiados: I. efeitos imediatos (até 12 meses). **Acta Oncológica Brasileira**, v. 9, p. 17-20, 1989.
31. SHUHEKO, Marina, LONGATTO FILHO, Adhemar; MAEDA, Marina Sakamoto; SHIH, Lai Wun Song; BORTOLAN, Jeni. Importância do "follow-up" de carcinomas do colo uterino irradiados: II. efeitos tardios (após 12 meses). **Acta Oncológica Brasileira**, v. 9, p. 45-50, 1989.
32. SILVA FILHO, A. M.; LONGATTO FILHO, Adhemar. **Colo uterino vagina**: processos inflamatórios, aspectos histológicos, citológicos e colposcópicos. Rio de Janeiro: Revinter, 2000.
33. TEIXEIRA, J. C.; MONTI, C. R., DECHAIN, S. F. M.; TEIXEIRA, L. C. Radiotherapy and prognostic factors evaluation in patients with squamous cell carcinoma of the uterine cérvix – stage IIIb. **Acta Oncológica Online**, [São Paulo], Hospital A. C. Camargo, v. 20, n. 1, jan./fev./mar. 2000 Disponível em: < http://www.hcanc.org.br/acta/2000/acta2k_3.html>. Acesso em: nov. 2003.
34. WACHTEL, E. The pronostic significance of the female genitale tract. **Acta Cytol**, v. 11, p. 35, 1967.

Selection of Breeding Materials with high Linoleic Acid and/or low Linolenic Acid Content in Soybean

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Abstract : In this research , one plant containing zero linolenic acid content was found with the "half - seed method" of capillary gas chromatography in the soybean strain 0358 - 5 -1-5. It was not reported in the world. The material will be very useful for breeding low linolenic acid content cultivated varieties and special for studying gene action for linolenic acid by the aids of biotechnology in soybean. [The Journal of American Science. 2006;2(3):29-32].

Keywords: Soybean; high linoleic acid; low linolenic acid; Lipoxygenase Null Mutants; half - seed method

1. Introduction

Soybean (Glycine max. L. Merrill) constitute the present world's third most important source of vegetable oil . They are widely adaptable and are grown under varied agroclimatic conditions. One of major goals for oilseed breeders to improve oil quality by selecting for higher linoleic acid and lower or no linolenic. It is well - known that linoleic acid is an essential fatty acid for human body and , therefore , any improvement in its content will be conducive to human health , whereas linolenic acid have has three double bonds sensitive to oxidation, thus resulting in its a unfavorable smell and taste.

Many people attach more importance to nutrition and the demand for good quality vegetable oil has kept increasing. In order to meet this demand , breeding of high yielding and good quality oilseed variety is very necessary.

In the experiment reported in this paper , a number of promising soybean lines were identified with the "half seed" method , which contain high linoleic acid and low/ or zero linolenic acid.

2. Materials and Methods

2.1 Plant materials. F4 self - pollination progenies of the combination "N98-9445A ×DongNong95018" were used in a field experiment. N98-9445A and DongNong95016, all characterized by high linoleic acid and low linolenic acid .

2.2 **Gas chromatography analysis.** Bulk samples of ten to twenty seeds of each plant tested were analyzed by gas chromatography. Fatty acid analysis was carried out according to the method of Lee et al [1]. A modified half - seed technique was carried out as detailed by Downey [2]. The harvested seeds of eight plants containing high linoleic and/or low linolenic acid content were subjected to half seed analysis. The seeds were steeped in water for half to one hour before they were separated to make it easier to separate the seed coat from the embryo. Then they were divided into two halves and the half without the embryo was used for half - seed analysis. The gas chromatography unit in this experiment was set as follows : Unit : Yanaco 6800. Column temperature : 200℃. Injection temperature: 200 ℃. Injection volume: 2 μl. Carrier gas and its flux: He; 40 ml/ min.

3. Results and Discussion

The breeding strategy for soybean generally aims at increasing the linoleic acid content to possibly 60 % and at the same time reducing the level of linolenic acid to the range of 3～5 % or less[3] . For realizing such a goal , the availability of proper breeding materials is of primary importance. With the mutant of soybean, Oro, Roy and Tarr succeeded in developing a new cultivar whose linolenic acid was 1.60～1.87% [4]. In our study , a unique plant material characterized by zero linolenic acid was identified with the half - seed technique. The composition of its fatty acid was found to be linolenic acid free and consist of palmitic acid 2. 99%, oleic acid 73.86% and linoleic acid 23.15% (Table 1). In their diallel mating of two soybean lines with distinctly different linolenic acid concentration (high vs. low), S. Pleines and W. Friedt (1989) used a material with very low linolenic acid content (2.99%) as parent and showed that linolenic acid content is mainly under the control of a nuclear gene of the embryo [5]. It is evident that the material 0358 - 5 - 1 - 5 will be very useful for selecting low linolenic acid and studying gene action for linolenic acid with the help of biotechnology.

Progress in selection for increased level of linoleic acid and reduced linolenic acid has been rather slow mainly due to a positive correlation between them as reported by many workers [6]. However, there is a hypothesis that the synthesis of linoleic and linolenic acid in summer turnip rape is controlled by two independently working enzyme systems. Based on this hypothesis, Jesson argued that it should be possible to reduce the linolenic acid content in rape and turnip rape oil below 5% [7] (1977). Up to now, many materials with low linolenic acid (<5%) have been developed, though no direct experiment evidence is available to support the hypothesis. The result in this study supported the assumption that linoleic acid and linolenic acid are positively correlated with each other (Table 2). But it is worth mentioning that for some individuals, such as 0358 - 5 - 1, striking difference existed between linoleic acid and linolenic acid contents, a fact that seems to support the hypothesis that the two fatty acids are conditioned by two independently working systems. In addition, as the data in the experiment suggest, a significant negative correlation is present between oleic acid and linoleic acid contents and between oleic acid and linolenic acid contents, which is in agreement with the results of other researchers.

Most of high linolenic and low linolenic acid plant has black or dark blue umbilicus In the descendants of this cross. It need to improvement for using in production. Take the material 0358 – 5 – 1-5 for example. Although it has the desirable characters of high linolenic and low linolenic acid , its small seed size and dark blue umbilicus limiting its application. There is a significant negative correlation between oleic acid and linoleic acid contents and between oleic acid and linolenic acid contents, which is in agreement with the results of other researchers. So some material had a linoleic acid content as high as 60.32%, But no materials with both high linoleic acid content (>60%) and low linolenic acid content (<3%) were found.

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Table 1. Fatty acid composition of F4 individual plant through half - seed selection

Plant Materials	C16 :0 (Pal)	C18 : 1 (Ole)	C18 : 2 (Lin)	C18 : 3 (Lnl)	Plant Materials	C16 :0 (Pal)	C18 : 1 (Ole)	C18 : 2 (Lin)	C18 : 3 (Lnl)
0358 - 4 - 1 – 1	3. 50	33. 78	42. 38	20. 34	0358 - 4 - 12 – 2 7	3. 38	39. 47	45. 56	11. 60
0358 - 4 - 1 – 2	3. 17	41. 76	42. 73	12. 34	0358 - 5 - 1 – 4	4. 28	70. 16	20. 76	4. 80
0358 - 4 - 1 – 3	3. 02	42. 76	42. 63	11. 59	0358 - 5 - 1 – 5	2. 99	73. 86	23. 15	0. 00
0358 - 4 - 1 – 4	3. 94	41. 25	42. 09	12. 72	0358 - 5 - 1 - 9	3. 74	72. 53	19. 78	3. 95
0358 - 4 - 1 – 5	4. 39	35. 23	50. 42	9. 95	0359 - 8 - 5 – 1	2. 88	71. 77	20. 56	4. 79
0358 - 4 - 1 – 6	4. 99	38. 88	45. 69	10. 44	0359 - 8 - 5 – 2	2. 63	79. 30	13. 73	4. 33
0358 - 4 - 7 – 1	3. 72	39. 43	44. 56	12. 30	0359 - 8 - 5 – 6	3. 19	74. 16	18. 92	3. 73
0358 - 4 - 7 – 3	2. 65	38. 34	44. 74	14. 27	0359 - 8 - 5 – 10	2. 14	73. 34	20. 83	3. 68
0358 - 4 - 7 – 7	4. 66	41. 08	45. 23	9. 04	0359 - 8 - 5 – 11	3. 13	71. 54	21. 73	3. 60
0358 - 4 - 7 – 8	5. 77	30. 81	52. 32	11. 11	0359 - 8 - 5 – 13	2. 94	61. 61	32. 45	3. 01
0358 - 4 - 7 – 9	3. 02	40. 64	42. 06	14. 28	0359 - 8 - 5 - 14	2. 35	74. 50	20. 48	2. 66
0358 - 4 - 12 – 3	3. 80	42. 66	43. 54	10. 00	0359 - 8 - 5 – 15	2. 66	77. 20	16. 79	3. 34
0358 - 4 - 12 – 5	3. 96	40. 79	42. 91	12. 33	0359 - 8 - 5 - 17	2. 44	74. 50	19. 07	3. 98
0358 - 4 - 12 – 7	5. 37	34. 50	46. 16	13. 97	0359 - 8 - 5 - 20	2. 15	70. 15	22. 97	4. 74
0358 - 4 - 12 – 8	3. 32	39. 33	46. 54	10. 81	0359 - 8 - 5 – 21	3. 48	79. 11	14. 13	3. 28
0358 - 4 - 12 – 18	3. 20	43. 83	45. 67	7. 31	0359 - 8 - 5 - 22	2. 61	80. 36	14. 23	2. 80
0358 - 4 - 12 – 19	3. 88	41. 91	46. 30	7. 92	0359 - 8 - 5 - 23	2. 35	75. 62	19. 01	3. 02
0358 - 4 - 12 – 21	3. 80	41. 20	44. 60	10. 41	0359 - 8 - 5 – 24	1. 99	82. 92	10. 9 2	4. 17
0358 - 4 - 12 – 23	2. 98	42. 85	44. 07	10. 09	0359 - 8 - 5 – 25	2. 23	67. 30	27. 52	2. 95
0358 - 4 - 12 – 24	3. 30	39. 19	47. 59	9. 30	0359 - 8 - 5 - 29	2. 79	72. 99	20. 23	3. 99

Table 2. The correlation coefficient of between oleic, linoleic and linolenic acid, linoleic & lin/ole* and lin/ole &lin/lin* for strain selected plants

Plant Materials	No. Sample	Oleic &linoleic	Oleic & linolenic	Linoleic & linolenic	Lin/ ole & linolenic	Lin/ ole & lnl/ lin
0358 - 4 – 7	10	- 0. 987**	- 0. 581	0. 451	0. 429	- 0. 451
0358 - 4 – 12	30	- 0. 952**	- 0. 595	0. 331	0. 528	- 0. 170
0358 - 5 – 1	30	- 0. 801	- 0. 677	0. 149	0. 311	- 0. 004
0359 - 8 – 5	30	- 0. 985	- 0. 404	0. 249	0. 262	- 0. 511
Total	100	- 0. 988	- 0. 827	0. 736	0. 771	- 0. 201

* Lin/ ole : linoleic and oleic acid ratio , lnl/ lin : linolenic and linoleic acid ratio.

References:

1. Lee , J . L. , Takayama , K. and Shiga , T. Breeding for the improvement of fatty acid composition in oileseed, L. I. Fatty acid composition in soybean oil of Asia and European varieties [J] . Bull. Nat. Inst. Agri. Sci. series 1974 ,D. 25 : 1 – 16 (in Japanese) .
2. Downey , N. N. and Harrey , B. L. , Methods of breeding for oil quality in rape[J] . Can. J . Plant Science 1963 ,(43) : 271- 275.
3. Rebbelen , G. and Thies , W. Brassica crops and Wild Allies-Biology and Breeding[J] . Japan Scientific Societies Press ,Tokyo , 1980 ,253 - 278.
4. Roy , N. N. and Tarr , A. W. Development of near – zero linolenic acid (18 :3) lines of oileed (*Brassica napus* L.)[J] . Z. Pflanzenzuchtg , 1986 ,(96) :218 - 223.
5. Pleines , S. and Friedt , W. Genetic control of linoleic acid concentration in seed oil of soy(*Brassica napus* L.) [J] . Theor. Appl. Genet. 1989 ,(78) : 793 - 797.
6. Roy , N. N. and Tarr , A. W. IXLIN - an interspecies source of high linoleic and low linolenic acid content (*Brassica napus* L.) Z[J] . Pflanzenzuchtg , 1985 , (95) :201- 209.
7. Jesson , R. Breeding for improved oil and meal quality in rape(*Brassica napus* L.) and turnip rape *(Brssica campestris* L.)[J] . Hereditas , 1977 , (87) :205 - 218.
8. Roy , N. N. and Tarr , A. W. Prospects for the development of oilseed (*B . napus* L.) with improved linoleic and linolenic acid content [J] . Plant Breeding , 1987 , (87) :89 -96.
9. Labana , K. S. , Banga , S. S. and Banga , S. K. 1993 , Monographs on Theoretical and Applied Genetics [J] 19 : Breeding Oilseed Brassica. 1993 ,76 - 93.
10. Okamoto , M. , Uesugi , R. , Kakihara , F. and Kato , M. Variation of fatty acid composition in amphidiploids strains preserved by self or sib - cross pollination form F1 hybrids (AABC) between *Brassica juncea* (AABB) and *Brassica napus* L. (AACC) [J] . The 7th Int. Congress of SABRAO and Int. Symposium WSAA. 1994 ,301 - 312.

Biodiversity of Mothronwala Swamp, Doon Valley, Uttaranchal

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Abstract: India is a hub of biodiversity, encompassing a wide spectrum of habitats from tropical rain forests to alpine vegetation and from temperate forests to coastal wetlands. Among the 25 hotspots India is considered as eighth hottest of hotspots extending from Western Ghats on one side and Eastern Himalayas on the other. India contributes significantly to this latitudinal biodiversity trend with mere 2.4% of the world's area. Wetlands are transitional zones between the terrestrial and aquatic environment. These habitats perform major ecological role in the biosphere. Many of the fossil fuels are known to be produced and preserved by the swampy environment of the carboniferous period. These are source, sinks and transformers of a multitude of chemicals, biological and genetic materials. These produce a rich collection of plants, many of which are potential for one, or more economic use these provide food, timbers, fuel, fodder and forage etc. India has a rich variety of wetlands habitats. Tropical swamp forests once formed an important part of vegetation and extended all along the base of Himalayas from Assam to Peshawar. The International Biological Program (IBP) states that: "A wetland is an area dominated by specific herbaceous macrophytes, the production of which takes place predominantly in the aerial environment above the water level while the plants are supplied with amounts of water that would be excessive for most other higher plants bearing aerial shoots". Doon valley is known for its swamps. There was a time when low lying areas of the valley were having a chain of swamps but human interference once started in the name of "Malarias Climate" still persists. The trees were cut at that time and the openings created resulted in the extinction of most of the swamps. Wetlands are one of the most productive ecosystems and thus subjected to human greed which is yet another reason for their extinction. The Mothronwala swamp is a "Hot Spot" of biodiversity due to its topographic and edaphic variations. Unfortunately these habitats have not been explored from ecological point of view. The fresh water swamp of Mothronwala is under threat due to human interference and other anthropogenic activities. The present work was carried out to explore the biodiversity of the swamp and suggest conservation and management strategies. [The Journal of American Science. 2006;2(3):33-40].

Key Words: wetlands, swamps, biodiversity, Mothronwala, conservation

Introduction

Diversity is a concept about range of variation or differences among entities. The term biodiversity is a contracted form of biological diversity. Biodiversity is the degree of variety in nature and nature itself and also is the variability among living organisms from all sources including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are a part. It includes diversity within species, between species and ecosystems. It is the most significant national asset and constitutes an enduring source for supporting the continued existence of human societies.

Wetlands are neither aquatic nor terrestrial, but are transitional zones. Swamps lie in the palustrine system of wetland. Swamps are marshy areas with typical habitats where water oozes out in perennial streams at constant level through out the year. They support characteristic vegetation on account of specialized edaphic conditions, as influenced by free water accumulation. Unfortunately these habitats have not been explored sufficiently from ecological point of view.

The Mothronwala swamp is a "Hot Spot" of biodiversity due to topographic and edaphic variations.

The only authentic record of the area available is in the old settlement documents preserved in the office of the District Collector, Dehradun. The earliest document available is one-dated 1862, and on a map the site is indicated as "land under water" and lies close to the Bindal River. A later record dates 1902 reveals that the river has changed its course and there is a wide gap between the present course of the river and the forest.

Local enquires made of the village elders have elicited the information that in the past, the swamp was much deeper and more inaccessible than at present. The villagers dreaded approaching the swampy zone. In a report on the Dehradun forests prepared by Dr. G. King and published in 1871, a reference was made to these areas and it was recommended that the forest department should drain the swampy places which would incidentally improve the health of the eastern part of Doon but nothing appears to have been done in this regard.

The swampy zones are located in between the ridges and are composed of innumerable pools with characteristics bubbling and small intercommunicating streams. The northern portion however is drier than the southern, which is slushier and consists of loose soil. Besides the pools and streamlets mentioned above there are two large streams with a swampy base, which originate from the extreme north of the forest and flow from the north to south. In Doon valley there are many patches of freshwater swamps, which are recognized as integral part of wetland ecosystems. Kanjilal (1901) first emphasized on vegetation and botanical value of swamps. The vegetation and soil texture of Mothronwala has been studied by Dakshini (1960a, 1960b, 1965, 1970, 1974). Deva and Aswal (1974) studied the taxonomy and ecology of Mothronwala swamp. Deva (1974), Srivastava (1978) and Ghildiyal (1989) studied the vegetation of other swamps of Doon valley that include Golatappar and Manu swamp. However, study relating to the Biodiversity of Mothronwala swamp has been left untouched, so the present study aimed to explore the biodiversity and give its conservation and management strategies.

Material and Methods

Study area

Mothoronwala, Dulhani-1 (new reserve) and Navada 10-14 (old reserve) of Lachiwala range about 5 Km from main city of Dehradun, at an elevation of 600m above sea level. It occupies an area of approximately 22 acres. The swamp lies at 30^0 15' 40" and 30^0 16' 45" N latitude and 78^0 1'and 78^0 2' 15" E longitude and lies to the South-East of Dehradun near the military township of Clement Town. On the East is the village of Mothronwala from which the swamp derives its name. On the north lies Banjarwala Tea Estate. On the West lies the Sushwa river, stream coming out of the swampy zone drains into the river that ultimately discharges into the Ganga through Rispana River. On the South is the Clement Town water works.

The swampy area of Mothronwala is humid and fairly green. The maximum rainfall ranges between 600 – 800 mm during the months of July-August and minimum is recorded during April - May. The maximum temperature reaches upto 40^0 C during the months of May and June whereas minimum of 2 - 3^0 C during December - January.

The ridge of Mothronwala swamp is about 10 - 11 m above the surrounding level. The slope along the ridge is approximately 20^0 - 30^0 .The northern part of the ridge is drier than the southern area, which is slushy. Inside the swampy area, the sub-soil water level is quite high and remains so through out the year. The slush in marshy place is knee deep. During rains the water infiltrate through the gravelly soil extending over a very

large area of the terrain oozes out here in a series of deep but narrow ravines giving rise to a number of streams which unites into a few main channels pour into the Suswa River.

Collection of aquatic flora and fauna

Clusters of algal filaments were collected from the swamp for the study of diatoms and algae present in them. Insects attached to stones were collected by a fine forceps. Insects inhabiting the shallow areas of the streams below stones were collected by enclosing $1m^2$ of the substratum with fine square-mesh netting cloth and sweeping the area completely. The insects were collected in cloth and picked up. The collected material was preserved in 4% formalin and identified.

Collection of terrestrial flora and macrophytes

Parts of different types of vegetation having flower, bud, node etc were collected and then pressed in newspapers and dried for identification. The herbaria were identified at Botanical Survey of India (BSI), Dehradun.

Results

Plant Diversity

Mothronwala swamp possesses peculiar vegetation due to topographic and edaphic varaiations. It has diverse and dense vegetation ranging from climbers and small herbs to tall trees. Indiscriminate human interference has led to the degradation of the swamp forest to a great extent leading a very small green cover. The original forest vegetation had dwindled to a larger extent and only two tree species namely *Shorea robusta* and *Dalbergia sisso* are left in the region. Other tree species like *Bischofia javanica, Celtris australis, Litsaea monopetala, Quercus leutrichophora, Toddalia asiatica* etc., could also be seen on the few places. Exorbitant growth of *Lantana camara* and other exotic weeds have replaced the larger part of the vegetation. The shallow streambeds often extending over vast area of the swamp are covered with original hydrophytic and amphibious communities *Calamus tenuis* is the most dominant species. Shrubs in the swamp reach to a maximum height of 2 – 3 m. A pure community of *Ipomoea fistulosa* dominates upper portion of the swamp and bank of channel. The villagers collect *Rorripa nasturtium aquaticum,* observed as patches along the stream for vegetable. The herbaceous vegetation of the ridge is very sparse. The dominating ground vegetation is *Parthenium hysterophorus* and the grass *Cynodon dactylon*. On the ridges small tree communities like *Ficus palnata* and *Pyrus paschia* were common. *Mallotus philippensis, Indigofera tinctoria*, were found at few places**.** Invasive weed *Lantana camara* occupies most of the area. The dominantly vegetation was *Parthernium hysterphorus* and few grasses like *Cynodon dactylon*. Small trees like *Desmodium, Indigofera tinctoria*, *Ficus palnata* could be seen on the slopes. The surface of the slope is almost covered with large number of herbs like *Ageratum conyzoides* (Table 1).

In the swampy zone, the plant diversity varies according to the habitat in pools and numerous streams usually macrophytes are found. Among shrubs *Ipomoea fistuosa*, *Lantana camara* etc are commonly found. *Polygonium barbatum*, *Oenanthe javanicam Desmodium trifolium* are seen along the streams and present on well-drained soils

The ground flora covers species like *Acorus calamus*, *Parthenium hysterophorus* etc. the livestock grazes the palatable species during the summer season, while the fern *Diplazium esculentum* locally known as lingora is collected for the vegetable in the region. *Calamus tenuis* is the most dominant at shallow

streambeds and *Ipomoea fistuosa* is dominant in the upper portion of the swamp (Table 2).

A total of 19 genera of algae belonging to three orders were found in the stagnant water of the swamp. 16 species belonging to Bacillariophyceae, 2 species of Chlorophyceae and 1 species of Myxophyceae were found. *Tabelleria* of Bacillariohyceae was found to be abundant. Amongst the Chlorophyceae *Spriogyra* was found to be abundant (Table 3).

Animal Diversity

Biodiversity is key factor for natural development of global ecosystem. The concern for biodiversity has emerged as a result of quantification of consumers and consumables. Among the animals *Lepus nigricollis* (Indian Hare) and *Susscrofa cristatus* (wild boar) were known to be dominant, *Rana tigrina* the only amphibian was found abundant. Four species of fishes also represented the animal diversity (Table 4). Leeches are found in large number during the rains. Water snakes were common in the streams. Among the macro-zoobenthos 13 species belonging to 5 orders were identified. Amongst the 13 species of macroinvertebrate present 5 species represented genera Trichoptera, 2 species of Ephemeroptera, 2 species of Odonata, 2 species of Coleoptera and 2 species of Hemiptera. Three species of Molluscs also represented the animal diversity of the swamp. Amongst the Trichopterans, *Planaria* was found to be abundant whereas *Hydropysche* was found to be rare. *Ephemeralla* of Ephemeroptera and *Gerris* of the order Hemiptera were also found abundantly (Table 5).

Table 1. Plant diversity of Mothronwala Swamp

Tree species
Shorea robusta
Dalbergia sissoo
Celtris australis
Ficus palmate
Sapium sebiferum
Solanum torvum
Indigofera tinctoria
Ficus religiosa
Caryopteris wallichiana
Pyrus pashia
Shrubs
Ardisia solanacea
Mallotus philippensis
Carrisa opaca
Zizyphus mauritiana
Murraya koenigii
Smilax glaucophylla
Plectranthes japonicus
Rubus niveus
Polygonum chinense
Weeds
Lantana camara
Parthenium hysterophorus
Eupatorium adenophorum
Herbs
Argemone mexicana
Solanum nigrum
Chenopodium album
Rungia pectinata
Grasses
Ageratum conyzoides
Cynodon dactylon
Cyperus kyllingia
Eleusine indica

Table 2. List of Aquatic Macrophytes of the swamp

Taxonomical Name	**Family**
Ranunculus sceleratus	Ranunculaceae
Rorripa nasturtium aquaticum	Brassicaceae
Sida acuta	Malvacea
Sida cordata	Malvacea
Ventilago denticulate	Rhamnaceae
Acer oblongum	Acoraceae
Acer pennata	Acoraceae
Pyrus pashia	Rosacea
Carallia integerrima	Rhizophoraceae
Oenanthe javanica	Apiaceae
Oldenlandia corymbosa	Rubiaceae
Inula cappa	Arteracea
Enhydra fluctuans	Arteracea
Ipomoea carnea	Convolvulaceae
Ipomoea fistulosa	Convolvulaceae
Bacopa monnieri	Scropulariaceae
Lantana camara	Verbenaceae
Allmania nodiflora	Amranthaceae
Polygonum barbatum	Polygonaceae
Commelina berghalensis	Commelinaceae
Narengaporphyrowm	Poaceae
Imperata cylindrica	Poaceae
Coix lachrymal jobi	Poaceae
Acorus calamus	Araceae
Calanus tenuis	Arecaceae
Pouzolzia pertendra	Urticaceae
Canna indica	Cannaceae
Cyperus iria	Cyperaceae
Cyperus globosus	Cyperaceae
Scirpus eractus	Cyperaceae
Justicia quinqueargularis	Acanthaceae

Table 3. Abundance of Algal components

Name	**Abundance**
Bacillaripophyceae	
Cymbella	++
Synedra	++
Pinnularia	++
Meridion	++
Diatoma	+
Achnathes	+
Gomphonema	++
Cocconeis	++
Melosira	+
Pinnularia	+
Nitzchia	+
Tabelleria	+++
Stauroneis	+
Flagilaria	+
Naviculla	++
Licmophora	+
Chlorophyceae	
Spirogyra	+++
Chlorella	+
Myxophyceae	
Oscillatoria	++

+++ Abundant, ++ Common, + Rare

Table 4. List of Fishes found in the stream flowing in the Swamp

Vernacular name	**Scientific name**
1. Kali Machi	*Barbus chilinoides*
2. Baan	*Mastacembalus*
3. Sewal	*Vphicephalus punctatus*
4. Potto	*Barbus ticto*

Table 5. Abundance of Macroinvertebrates

Name	Abundance
TRICHOPTERA	
Molanna	++
Hydropsyche	+++
Plannaria	+
Economus	+
Hydroptila	+
COLEOPTERA	
Amphizoa lecontes	++
Anchycetus	+
MOLLUSCS	
Gyraulus	+++
Cerithidea	+++
Lymnaea	+++
HEMPITERA	
Gerris	++
Hespercorixa	+++
EPHEMEROPTERA	
Heptagenia	++
Ephemerella	+++
ODONATA	
Enallagma	++
Agrion	+

+++ Abundant, ++ Common, + Rare

Discussion

The threats to wetlands may be divided into two broad categories: natural threats and anthropogenic threats, which may be direct or indirect. Natural threats include eutrophication, erosion, storm damage, drought or biotic interference other than by man, which may lead to destruction of wetlands. The human intervention by drainage and reclamation for agriculture and urban construction stop them to play their usual ecological roles. Ecological degradation of wetlands together with pollution has resulted in the loss of flora and fauna.

The fresh water swamp of Mothronwala is under great environmental stress and has been degraded to a great extent during the last few decades. The major portion of the swamp has been encroached upon by the human settlements, agriculture, cultivation and related developmental activities. Forests felling are common on the ridges. The villagers have occupied the peripheral area of cultivation of various fodder species. As the cantonment is in the close vicinity of the swamp, the area is being exploited to meet out the various needs of the military persons. A water pump has been installed inside the swamp to pull out the water to be used for drinking, bathing and other domestic purposes.

Lopping of trees by people from neighboring village results in the deformity of some of the trees with the consequent effect on the ground floor vegetation. Invasion of exotic weeds like *Lantana camara, Parthenium hysterophorum, Ageratum conyzoides, Ipomoea* has drastically changed the vegetation of the swamp. Plant species like *Shorea robusta, Bombax ceiba, Grewia oppositifolia, Toona ciliata* are used for fodder, fuel and timber by villagers.

Cattle trampling is another big biotic factor responsible for reduced vegetal cover of the region. Grazing is also a factor to be considered particularly on the slope and the ridges. Leasing out of medicinal plants like *Centella asiatica, Bacopa monnierii, Berchemia floribunda, Desmodium triangulare Cassia pumila, Acorus calamus etc*. have caused the depletion of these species from the swamp area. In Mothronwala swamp, the ecological succession is resulting into conversion of aquatic region to terrestrial and is also contributing to the shrinkage of waterbed area. Erosion of the exposed slopes is responsible for the alteration in vegetational cover from season to season. Higher deforestation rate results in the loss of topsoil, which is drained off with rainwater and settles down in the stream. This result in

rise of soil level in swamps making them much shallower with reduced water spread area.

Strategies for the conservation of the swamp

Wetlands are the sources sinks and transformers of chemical, biological and genetic materials. They play a significant role in environment by providing a unique habitat for a wide variety of flora and fauna. However, over a period of time these natural heritages are continuously disturbed by human interference and over exploitation of biological resources available in them or in nearby locations. Since last few decades efforts have been made at national and international level to assess the status, management and conservation of wetlands with growing awareness the importance of these fragile ecosystems have been realized throughout the globe (Chatrath, 1992).

The long term solution to the problem of protecting wetlands lies in educating the masses. Unless people realize the need to safeguards wetland ecosystem and are made aware of how they can contribute to this effort, there is little hope for the survival of these ecologically valuable and vulnerable habitats. The fresh water swamp of Mothronwala is under threat due to human interference and other anthropogenic activities. As a consequence, some measures are of utmost importance to check their further deterioration like the knowledge of the physical dimensions of these fresh water swamps by way of field surveys and other appropriate techniques like remote sensing etc. should be gained.

Inventory of both flora and fauna in these swamps should be made and rare, endangered and economically important species should be given top priority for their protection. Since deforestation in the catchment area due to human interference, has adversely affected these swamps. It is necessary to go for large scale afforestation in these areas.

Sincere efforts should be made to check the soil erosion from slopes, which lead to siltation in these swamps. It can be done by constructing check dams in high reaches, at different places and initiating afforestation in these areas. There should be a regular testing and monitoring of the water quality of these swamps. The water samples need to be taken from the disturbed areas along the stream at regular intervals to judge the adverse effects of human activities. State Pollution Control Board situated locally should be entrusted with such responsibility.

There should be a complete ban on all construction activities up to a specified distance, say about 100m or more from the swamp. This can be ensured by making a clearance mandatory from the state environment department before undertaking any construction activity in the vicinity of the swamp. Efforts should be initiated by the State Forest Department to protect these swamp forests from further destruction by enforcing strict laws and warding heavy penalties on defaulters who are harming these ecologically sensitive zones by over exploitation of resources, cutting and lopping, diversion of water for irrigation and agriculture and urban land use.

To make people aware of the importance and threats to wetlands and their conservation, various government institutions, NGOs and media (both print and audiovisual) should take the lead and make it a mass movement. Local communities should be involved to ensure sustainability of conservation effort under taken by the government agencies. For this they can be involved in decision-making processes required for management and conservation of wetlands.

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References

1. Abbasi, S.A. 1997. *Wetlands of India: Ecology and Threats*. **1**: 3-12.
2. Anonymous. 1990. *Wetlands of India: A Directory*, MOEF, Govt. of India, New Delhi.
3. Champion, H.G. 1936. A preliminary study of forest types of India and Burma. *India Forest Rec*. N.S. **1**: 1-286.
4. Beard, J.S. 1955. The classification of tropical American vegetation types. Ecology 36: 89-100.
5. Champion, H.G. and Seth, S.K. 1968. *The Revised Survey of the Forest Types of India.* Manager of Publications, New Delhi.
6. Chatrath, K.J.S. 1992. *Wetlands of India.* Ashish publishing house. New Delhi.
7. Dakshini, K.M.M. 1960a. The Vegetation of Mothronwala Swamp Forest: A preliminary survey. *Bull. Bot. Surv. India*. **2**: 57-59.
8. Dakshini, K.M.M. 1960b. The Vegetation of Mothronwala Swamp Forest (plant communities of swamp zone). *Indian Forester*, **86**: 728-733.
9. Dakshini, K.M.M. 1962. Mothronwala swamp forest, taxonomy and ecology. Ph.D. Thesis, Agra University, Agra India.
10. Dakshini, K.M.M. 1965. A study of vegetation of Mothronwala Swamp Forest, Dehradun, India. *J. Ind. Bot. Soc*., **44**: 441-448.
11. Dakshini, K.M.M. 1968. Conservation of natural vegetation from the point of view of productivity of vegetational stand. Advise note on symposia and discussion 88th. *Indian Sci. Cong*. Varanasi. 12-13.
12. Dakshini, K.M.M. 1970. The Flora of Mothronwala Swamp. *Jour. Bomb. Nat. Hist.Soc*. **67(2)**: 176-186.
13. Dakshini, K.M.M. 1974. The Flora of Mothronwala Swamp. *Jour. Bomb. Nat. Hist. Soc.* **71**(2): 235-243.
14. Deva, Som and Aswal, B.S. 1974. Taxonomy and Ecology of Mothronwala swamp: A Reassessment. *Indian Forester* (100): 12-19.
15. Deva, Som and Srivastava, M.M. 1978. An Ecological Study of Vegetation of Golatappar Swamp. *Indian Forester* **1(1)**: 44-52.
16. Duthie, J.F. 1903. *Flora of Upper Gangetic Plain*.Calcutta
17. Ghildial, J.C and Srivastava, M.M. 1989. The vegetation of Manu Swamp: A Tropical fresh -water swamp forest. *Indian Forester* **115(3)**: 183-191.
18. Kanjilal,U. 1901.Swamp forest in Dehradun,N.W Province.*Indian Forester* ,**27:** 228-230
19. Kanjilal, U.1934. *Flora of Assam.Shillong* B**(4)**-2
20. Kanjilal,U. 1956. *Forest Flora of the Chakrata Dehradun and Sharanpur Forest Divisions*, Uttar Pradesh.
21. King,G. 1871. Report on Dehradun Forests.Nr.**7:**3-17.Allahabad.
22. Rao, R. R. 1996. Conservating the Hot Spots of Biodiversity in India. In: Ramakrishan, P.S. *et.al*, (eds.)*, Conserving Biodiversity for Sustainable Development,* Indian Nat. Science Academy. New Delhi, 246 pp: 95-107
23. Richards, P.W.1957.*The Tropical Rain Forests*, Cambridge Univ. Press
24. Walton, H.G. 1911. Dehradun Distt. Gazetters of U.P. Vol. **1.** Allahabad

Application of Fuzzy-hierarchal Integrated Evaluation Method on the Oasis Ecosystem Stability

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Abstract: The factors influenced on the oasis ecosystem stability involve many aspects and the relationships among factors have the characteristic of uncertain and hierarchal. This paper takes the irrigation area of the north of Ningxia (that draws water from the Yellow River) as an example to compare the oasis ecosystem stability of ten calculated units with the method of fuzzy-hierarchal integrated evaluation. The results show that this method is scientific and rational, and is also an effective method to evaluate the oasis ecosystem stability. [The Journal of American Science. 2006;2(3):41-47].

Keywords: fuzzy; hierarchal; oasis; ecosystem; stability

1. Introduction

The oasis ecosystem stability refers to the situation in which the energy flow, material flow, human flow and information flow of the oasis ecosystem can be kept in a good cycle, the existing surroundings of lives in oasis are optimized continuously, and the functions of oasis can also be kept to develop sustainable. Because the basic character of stability is to promote the sustainable development and sustain a good cycle of system, the essence connotation of oasis stability is the sustainable development of oasis ecosystem. [1] From the definition of the oasis ecosystem stability, we can know that the factors influenced on the oasis ecosystem stability involve many aspects and these factors have the characteristics of dynamic and continuous because of they will change themselves in difference time and space.

The oasis is a place with centralized human activities and high developed productivity. The primordial oasis is the long evolutive result of nature, and various species, resources depends on each other on a harmonious state. But this primordial nature balance is broke down by the modern human beings. Because of the limitations of knowledge and technologies, some irrational development and utilization of resources in oasis are appeared, especially water resources, which plays a key role on the subsistence and development of oasis. As a result, the ecosystem of oasis begins to degrade gradually, and further to effect on the prosperity and sustainable development of oasis. Therefore, it is urgent to study the crucial factors that influence on the stability of oasis ecosystem from the view of nature and human, then to make out the corresponding evaluation standard to direct the human activities and make sure the healthy stability and sustainable development of oasis ecosystem.

2. The Establishment of Evaluation Indexes

Because the involved features of oasis ecosystem stability are very complex, the evaluation index system

must follow the principles of science, hierarchy, integrated and predication.

Water, land, biology and environment are the four main basic feathers to form the oasis system. Moreover, they are the key feathers who have an important effect on the sustainable development of the oasis ecosystem stability. The human activities are all related to these four feathers and the changes of these feathers are also the reflections and results of human intervention. Therefore, we can make them as the first control indexes of the oasis ecosystem stability evaluation, then to classify them further according to their importance degree. The indexes system is shown in Figure 1.

3. Fuzzy-hierarchal integrated evaluation model

From the evaluation indexes system of oasis ecosystem stability, we can know that the system can be decomposed, so it has the characteristic of hiberarchy. The indexes are related and depended on each other and the relationships among them have uncertain and fuzzy characteristics. This paper evaluates the oasis ecosystem stability by establishing the fuzzy-hierarchal integrated evaluation model. The following is the process of the whole evaluation:

(1) Construct judge matrix $\lfloor u_{ij} \rfloor_{k\times k}$ of each level according to the importance degree $f_{uj}(ui)$ of each index. Table 1 is the judge value of the importance degree of each index.

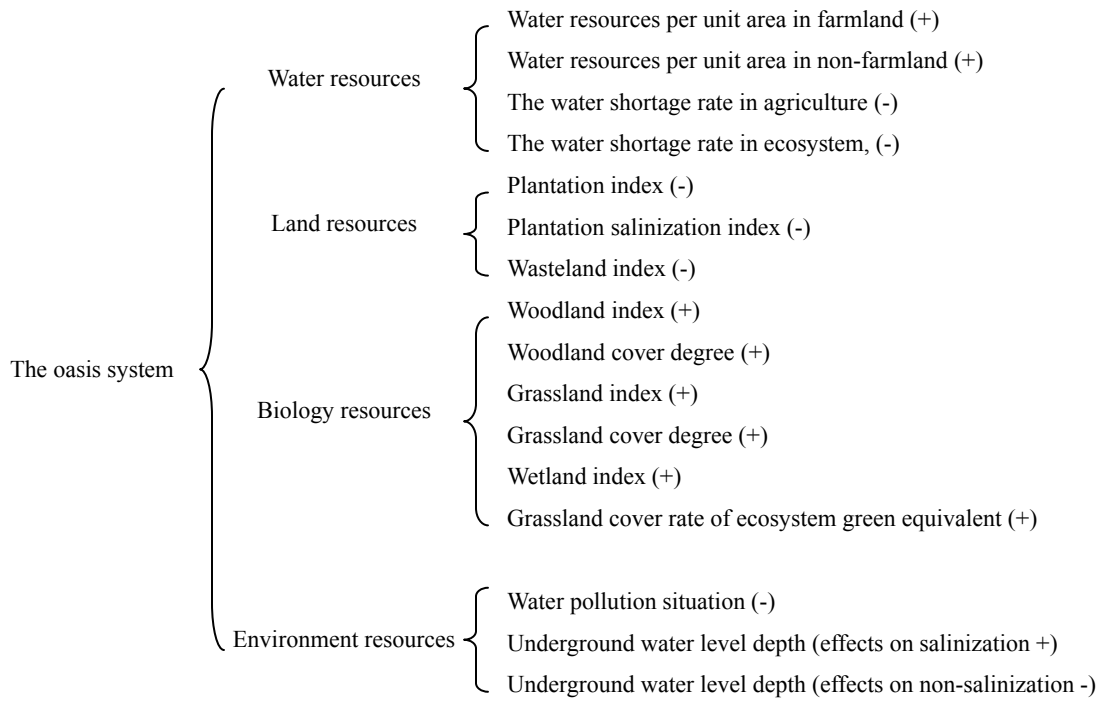

Figure 1. Evaluation indexes system of oasis ecosystem stability

Notes: "+" means the positive index and " –" means the negative index

Table 1. The judge value of the importance degree of index

u_{ij}	1	3	5	7	9	2、4、6、8
$f_{uj}(ui)$	equal important	a little more important	obvious important	strong important	absolute important	between each important degree

(2) Compute the largest eigenvalue $\lambda_{\max}$ and eigenvector ξ of each judge matrix, and to check up the consistent of matrix $\lfloor u_{ij} \rfloor_{k\times k}$.

(3) If the matrix is consistent, we can calculate the fuzzy weight vector $\underset{\sim}{A}$ of each index in the lowest level.

(4) Ascertain the fuzzy matrix of scheme level[2]

Suppose the decision scope V is a collection of schemes (oasis stability evaluation region).

$V =$ {region 1， region 2， …， region m}$= \{v_1, v_2, ..., v_m\}$

The indexes collection which has an important effect on the oasis ecosystem stability is that:

$u = \{f_1, f_2, \ldots, f_n\}$

So, the index vector of each region is as follows:

$v_j = (f_{1j}, f_{2j}, \ldots, f_{nj})^T$， $j = 1, 2, \cdots, n$

If the value of index i in the scheme j is considered as f_{ij}, we can get the index value matrix F with m regions and n indexes:

$$F = \begin{pmatrix} f_{11} & f_{12} & \cdots & f_{1m} \\ f_{21} & f_{22} & \cdots & f_{2m} \\ \cdots & \cdots & \cdots & \cdots \\ f_{n1} & f_{n2} & \cdots & f_{nm} \end{pmatrix}$$

When the index f_{ij} can be calculated, the r_{ij} is calculated as:

$$r_{ij} = \begin{cases} 0.1 + \dfrac{f_{i\max} - f_{ij}}{d}, & \text{when } f_{ij} \text{ is a negative index} \\ 0.1 + \dfrac{f_{ij} - f_{i\min}}{d}, & \text{when } f_{ij} \text{ is a positive index} \end{cases}$$

In which, d is the grade difference value and can be calculated as $d = \dfrac{f_{i\max} - f_{i\min}}{1 - 0.1}$, r_{ij} is the evaluation value of the index i in the region j

An evaluation fuzzy matrix is formed by n evaluation values of m regions.

$$\underset{\sim}{R}=\begin{bmatrix} r_{11} & r_{12} & \cdots & r_{1m} \\ r_{21} & r_{22} & \cdots & r_{2m} \\ \cdots & \cdots & \cdots & \cdots \\ r_{n1} & r_{n2} & \cdots & r_{nm} \end{bmatrix}$$

(5) The integrated fuzzy integrated evaluation results can be got by weighted average model $M(\bullet,+)$:

$$\underset{\sim}{A}\circ\underset{\sim}{R}=\underset{\sim}{C}=(c_1,\ c_2,\ldots c_m)$$

4. Model application

The irrigation area of drawing water from the Yellow River in Ningxia is located in the northwest of China with few rain and strong evaporation, and its ecosystem is very fragility. For recent years, the contradiction between water supply and demand is becoming more serious, and the human activities are more frequent and strong which result in the degradation of oasis ecosystem stability. Therefore, it is necessary to apply the model into this region so as to sustain the harmonious development of oasis soc-economy and eco-environment.

In the model application, the irrigation area is divided into ten calculated units according to the administrative districts. Under the condition of 50 percent of inflow frequent of the Yellow River in 2000, the ecosystem stability of ten units is evaluated and compared.

4.1 Compute fuzzy weight vector $\underset{\sim}{A}$

Aiming at the actual situation of irrigation area, the important degree among indexes is compared to construct a judge matrix. With the software of Matlab, the eigenvalue and eigenvector of corresponding matrix are calculated, and then the matrix's consistence is checked up. At last, the fuzzy weight vector $\underset{\sim}{A}$ is got as:

$$\underset{\sim}{A}=(0.116,0.088,0.116,0.17,0.075,0.013,0.027,0.036,0.036,$$
$$0.023,0.023,0.057,0.057,0.097,0.041,0.026)$$

4.2 Construct the fuzzy matrix of scheme level

The index values of each single unit in irrigation area are as Table 2 and Table 3 [3]-[5]:

Table 2. The index value of each calculated unit in irrigation under the condition of 50 percent of inflow frequent of the Yellow River in 2000

Unit	Water resources per unit area in farmland (m^3/mu)	Water resources per unit area in non-farmland (m^3/mu)	Water pollution (t/a)	Plantation index	Plantation salinization index	Woodland index	Woodland cover degree	Wetland index
Zhongwei City	671.85	231.23	8817.68	0.31	0.14	0.04	0.34	0.11
Zhongning County	643.80	324.02	5338.07	0.39	0.08	0.03	0.32	0.21
Qintongxia City	604.29	228.32	5224.65	0.41	0.08	0.02	0.31	0.22
Yongxing County	604.26	235.11	5751.90	0.47	0.09	0.02	0.28	0.07
Yinchuan City	568.18	263.09	6678.23	0.51	0.13	0.03	0.32	0.09
Helan County	610.53	294.04	4493.50	0.58	0.17	0.00	0.35	0.06
Pingluo County	579.54	255.15	8297.57	0.47	0.17	0.03	0.31	0.16
Shizuishan City	570.38	180.56	4546.30	0.40	0.22	0.02	0.35	0.12
Wuzhong City	651.10	185.33	7572.40	0.41	0.22	0.04	0.32	0.12
Lingwu City	664.94	277.13	10129.90	0.47	0.18	0.03	0.34	0.15

Table 3. The index value of each calculated unit in irrigation under the condition of 50 percent of inflow frequent of the Yellow River in 2000

unit	Grassland cover degree	Wetland index	Wasteland index	Grassland cover rate of ecosystem green equivalent	The water shortage rate in agriculture	The water shortage rate in ecosystem	Underground water level depth (m)	Underground water level depth (m)
Zhongwei City	0.30	0.01	0.43	0.58	0.00	0.00	2.32	2.32
Zhongning County	0.26	0.01	0.23	0.56	0.00	0.00	1.88	1.88
Qintongxia City	0.29	0.01	0.24	0.54	0.00	0.00	1.70	1.70
Yongxing County	0.43	0.03	0.34	0.60	0.00	0.00	1.61	1.61
Yinchuan City	0.47	0.04	0.20	0.69	0.00	0.00	1.57	1.57
Helan County	0.42	0.08	0.20	0.67	0.00	0.00	1.32	1.32
Pingluo County	0.30	0.03	0.21	0.57	0.00	0.00	1.13	1.13
Shizuishan City	0.48	0.03	0.31	0.64	0.00	0.00	1.46	1.46
Wuzhong City	0.45	0.01	0.30	0.63	0.00	0.00	2.19	2.19
Lingwu City	0.33	0.01	0.23	0.60	0.00	0.00	2.03	2.03

The fuzzy matrix $\underset{\sim}{R}$ is got with the method of the fuzzy matrix of scheme level calculation.

4.3 Fuzzy integrated evaluation

By the above formulation:

$\underset{\sim}{A} \circ \underset{\sim}{R} = \underset{\sim}{C} = (c_1, c_2, \ldots c_m) = (0.106, 0.12, 0.09, 0.09, 0.10, 0.124, 0.079, 0.092, 0.098, 0.101)$ So,

the priority order of oasis ecosystem stability of ten calculated units in irrigation area is: Helan County > Zhongning County > Zhongwei City > Lingwu City > Yinchuan City > Wuzhong City > Shizuishan City > Yongning County > Qingtong City > Pingluo County.

5. Oasis ecosystem stability evaluation in each calculated unit

With the method of fuzzy integrated evaluation, we can compare the oasis ecosystem stability of each unit in irrigation area of Ningxia. However, the ecosystem stability of each unit is still not be ensured, so it is need to introduce the corresponding transformation function to transform the evaluation value of each calculated unit, and compare it with the integrated evaluation standard of oasis ecosystem stability. On these results, the oasis ecosystem stability of each unit in irrigation area can be determined.

The integrated evaluation standard of oasis ecosystem stability is as Table 4[6]-[7].

In the process of model calculation, all experts are think the ecosystem stability of Ningxia is in a good situation in 2000. So the index transformation function can be constructed as: $y = -0.245 + 15.61x - 66x^2$. Combing with the fuzzy integrated evaluation results, the oasis ecosystem stability of each unit in irrigation area can be determined as Table 5.

Table 4. The integrated evaluation standard of oasis ecosystem stability

The oasis ecosystem stability	The integrated evaluation standard
Best	>=0.8
Better	0.6～0.8
Mediocre	0.4～0.6
Worse	0.2～0.4
Worst	<0.2

Table 5. The oasis ecosystem stability of each unit in irrigation area of Ningxia

Unit	Zhongwei City	Zhongning City	Qingtongxia City	Yongning County	Yinchuan City	Helan City	Pingluo County	Shizuishan City	Wuzhong City	Lingwu City
Integrated evaluation value	0.668	0.678	0.624	0.625	0.655	0.676	0.578	0.633	0.652	0.658
Stability situation	Better	Better	Better	Better	Better	Better	Mediocre	Better	Better	Better

From the results, we know that in 2000, when the inflow frequent of the Yellow River is 50 percent, expect Pingluo County, the ecosystem stability of other Counties or Cites in Ningxia irrigation are all better, but are all at the lower limit of good state.

6. Conclusions

The oasis ecosystem stability is a complex problem involving various feathers of various aspects and the stability presents difference changes along with the difference of time and space. With the method of fuzzy-hierarchal integrated evaluation, this paper discusses the oasis ecosystem stability and gets the scientific and rational results, so this method can be adopted and popularized. In modern days, human activities have become the dominant factor to determine whether or not the development of the oasis ecosystem stability is healthy. Therefore, the future study on the oasis ecosystem stability shall be paid more attention to the human activities and their effects on the oasis stability.

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References

[1] Deling Han. Discuss on the Oasis Stability[J]. Journal of Ningxia University. 1999:6.

[2] Jijian Xie, Chengping Liu. The Method and Application of Fuzzy Math. Wuhan: Science and Engineering Publishing Company in Center China. 2002:5.

[3] Yuansheng Pei, jianhua Wang, Luo Lin. Analysis of effect of South-to-North Water Transfer Project on Aquatic Ecosystems of Haihe River Basin[J]. Acta Ecologica Sinica 2004;24(10):2115-2123.

[4] Jinping Zhang, Zhangjing, Suyan Sun. Application of Grey Correlation Analysis in Oasis Ecosystem Stability Evaluation[J]. Resources Science 2006;28(4):196-198.

[5] Yuansheng Pei, Suyan Sun Chuiyu Lu. Study on Oasis Ecosystem Stability Prediction[J]. Journal of Hydraulic Engineering 2006; 38(4):23-25.

[6] Shishen Yang g. Study on the model of grade division of natural disaster and comparison of disastrous conditions[J]. Journal of Natural Disasters 1997;6(1):9-12.

[7] Yaning Chen, Siquan Yang. The application and model of grey association for evaluation of natural disaster[J].Progress in Geography 1999; 18(2).158-162.

Study on Transfer and Transformation of Nitrogen and Phosphorus in Agriculture Ditch under Rainfall Runoff

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Abstract: TN and TP in agriculture ditch are measured and analyzed under rainfall runoff in this paper, and in order to intercept and wipe off effectively nitrogen and phosphorus from agricultural soil, the transfer and transformation and the space-time distributing of nitrogen and phosphorus are also studied. The result shows that, the agriculture ditch changes as outside condition (rainfall), otherwise it has the ability of anti-jamming and restoration and can restore the transfer and transformation of nitrogen and phosphorus. Because of this characteristic, TN concentration variation along cross section was according to the cubic equation and TP concentration variation is on the whole the descending variation of the exponential curve along cross section, and the concentration variations of both TN and TP are the cubic equation with time. [The Journal of American Science. 2006;2(3):58-65].

Keyword: nitrogen and phosphorus; agriculture ditch; transfer and transformation; rainfall runoff

Introduction

Nitrogen and phosphorus are important life elements, and main component which is not substituted for life support system, and also the fundamental element which advances sustainable development of agriculture (Yan, 1999). The use of nitrogen and phosphorus fertilizer is one of effective measures which comes true increase of Chinese foodstuff. However, the over-fertilization with nitrogen and phosphor also brings non-point source pollution. The recent research shows that, the pollution load of nitrogen and phosphorus has accounted for over 50% of that in water body and seriously affects water body (Canter, 1986). Because of the influence of non-point source pollution, 63.6% of lakes in China have suffered eutrophication, and the concentration of TN and TP in lakes such as Tai Lake, Chao Lake, Dianchi Lake and so on where existed some agriculture high yield areas is about ten times as high as that in 1980s and over 50% of nitrogen and phosphorus pollution load comes from agriculture non-point source pollution (Coote, 1982). To be the channels by which nitrogen and phosphorus enter into water body, some researches have been carried out in agriculture ditch, however, only a few attempts have been made to study on the intercept mechanism and the transfer and transformation of nitrogen and phosphorous (Jorgensen, 1983; Tiessen, 1995). Therefore, it is very important to study the transfer and transformation of Nitrogen and Phosphorus in agriculture ditch to control agriculture non-point source pollution. This paper chooses Jiaxing town in Zhejiang province in southeast of China as the

study area and the variation of TN and TP in rainfall are mainly studied.

1. Materials and Methods

1.1 Site description

The Shuangqiao farm which was founded in Nov. 1949 in Jiaxing town in Zhejiang province of China is chosen as research area. The farm is also agriculture demonstrate garden in Zhejiang province. The area, in which field is agglomerate and ditch system is reticulate and ditch gradient is smaller than 0.2%, locates in the plain of Hangjia Lake. The ditch keeps perennially some water and is dry in December to next March. The period of July to October when the rainfall is more typical and rainfall is between 50mm to 100mm is chosen as the research period. This rainfall is about 85mm, maximal rainfall intensity is about 40mm/h and typical rainfall time is about two hours in the area and is used in this paper.

1.2 Methods

The distribution of ditch is showed in Figure 1. The cross sections are set per 30m along the stream direction and the space between the last two sections (the 5th and 6th section) is 100m. The total length of Ditch Two is 350m. After rainfall, the water was sampled daily and the velocity and quantity of flow of five days were measured which are showed in Table1. The samples were collected with 200ml PVC bottle , kept in 4℃and was measured the next day. Concentration of TN and TP are analyzed using standard methods (SEPAC, 2002).

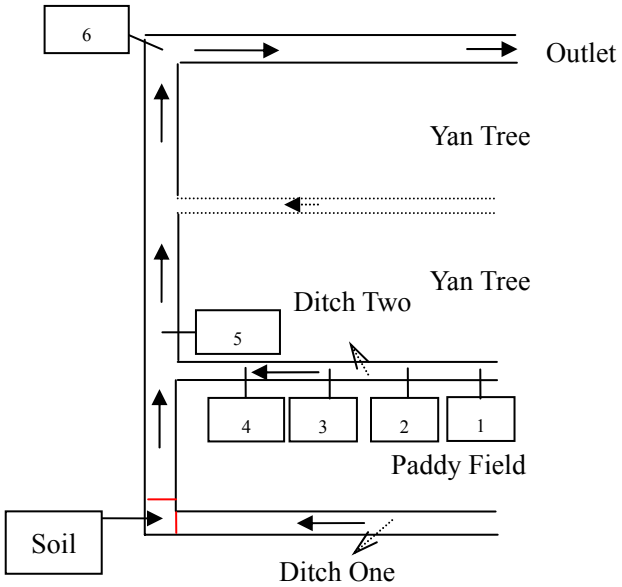

Figure 1. Distribution of cross section in ditch

Table 1. Velocity and quantity of flow in five days after rainfall

time	1st day	2nd day	3rd day	4th day	5th day
V/(cm·min^{-1})	2.5	1.6	0.8	0.3	0.2
Q/(L·min^{-1})	0.625	0.4	0.2	0.075	0.05

2. Results and Discussions

2.1 Variation of TN and TP concentration along ditch section

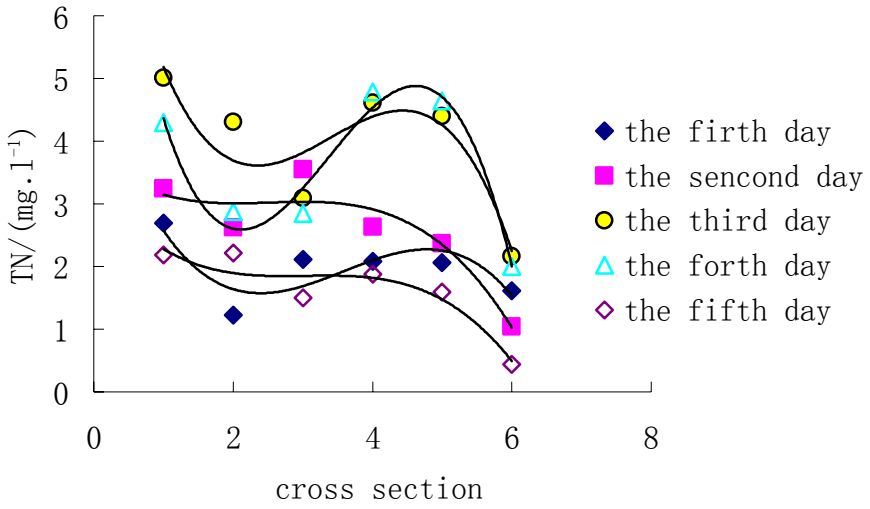

Figure 2. Variation and its simulation of TN concentration along cross section

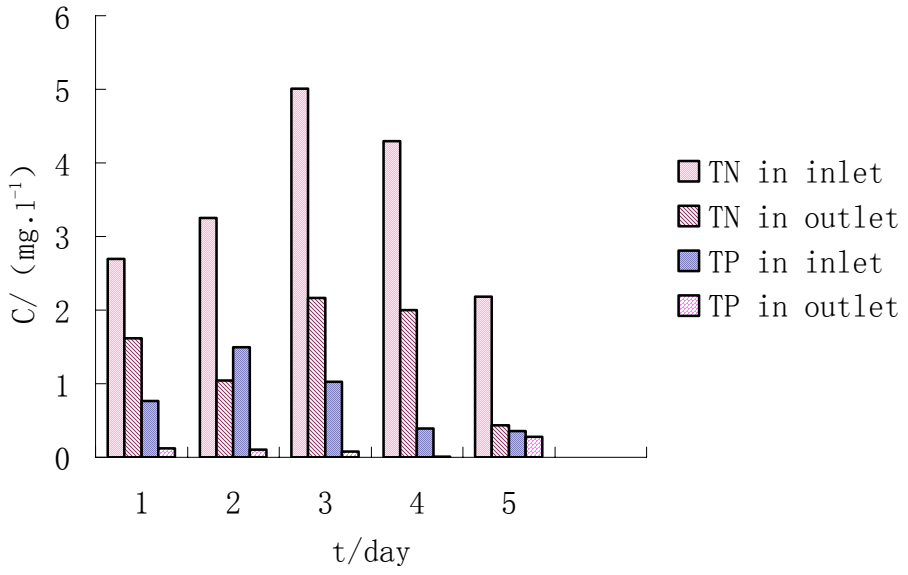

Figure 3. Contrast of TN and TP concentration in inlet and outlet

The transfer and transformation of nitrogen and phosphorus is very intricate especially in rainfall and its intercept mechanism is hardly studied, therefore, the variation of TN and TP concentration may be help to

open out the transfer and transformation of nitrogen and phosphorus along the ditch. From the Figure 2, the TN concentration in inlet and outlet were between 2 to 5mg·l^{-1} and between 1 to 3.5 mg·l^{-1} respectively. From Figure 2 and Table 2, the simulation result of TN along the ditch indicated that TN concentration variation along cross section was according to the cubic equation and the correlation coefficient is about 0.6-0.9. By comparing TN concentration in inlet with that in outlet (Figure 3), TN concentration decreased at a rate of 40 to 70% which indicated that the agriculture ditch can intercept nitrogen even if under the condition of rainfall. In rainfall, TN concentration variation was not simple beeline but cubic equation. TN concentration along cross section was not degressive, inversely, it increased a little especially at the 5th section. This may be that there is a big branch ditch. In short time, the runoff was large which caused large loss of nitrogen, so the TN concentration in main ditch increased.

Figure 4 showed the variation and its simulation results of TP concentration along cross section. From Figure 4, the TP concentration in inlet was 0.3-1.5 mg·l^{-1}and that in outlet was 0.01-0.3 mg·l^{-1}, and the variation of TP concentration along cross section was degressive. Combined with Table 2, the simulation result indicated that TP concentration had the descending variation of the exponential curve on the whole. Comparing the TP concentration in inlet with that in outlet in Figure 2, we can find that the agriculture ditch can intercept effectively phosphorus element and the phosphorus decreased at the rate of 20%-80%. The TP concentration variations along cross section at different time were alike which indicated that TP concentration variation along cross section was very regular, what's more, the change was the descending variation of exponential cure on the whole, but it had a little change as time gone.

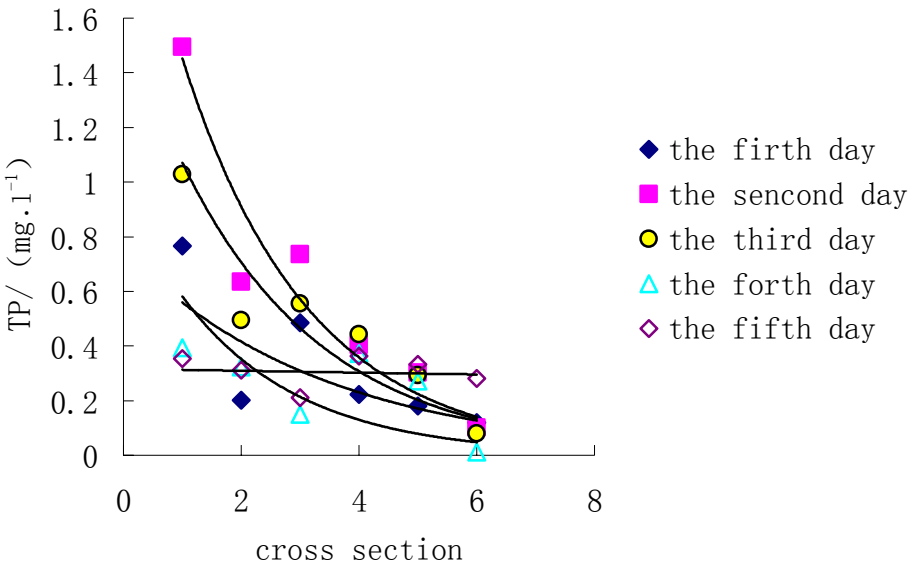

Figure 4. Variation and its simulation of TP concentration along cross section

Table 2. Simulation result of TN and TP concentration along cross section

time	TN		TP	
	Simulation equation	R^2	Simulation equation	R^2
1st day	$y = -0.1031x^3 + 1.1099x^2 - 3.5416x + 5.1067$	0.6714	$y = 0.7514e^{-0.2959x}$	0.6472
2nd day	$y = -0.0509x^3 + 0.3865x^2 - 0.9413x + 3.7536$	0.867	$y = 2.3173e^{-0.4669x}$	0.9023
3rd day	$y = -0.1938x^3 + 1.9696x^2 - 6.0427x + 9.4496$	0.8278	$y = 1.6241e^{-0.4164x}$	0.8144
4th day	$y = -0.293x^3 + 2.9582x^2 - 8.5826x + 10.281$	0.9541	$y = 0.9568e^{-0.4996x}$	0.4618
5th day	$y = -0.008x^3 + 0.0987x^2 - 0.5048x + 2.6615$	0.6768	$y = 0.3163e^{-0.0111x}$	0.0109

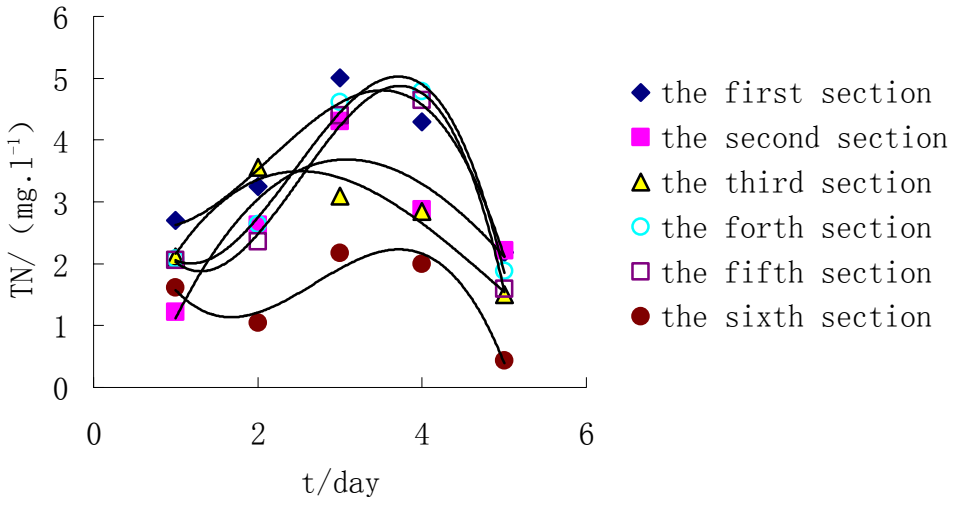

Figure 5. Variation and its simulation of TN concentration with time

2.2 Variation of TN and TP concentration with time

From the Figure 5, TN concentration of every section on the first day was low at 1-3 mg·l^{-1}, increased on the second day, and reached maximum on the third day or forth day, then fell gradually, at last, stabilized at 1-2 mg·l^{-1}. The simulation result indicated that, TN concentration variation with time matched the cubic equation well and the peak value occurred generally on the third day or forth day. The sediment sorption, nitrification-denitrification, macrophyte uptake, infiltration were the main processes by which nitrogen in the agriculture ditch was intercepted (Copal, 1999; Jansson, 1994). After rainfall, a lot of particulate nitrogen (PN) combined with the clay in the process of the transfer and transformation and then deposited, so the sediment can intercept lots of PN. Nitrification-denitrification was the main process that can wipe off nitrogen from water body. The agriculture ditch had the condition of nitrification-denitrification (aerobic area and anaerobic area, enough carbon and

nitrogen nutrients, and so on) (Schade, 2002), so nitrogen may be transformed into N_2, NH_3, N_2O and wiped off from water body. In growth season, the emergent microphyte and macrophyte in ditch had a great deal uptake to inorganic nitrogen. Macrophyte root released oxygen at the root zone and the nitrification-denitrification of sediment was strengthened, so nitrogen in ditch was transformed into gas and escaped from water body. But, from Figure 5, TN concentration increased only at the beginning and reached maximum and then fell, which showed that the ditch system was not stable. The convergence of rainfall runoff in short time affected the nitrogen transfer and transformation, but the effect disappeared gradually with time which showed that the ditch system had the ability of anti-jamming and restoration and can gradually restore stable in certain time.

From Figure 5 and Table 3, TN concentration of every section on the whole reached the maximum on the third day or forth day after rainfall. The result provided the optimal control time for control efficiently the loss of nitrogen. The same variation of TN concentration of every section indicated that nitrogen transfer and transformation in the agriculture ditch was not affected by space and location (cross section).

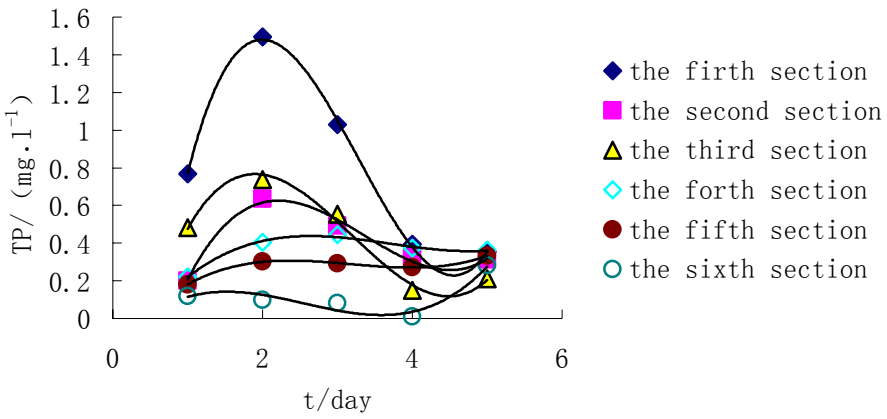

Figure 6. Variation and its simulation of TP concentration with time

Figure 6 describes the variation of TP concentration with time at different sections. On the first day TP concentration of every section was low at 0.1-0.7 $mg \cdot l^{-1}$, while on the second day increased to maximum and then fell smoothly. From the simulation result of Figure 6 and Table 3, we can see that the variation of TP concentration was the cubic equation with time and the correlation coefficient was great. Contrast to nitrogen, the sorption was the main process of transfer and transformation of phosphorus (Jorgenson, 1996). During rainfall, because of the convergence of rainfall runoff as well as the in-stabilization of ditch, the phosphorus was not clearly transformed and the TP concentration increased and then reached maximum, but the absorption of the particle matter in water body and sediment and the ability of anti-jamming and restoration of the agriculture ditch made the transformation of phosphorus be resumed and the TP concentration reduce. Another reason that phosphorus decreased was that phosphorus decomposed in anaerobic condition and transformed into PH_3 then escaped from water body (Jiang, 2004).

The simulation result of Figure 6 and Table 3 showed that the TP concentration of every section

reached the maximum on the second day after rainfall, so the second day after rainfall was the optimal time of controlling efficiently phosphorus loss. The similar TP concentration variation at different sections with time indicated that the agriculture ditch can intercept efficiently phosphorus and the interceptive variation was not affected by the space and location (cross section).

Table 3. Simulation result of TN and TP concentration with time

section	TN		TP	
	Simulation equation	R^2	Simulation equation	R^2
1st section	$y = -0.217x^3 + 1.3956x^2 - 1.7743x + 3.2231$	0.9394	$y = 0.149x^3 - 1.4627x^2 + 4.0544x - 1.9703$	0.999
2nd section	$y = 0.0418x^3 - 0.8926x^2 + 4.3077x - 2.3373$	0.8497	$y = 0.0614x^3 - 0.^{6186x2} + 1.8348x - 1.0714$	0.9848
3rd section	$y = 0.0667x^3 - 0.9847x^2 + 3.6887x - 0.6132$	0.937	$y = 0.0749x^3 - 0.7175x^2 + 1.9144x - 0.7947$	0.9865
4th section	$y = -0.3759x^3 + 2.7597x^2 - 4.9575x + 4.629$	0.9932	$y = 0.0168x^3 - 0.1868x^2 + 0.6346x - 0.2452$	0.9899
5th section	$y = -0.4191x^3 + 3.1642x^2 - 6.1096x + 5.3932$	0.9928	$y = 0.0177x^3 - 0.1685x^2 + 0.5007x - 0.1685$	0.999
6th section	$y = -0.0071x^3 + 0.2583x^2 - 0.8749x + 2.1543$	0.8421	$y = 0.0283x^3 - 0.2169x^2 + 0.4634x - 0.1607$	0.9318

Conclusion

1. The agriculture ditch in itself is instable and will change under the influence of outside conditions (rainfall), but it has the ability of anti-jamming and restoration and can restore the transfer and transformation of nitrogen and phosphorus. This is an important characteristic of agriculture ditch.

2. The agriculture ditch can still intercept efficiently nitrogen and phosphorus in rainfall. Because of the convergence of outlet along cross section, the TN and TP concentration change along the cross section. TN concentration variation along cross section was according to the cubic equation and TP concentration variation is on the whole the descending variation of the exponential curve along cross section.

3. The concentration variation of both TN and TP are the cubic equation with time. The peak vale of TN concentration of every section was on the third day or the forth day. The variation of TP concentration is more relatively stable and the variation trend is on the whole alike. Furthermore, the peak vale of TP concentration was mostly on the second day. Therefore, this indicates that the decomposing process of every section in agriculture ditch has the same trend on the whole and the variation trend will not change a lot by space and location (cross section).

4. The TN and TP concentration variation of every section indicate that TN concentration reached maximum on the third or forth day however the peak vale of TP concentration was on the second day. Therefore, it provides the optimal control time for

controlling efficiently the loss of nitrogen and phosphorus after rainfall, namely, we can control efficiently nitrogen element on the third or forth day and control phosphorus element on the second day after rainfall.

Because of the particularity and complexity of agriculture ditch, each researcher has the different opinion to the interception of nitrogen and phosphorus and takes the different study method and means, but the fact that agriculture ditch can intercept nitrogen and phosphorus has been affirmed. With the continuous research, the transfer and transformation of nitrogen and phosphorus will be further clarified.

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References

1. Yan Weijin. Phosphorus and nitrogen transfers and runoff losses from rice field wetlands of Chaohu Lake. Chinese Journal of Applied Ecology. 1999;10(3):312~316.
2. Canter LW. Environmental impacts of agricultural production activities. Lowis Publishers, Inc. 1986.
3. Coote DR, MacDonald EM, Dickinson WT, et al. Agriculture and water quality in the Canadian Great Lakes BasI. Representative agricultural watersheds. J Environ Qual. 1982;11:473~481.
4. Jorgensen SE, Mitsch WJ. Application of ecologic modeling in environmental management, part B. Elsevier Scientific Publishing Company. 1983.
5. Tiessen H. Phosphorus cycles and transfers in the global environment. SCOPE, Newsletter. 1995;47:1~4.
6. Jiang Cuiling. Purification Capacity of Ditch Wetland to Agricultural Non-point Pollutants. Environment and Science. 2004;2(25):200.
7. Copal NB. Natural and constructed wetland for wastewater treatment: potentials and problems Wat Sci Tech. 1999;40(2):27~35.
8. Jansson M, Andersson R, Berggren H. Wetland and lakes as nitrogen traps. AMBIO. 1994;23(6):320~325.
9. Schade JD, Marti E, Welter JR, et al. Sources of nitrogen to the riparian zone of a desert stream: implications for riparian vegetation and nitrogen retention. Ecosystems. 2002;5:68~79.
10. Jorgenson SE, Nielsen SN. Application of ecological engineering principles in agriculture. Ecological Engineering. 1996;7:373~381.
11. State Environmental Protection Administration of China. Monitoring and Analytical Method for Water and Wastewater, the 4th version, Beijing: Environmental Science publishing of China, 2002.

Consulting Market Evolution and Adjustment of Hydropower Project in China

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Abstract: This paper establishes a model of evolutionary game theory in the condition of unconstrained market to illustrate the basic evolutionary law of consulting market according to the developing process of Chinese consulting market in hydropower project construction. Through analyzing the parameters, this paper proposes some strategies and methods to optimize the market behavior. Then some market constraint such as punishment laid on consulting company for violation behavior by chief department within certain industry is added into the model. Through improvement and further analysis of the model, it shows that the consulting of chief department is crucial in the process of creating good market environment and retreating from "lock" state. It also puts forward some beneficial suggestions on how to establish a regular consulting market with a healthy developing cycle. [The Journal of American Science. 2006;2(3):66-73].

Key words: evolutionary game theory; consulting market; incomplete information

Contents

1. Introduction

Since the implementing of project construction consulting (supervision) system in 1988, it has been playing a more and more important role in project construction. Meanwhile the consulting system has drawn much more social attention and gained universal recognition. On the whole, the result of its implementation is remarkable but there are still some problems. Some related investigation shows that the consulting system in our country is still in its primary stage. There are two main problems. Firstly, the construction consulting market is not regular and its competitive system is not complete. Secondly, the management level is not high enough and consulting engineers are not fully qualified (Guohui Jiang, 2005). So, what is the inner mechanism that causes such a phenomenon? How to solve the problem and form a good consulting market criterion? This paper just uses the evolutionary game theory to analyze the developing rules of consulting market in order to find out the ultimate reasons causing the existing problems. It also proposes some corresponding methods to achieve healthy and regular development of construction consulting market in our country.

Evolutionary game theory is one kind of game theory model, which is about mutual reaction of behavior strategy and iterative process. Its basic

principle is the theory of "survival of the fittest" in biological evolutionism. In the model, every behaving individual can choose different behavior strategies so as to gain corresponding "payment" and "adaptation degree". After a period of iteration, the adopting range of one kind of behavior can cause changes in its adaptation degree, which can make the behaviors of one individual begin to evolve according to the "survival of the fittest" principle.

The evolutionary game theory believes that the limited rational economic subject hardly knows whether it is in advantageous state or not exactly. Instead, it uses the most advantageous strategy to imitate gradually so as to reach a balanced state. Suppose there are many participants in one system. Then every game is going to carrying out stochastic sampling from all the participants. The selected participant will take part in the element game and can gain interest. Then the above process will be repeated. The evolutionary game theory is just the tool for analyzing such a process above. And it studies how the participants choose and adjust strategies during the whole evolutionary process, whether there is a stable balance point (Zhaohan Sheng , 2002) or not and how to explain the point.

In our present project construction consulting market, the consulting companies usually adopt the way of bidding to obtain commission contract. Whether a project legal entity decides to consign a task to a consulting company or not depends on the qualification and efforts of consulting engineers appointed by that company. If the consulting engineer is not quite qualified or even cooperates with the contractor to deceive the project legal entity, it will do much harm to the legal entity's interests. The large or middle size project legal entities often request the consulting companies to consign qualified consulting engineers according to certain promise. Although some entities adopt appointment guarantee, problems such as lower bail still exist. Some consulting companies often pretend to consign a high qualified consulting engineer when submitting a tender, but just send a common one in practice. They abuse the promise in order to gain consulting contract, which can make much incommodiousness to the management of legal entities and even can make them suffer some loss that is not deserved. In addition, in the incomplete information consulting market it is really very difficult for the project entities to choose a satisfied consulting engineer with high qualification. What they can only do is to surmise the general situation of the market through "study" and then revise their own behavior strategies to enhance the effectiveness of the consulting commission. Some consulting companies even use the way of using high-qualified consulting engineer to bid and appointing low-qualified personnel to gain extra income because of false information. As sometimes information is not complete, it often affects the choice of behavior strategies for both the project legal entity and consulting company. As a result, the market will be locked in an unhealthy state.

2. Establishment and Analysis of the Model

Suppose it happens in a natural market, which means a market without any constraint. The project legal entity and consulting company will begin to discuss the strategies. Suppose the legal entity will tend to choose a higher qualified consulting engineer for the sake of his own interest but during the negotiation process two kinds of performances will appear (Yuyin Yi, 2003 & Tiaojun Xiao, 2004): ① complying with appointment guarantee (B_1); ② violating appointment guarantee (B_2). Likewise, suppose the consulting company will select a high qualified consulting engineer and make up an appointment at first. But in fact two kinds performance will take place as well: ① complying with his promise (E_1); ② violating his promise (E_2).

Suppose the consulting company really violate his promise and send unqualified engineer after signing the contract. This hypothesis is quite reasonable. More interests can be attained if the consulting company

appoints unqualified consulting engineer. So they will tend to violate his promise if there is not any other constraint. Suppose three situations: E_1 and B_1 can not reach an agreement and both of them will suffer loss to some extend; B_1 and E_2 sign the guarantee contract and both attain deserved interests; E_2 and B_2 share the same strength so it is half to half to assume appointment guarantee or to pursue promise contract. Table1 shows the income matrix of project legal entities and consulting companies.

Table1. The income matrix of project legal entities and consulting companies'

Consulting company / Legal entity		Complying with promise	Violating promise
		E_1	E_2
Complying with appointment guarantee	B_1	$-C_2$, $-C_1$	G_B, G_E
Violating appointment guarantee	B_2	G_B-E, G_E+E	$G_B-E/2$, $G_E+E/2$

In Table1, $C_1>0$ equals to the legal entity's loss when no agreement is reached; $C_2>0$ equals to consulting company's loss when he fails to attain consulting contract; $G_E>0$ equals to consulting company's income when he gets the consulting contract; $G_B>0$ equals to legal entity's income (Since the legal entity consigns consulting company, which can reduce his own amount of managers, he gets this kind of income); $E>0$ equals to the consulting company's extra income if he do not assign high qualified consulting engineer.

Suppose among all the legal entities the proportion of those who adopt strategy B_1 is p and those who adopt strategy E_1 is q. According to the Malthusian dynamic equation, which means the increasing rate of strategies equals to its adaptation degree, if the adaptation degree of one strategy is higher than the average level the strategy will increase (Friedman D, 1998). The dynamics equation is as followings.

$$\dot{p} = p(1-p)(1,-1).A(q,1-q) \qquad (1)$$

$$\dot{q} = q(1-q)(1,-1).B(p,1-p) \qquad (2)$$

In the two equations,

$$A=\begin{bmatrix} -C_2 & G_B \\ G_B-E & G_B-E/2 \end{bmatrix}$$ and

$$B=\begin{bmatrix} -C_1 & G_E+E \\ G_E & G_E+E/2 \end{bmatrix}$$ refer to the income matrix of legal entities and consulting companies. So the copy dynamic equation of consulting market is as following.

$$\dot{p} = p(1-p)\left[E/2-(G_B-E/2+C_2)q\right] \qquad (3)$$

$$\dot{q} = q(1-q)\left[E/2-(G_E+E/2+C_1)p\right] \qquad (4)$$

Proposition1: if $G_B+C_2>E$, system (3)~(4) indicate that in plane M=$\{(p, q); 0\le p, q\le 1\}$ there are 5 balance points, which are unstable point (0, 0) and (1, 1), stable point (0, 1) and (1, 0), saddle point.

$$F=\left(\frac{E/2}{G_E+E/2+C_1},\frac{E/2}{G_B-E/2+C_2}\right)$$

Proof: only this situation $G_B+C_2>E$ is taken into consideration. If $G_B+C_2\le E$, the following equation can be achieved:

$$\dot{p} = p(1-p)\left[E/2-(G_B-E/2+C_2)q\right]$$

$$= p(1-p)\left[(1-q)E/2+(E-G_B-C_2)q\right]\ge 0$$

So, in plane M, p will increase from 0 to 1 monotonously. At this time all the legal entities will comply with the appointment guarantee contract, other wise they will be forced to exit the market. This kind of market state can not be maintained, so it is not taken into account in this paper.

If $G_B+C_2-E>0$, obviously system (3)~（4） contain 5 balance points: (0,0), (1,1), (0,1), (1,0), and *F*. Then the partial stable analysis (Xiaoxin Liao, 2000) will be carried out and such a conclusion will be reached: （0,0) and (1,1) are unstable source outward point; (0,1) and (1,0) are stable inward point; *F* is saddle point. The proof finishes now.

According to proposition1, phase of system (2) is showed as following Figure 1.

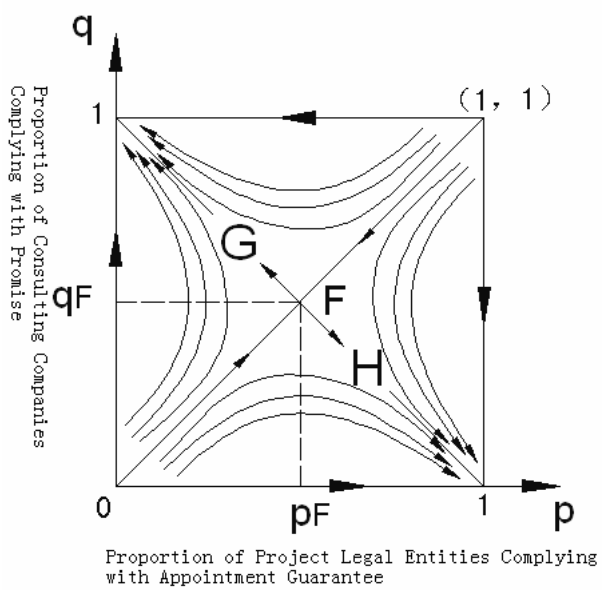

Figure 1. Evolution Rule of Consulting Market without Constraint

After analyzing Figure 1, we know if the initial state is within region G the system will converge at point (0,1), that is to say, all the legal entities fail to comply with the appointment guarantee contract, while all the consulting companies assume promise contract. So it will be evolved that the consulting companies will not dispatch high-qualified consulting engineers or even do not dispatch at all. If the initial state is within region H (the right down area of the line through point (0,0), (1,1) and F) the system will all converge at point(1,0) , namely all the legal entities comply with appointment guarantee contract, while all the consulting companies fail to comply with promise contract. So it will be evolved that consulting companies will surely dispatch high qualified consulting engineers.

Thus after a long-term "natural" evolution the result will be entirely different. Two kinds of market will appear. One is a normative market, in which legal entities assume appointment guarantee contract and consulting companies dispatch highly qualified consulting engineers based on the contract. The other one is a market whose behavior is not standard, in which legal entities carry out appointment guarantee contract but consulting companies do not dispatch highly qualified consulting engineers. Meanwhile, both the two markets are in stable state in the evolution. If any participant performs oppositely, he will not survive due to the selection of the market.

Although the normative consulting market is what we expect, it is not quite easy to make the consulting market develop in a fine circulatory direction. In the following part of this paper, we will analyze the parameter of the model to figure out its influence on market evolution and try to optimize the consulting market.

3．Parameter Analysis and Control

Suppose the initial condition of the system is stochastic and is distributed uniformly within plane M={(p，q)；$0\leq p$，$q\leq 1$}. The purpose of parameter analysis is to confirm whether the change of parameter can cause reduction of the G region and expansion of H region and meantime force point *F* move left upward. If these changes appear the system is supposed to be stable at point (1,0). In the following part of this paper, the influence of some parameter's changes on consulting market performance is discussed and some valid control methods are proposed.

1) Legal Entity's Income G_B for Consigning Consulting Company

At present, the consulting fee of domestic large and middle scale projects is mostly charged according to

its proportion in the whole project investment Generally, it is based on the No.497 Document issued by Chinese Price Bureau and Ministry of Construction jointly in 1992 and *Market-set Price Range of Hydropower Project construction supervision* published by the Hydropower Construction Supervision Branch which belongs to China's Construction Supervision Association in 2003. But different rate of consulting fee often can give different impact on market performance. When G_B increases (the consulting fee rate falls), coordinate p_F of saddle point F dose not change but q_F reduces, which means that saddle F moves down plane M so region H will reduce but region G expands. On the other way round, when G_B reduces (consulting fee rate increases), region H expands but region G reduces. It shows that if the consulting fee rate increases within a proper scope, the consulting companies will get more normal income. So the possibility for consulting companies not to dispatch qualified consulting engineers will decline and the market performance tends to be more rational. If the consulting fee rate falls, consulting company's normal income will be reduced so they will tend to dispatch consulting engineers without high qualification.

2) Extra income E

When E increases, p_F and q_F will go up as well. But q_F rises more than p_F, so saddle point F will move to the upper right corner of M region. Then region H expands while region G reduces. When E decreases, region H reduces while region G expands. Apparently if a consulting company does not dispatch high-qualified consulting engineer it will gain more extra income. So consulting companies will more likely tend to comply with the promise contract. But actually at this time, the possibility for project legal entities to comply with appointment guarantee contract increases as well. Then it is obvious that the self-adjustment function of the market has come into strong effect, which can contain the consulting companies' negotiating motivation.

3) Consulting companies' loss C_1 when agreement is not reached

When C_1 increases, p_F decreases but q_F keeps stable, namely point F will move to the left of plane M. So the area of H expands but the area of G reduces. Whereas when C_1 decreases, the area of H reduces but the area of G expands. It is obvious that the increasing of loss C_1, which results from failing to achieve the contract, will be effective on reducing the possibility of consulting companies to comply with contract and not dispatch high qualified consulting engineer. In fact, the consulting companies' loss, which results from failing to achieve contract, is tightly related with the scale of consulting market and construction market. If the ongoing projects in construction market are limited, the project legal entities will tend to monopolize the market. So it is not easy for the consulting companies to attain consulting work and the loss, which results from failing to achieve contract, will increase. Under this situation, consulting companies have to extend their business to other consultation fields so as to reduce their loss resulted from failing to achieve contact.

4) Legal Entities' Loss C_2 When Contract is not Achieved

When C_2 increases, p_F keeps stable but q_F decreases, which shows that point F will move down plane M. So region H will reduces but region G will expand. Whereas when C_2 decreases, region H will expand but region G will reduce. So when the project legal entities' loss, which results from failing to achieve contract, increases, it is more difficult for them to ask for appointment guarantee contract. Then the possibility for consulting companies to assume promise contract will increase. The project legal entities' loss which results from failing to achieve the contract is related with the scale of consulting market. If the market is small, the consulting companies are

limited. At the same time, it tends to be difficult for the project legal entities to find proper consulting companies, so their motivation of complying with appointment guarantee will decrease.

All in all, the consulting market holds its own operating disciplinarian and this model just reflects the basic operating disciplinarian. Based on such kind of disciplinarian, the consulting market can be controlled limitedly and will go ahead in a much better direction.

4．Improvement of the Model

In this model discussed above, the management and supervision of charge department within this industry is not involved, so the consulting companies will tend to not dispatch high qualified consulting engineers in order to gain extra income. If the charge department is involved and can give certain punishment for the consulting companies' violating behavior, when any violation happens project legal entities can appeal to charge department. If any violating behavior is testified, charge department can order the consulting companies to correct their behaviors and give certain punishment. Under this situation, suppose the consulting company's loss is R and the project legal entity's cost for supervising the consulting company's performance is K>0. So the income matrix of both them is showed as Table 2.

Table 2. Improved Income Matrix of Strategy Choice

Consulting company / Legal entity		Complying with promise	Violating promise
		E_1	E_2
Complying with appointment guarantee	B_1	$-C_2$，$-C_1$	G_B，G_E
Violating appointment guarantee	B_2	G_B-K，G_E-R	$G_B-K/2$，$G_E-R/2$

The dynamics equation of consulting market becomes as following:

$$\dot{p} = p(1-p)\left[K/2-(G_B-K/2+C_2)q\right] \quad (5)$$

$$\dot{q} = q(1-q)\left[-R/2-(G_E+R/2+C_1)p\right] \quad (6)$$

Proposition 2: If $K<G_B+C_2$, the system (5)~(6) indicate that there are 4 evolution game balance points in the plane M={(p，q)；$0\le p$，$q\le 1$}, which are saddle point(0,0) and (0,1), inward point(1,0) and source outward point(1,1).

Proof: as the same reasons in proposition1, here only $K<G_B+C_2$ is taken into consideration. When $K<G_B+C_2$, it is obvious that system (5)~(6) only contain 4 stable points in plane M={(p, q)；$0\le p$, $q\le 1$}, which are (0,0), (0,1), (1,0), (1,1). According to dynamics analysis, point (0, 0) and (0,1) are saddle points; point (1,0) is stable inward point; but point (1,1) is unstable source outward point.

From proposition2, the phase diagram of system (5)~(6) is showed in Figure 2.

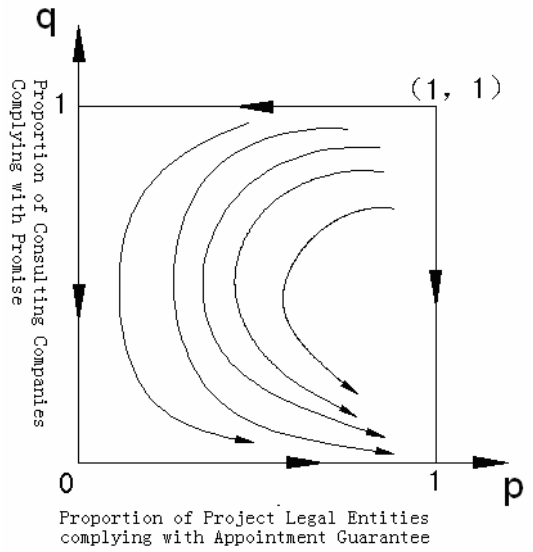

Figure 2. Evolution Law of Consulting Market under Supervision of Charge Department

In Figure 2, setting out from any original state in

plane M, the system will converge at point (1,0). Under this situation all the project legal entities will comply with appointment guarantee contract or promise contract, and meantime they will adopt some ways of supervision to protect their interests. And all the consulting companies will dispatch high qualified consulting engineers according to the contract. So the consulting companies will tend to perform more legally and rationally under the threatening of punishment. And the consulting market will evolve into a normative market eventually. But this punishment is closely related with the rate of project legal entities' appeal and the strength of law enforcement. But if some project legal entities are over-tolerant or the charge department cannot execute the law strictly, the violation behaviors will also increase.

5. Conclusion of the Model and its Enlightenment

In Fig1, if the initial state is within region G the consulting market will evolve into a nonstandard market. If it is within region H the market will evolve into a more rational and complete one. Obviously, the evolving path of consulting market sensitively depends on its initial state in some extend. And the evolution of consulting market has the nature of path dependence. So if the initial state comes into region G for some accidental reasons, the market will tend to be inefficient and even go into an unhealthy "lock" state at last. So the project legal entities should strengthen self-protected consciousness and be stricter when selecting consulting companies. It will be much more helpful to request consulting companies to provide the name list of consulting engineers and a complete appointment guarantee contract. If any violation behavior happens, quick notification to charge department is also necessary. If so the market will come into a healthy circle and ends in a rational and complete one.

If the system comes into a sub-excellent or inefficient state, its recovery path depends on the nature of all the factors that form its self-enforcement system. In the consulting market the self-enforcement system for income increasing results from its own effect, such as cooperation effect. As long as the communication between charge department, project legal entity and consulting company is strengthened so as to reach some agreement, the recovery path is realized. As showed in Fig2, under supervision of charge department the consulting market will develop itself along a healthy circular path and evolve into a more normative market. Even though the market begins to go along an inefficient path, it can also get out from the "lock" state and achieves recovery path as long as the charge department executes the law rigidly and be unanimous with project legal entity.

Since the reform of project construction management system still stays in its beginning tage in our country now, many violating behaviors have come out. In order to make the market more normative and evolve along a healthy path, the selection of a definite project management system is necessary. Through study effect, cooperation effect and supervision, the system for income increasing is established. Then after a complete selection by the market, a more clear and inspiring project management system with constraint function comes into being and replaces the former one so as to avoid "closedown" state. In such a circular process, income will increase gradually and the consulting market will finally go along a healthy path and evolve into a normative market.

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References

4. Guohui Jiang. Water Recourse Project Supervision. China: Beijing. Water Power Press. 2005.
5. Zhaohan Sheng, Depeng Jiang. Evolutive Economics, China: Shanghai. Sanlian Bookshop. 2002.
6. Yuyin Yi, Tiaojun Xiao. Evolution and Control of Credit Market of China. Journal of Southeast University 2003; Vol. 33 (4): 483~486.
7. Tiaojun Xiao. Game Theory and its application. Shanghai Sanlian Bookshop 2004.
8. Friedman D. On Economic Applications of Evolutionary Game Theory. Journal of Evolutionary Economics 1998(8): 15～43.
9. Xiaoxin Liao. Theory and Application of Stability for Dynamical Systems. Beijing: National Defence Industry Press 2000.

Functional Implications of the Universal Theory of Relativity

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Abstract: It is interesting to note that in the natural sciences scientists are not interested in the universe as a whole including themselves, but direct their attention to some parts of the universe and make that the object of their studies. It is only with the development of the Universal Theory of Relativity (UTR) by this author that for the first time we have come to a stage where we can examine the universe as a whole. Normally we make relative studies among different parts of the universe. In the UTR, it has become possible to examine the universe as a whole in relation to the preferred frame that surrounds it and has unknown or unlimited properties. . [The Journal of American Science. 2006;2(3):74-84].

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Structure of the Universe

It is interesting to note that in the natural sciences scientists are not interested in the universe as a whole including themselves, but direct their attention to some *parts* of the universe and make that the object of their studies. It is only with the development of the Universal Theory of Relativity (UTR) by this author that for the first time we have come to a stage where we can examine the universe *as a whole*. Normally we make relative studies among different parts of the universe. In the UTR, it has become possible to examine *the universe as a whole in relation to the preferred frame* that surrounds it and has *unknown* or unlimited properties.

There have been several questions that have been baffling physicists throughout the history of science. A great number of solutions have been proposed but none has been fully convincing. Some of these questions are:

1. Why do the planets revolve round the Sun? Why does the gravitational pull of the Sun not make these planets ultimately unite with it? What carries the centrifugal forces that make the planets orbit round the Sun in a specific manner and does not allow them to fall into it?

2. Why do all planets and minor planets revolve around the Sun in slightly ellipsoid orbits in almost the *same plane* and in the *same direction* in which the Sun rotates? And why most of the planets, minor planets, satellites (with few exceptions) spin in the *same direction* in which the Sun rotates?

3. Why does the ring of the Saturn always point in the *same direction* in reference to the fixed stars?

4. Why is the universe uniformly homogenous on a large scale? As Hawking says: *"Why does it look the same at all points in space and in all directions?"*

5. Why is the temperature of the microwave background radiation almost the same?

6. Why does the universe seem to be ever expanding at just above the critical rate? Or what does prevent the universe from collapsing owing to its own gravity?

7. Why was the early universe so hot?

8. How did the universe originate and how would it come to an end?

9. What causes energy-mass equivalence ($E=mc^2$)

10. What causes quasars to have so much energy as they seem to possess?

11. What is the reality of Dark Matter and Dark energy?

12. What is the nature of different forces of nature and how they work?

Let us first analyse the solar system. When we study the revolutionary and rotational motions of the different types of bodies that comprise the solar system, namely the Sun, the Planets, the Minor Planets and Satellites, the following important facts emerge:

1. *All* planetary orbits are *almost* circular and lie in the *same* plane. These orbits tend to be more elliptical in case of some of the planets than the others. Another important feature is that the planets move around the Sun in the *same* direction as the Sun itself rotates.

Except the orbit of Pluto, which has a tilt of 17 degrees, all the planets have a tilt of less than 7 degrees.

2. The satellites also move about the planets in *almost* circular orbits, *mostly* in the *same* plane.

3. The terrestrial planets (Mercury, Venus, Earth and Mars) are relatively small, dense and near the Sun. The giant planets, on the other hand, that include Jupiter, Saturn, Uranus and Neptune, are relatively large, though of low density, and are farther from the Sun.

1. Most of the planets also spin about their own axis in the *same direction* as they rotate about the Sun. The exceptions are Uranus and Venus. The axis of Uranus, nearly in the plane of the elliptic, is tilted over so far that it rotates in a retrograde manner. The Venus rotates extremely slowly in the retrograde fashion.

5. Most of the satellites also revolve in the *same direction*. These include Moon, the Earth's satellites, the two satellites of Mars, about a dozen satellites of Jupiter and 9-10 satellites of Saturn. However, some satellites revolve in the retrograde directions.

6. All the asteroids which number more than 1700 revolve around the Sun in the *same plane* and in the *same direction* as the planets. Most of them move in near circular orbits. But some of them move in elongated orbits.

Now, this is a unique situation. There must be some reason why all the bodies - planets, asteroids, comets and satellites, should orbit the Sun in the *same plane* and why an overwhelming majority of them should spin in the *same direction* in which the Sun rotates. Why did they not occupy orbits in different planes, which could have made their places even safer? Then there would have been lesser chances of their orbits crossing one another. Nebular Hypothesis tries to provide an answer, but the rotation of the whole Uniglobe proposed by this theory provides a more plausible answer. Even if the nebular hypothesis were correct, this we have to find an explanation of *why all the components of the gases that made the solar system would align in a way that would produce a system like what we see today*. The Sun's gravitational pull is almost the same in all the innumerable planes possible having tilts from 0 to 360 degrees. Does this not point to the possibility of some *major external factor* trying to keep them in the same plane and make them revolve and rotate in the same direction? If the Uniglobe *as a whole* is also rotating on its axis, the solar system in its entirety must be moving with a great speed in a particular direction. This motion would *almost* be linear considering the huge size of the universe. It is only natural for the individual revolutions and rotations of the bodies belonging to the solar system to correspond to the direction of the movement of the portion of the Uniglobe in which the solar system exists.

Let us try to understand this with the help of an analogy. Let us suppose, a big stone is thrown in a still water. It will sooner or later sink to the bottom and the path travelled by it will be almost *vertical*. If on the other hand, an object is thrown in a fast moving river, it will also move in the direction in which the river is flowing. This movement will be opposed by the gravitational pull of the Earth, which will try to make it sink down. How long the object takes to settle down on the bottom of the river, and what path it follows, will obviously depend on the *depth* of the water and the *speed* of the river. The greater the depth of the water and the faster the speed of the river, the greater will be the time taken by the object to settle down; the bigger will be the distance it covers after its fall in the river. The path travelled by the stone before touching the bottom would be a part of a circle or an *ellipse*. If several stones are thrown at different points in a fast flowing river, the paths followed by them before touching the ground would all correspond to the direction of the flow of the river. It can also bee seen that an object with greater density will cover lesser distance before settling down than one with smaller density will.

Now, let us suppose, instead of one stone, two stones, one much heavier than the other, mutually connected with a long cord, are thrown together in the river. What path would they follow before settling down at the bottom? The direction of the flow will try to take both of them forward with the same speed but the heavier stone will move slower than the lighter stone. Had they not be connected with a rod, the lighter stone would have left the heavier stone far behind. But being connected with the rod, this cannot leave the company of the heavier stone. The lighter stone will then go down towards the base of the river as if it is moving around the heavier stone. We can also visualise the case of mutually connected stones of varying sizes, one stone greatly heavier than others, in a fast flowing river. But the analogy is not complete because, in the case of a river, the attraction between the Earth and the stones is very large. Had this attraction been zero and the depth of water immensely large and the attraction between stones appreciable, the system would have perhaps been more comparable with the solar system.

In the solar system, the planets are able to move round the Sun because, on the one hand, they are being *pulled* (directly or through the warping effect) by the gravitational pull of the Sun, and on the other hand, they are being kept at a specific distance due to an *opposite pull* generated by the inertia consequent of the high velocity of the zone. Scientists, till now, have believed that the centrifugal force is supplied by distant rotating

bodies. Heisenberg says: *"Since the centrifugal forces had to be considered as due to physical properties of empty space, as had been discussed before, Einstein turned to the hypothesis that the gravitational forces are due to properties of empty space."* He then says*, "The centrifugal forces in a rotating system must be produced by the rotation (relative to the system) of distant masses".* The UTR suggests that the centrifugal forces are the effect of the rotation of the universe as a whole, of which empty space is also a part. Without the rotation of the Uniglobe, centrifugal forces could in fact not have been sustained, as the influence from distant objects will take time to reach the rotating systems. The presence of the rotation in the rotating system is clearly due to the combined effect of the rotation of the universe, and the centripetal force created by the gravitational effects of the larger body on the small body, whatever way they operate. The former tries to carry the rotating body along with it; the latter curves it towards the bigger object. In fact if the centrifugal force would have been provided by the distant masses, the combined effect of the centrifugal force and the centripetal force would have led ultimately to the suspension of the body *in between those distant objects and the massive body,* rather than its rotating around it. Furthermore, with the expansion of the universe, the centrifugal force would have gradually weakened were the universe expanding, as is commonly believed, the attraction by distant bodies, if any, would constantly become weaker. And because the universe is said to be expanding at a rate of about 75000 Kms/Sec, (much more in the distant regions) this weakening of the attraction by the distant bodies would grow at a considerable rate. This means the orbits of the planets must have kept on contracting at a regular rate. Einstein's idea that the planets only follow the nearest body on a curved path, because the space-time is also curved is also not convincing. Describing Einstein's theory of gravitation, Hawking *says "Particles try to follow the nearest line to a straight path in a curved space, but because space time is not flat, they appear to be bent as if by a gravitational field".* This theory could have been better appreciated if the Sun would have been at absolute rest but the Sun is also moving at a considerable speed, about 360 Kms/sec; so the field around the Sun is not continuously constant but is moving around in a particular direction. It can be argued that, in an expanding universe, the curving effect due to Sun on space-time would also have been constantly increasing. Now, if the particle tries to follow the nearest thing, it must constantly be in the know of the position of that thing, which requires regular communication. Suppose the particle tries to follow the nearest object. Had there been no inertia, they would have continued at the same distance between them in a parallel line. Or they could have moved in spherical orbits in a way that the distance between them would have always remained almost the same. But due to inertia (created by the rotation of the universe), the bigger particle cannot move as fast as the smaller particle. In this case, it becomes imperative that the much *smaller particle rotates round the bigger* particle because that alone would maintain the distance between them to within a specific range.

A very interesting fact that further proves the rotation of the Uniglobe is the *direction of the rings of Saturn.* What is important about the position of the ring plane of Saturn is that, viewed from the Earth and the Sun, the tilt of the rings is continually changing. Twice in each Saturnian revolution, on alternate interval of 13 years 9 months and 15 years 9 months, the plane of the rings passes through the Sun. A few months before and after each such occasion, the plane of the ring must pass through the Earth. This is due to the fact the Earth is near the Sun, as viewed from the Saturn. However, what is most interesting about the plane of the rings of the Saturn, from the point of view of this theory, is that it remains in an *almost fixed position with reference to the fixed stars*. What does this prove? As the rings are mostly in gaseous forms and are not firmly fixed to the surface of the Saturn, they try to remain in the *plane of the rotation of the universe*. Had this not been the case, there is no reason why they should have been continually tilting as viewed from the Sun. Their position as viewed from the Sun, should have remained constant but, in reference to the fixed stars, it should have been continually changing.

It is also interesting to note that *the planets nearer to the Sun rotate around it with higher speed than farther ones*. This is usually explained by the argument that in order to avoid falling in the sun, and keep revolving in the orbit, the nearer planet has to increase its speed because it experiences greater pull from the Sun. The question again arises: why should the planet avoid falling in the Sun? Why should it be so conscious of its decision that it increases its speed only to avoid falling? Who tells it to increase this speed? If planets move in the orbits only due to a warped space, not due to the attraction of the Sun, and they simply follow a linear path in a geodesic, as explained by Einstein, then there is no need at all for the closer planets to increase their speed. Whether their speed is slow or fast, they would follow the curved path. This is explained better by the UTR.

As I had discussed earlier that the planets lying closer to the Sun move faster than those lying farther. This is said to be due to the fact that, in order to avoid falling in the Sun, the closer planets have to increase their speed. Einstein's theory of General Relativity argues that the planets seem to be revolving around the Sun only because the space in the vicinity of the objects becomes warped. Planets revolve not due to attraction

by the Sun, but because they just follow the curved path. If this is so, and planets are not attracted by the Sun, why do they have to increase their speed in order not to fall in the Sun. The distance from the Sun should not have any effect on the speed of the revolution of the planets, because they just follow a curved path. If someone argues that the nearer planets revolve faster on account of the greater curvature of path, this would be an erroneous conjecture. The curved path is not like a steep path. The more steep a path is, the greater the speed of the vehicle, because of the increasing proximity to the centre of gravitation. But that is not the case with a curved path. In fact vehicles tend to become slower on a curved path. *The more a path is curved the slower will be the speed of the vehicle*. This is a fact, which we observe daily in our life. The motion of planets is better explained by the UTR. As discussed above, motion is the fundamental property of the universe, and all its constituents and every particle tries to achieve the highest speed possible in the direction of the periphery of the universe, because the universe is rotating as a whole on its axis. Its speed however is hindered by its own mass and the presence of bodies around it. A planet does not want to fall in the earth, because it is trying to achieve the fastest speed possible along with the motion of the universe as a whole. The closeness of the Sun wants to pull it towards it. So the speed relative to Sun will increase but it will not fall in the Sun.

Rotating versus Expanding Universe

The UTR is different from the current theory of Physics in that while the latter is based on the *continuous expansion* of the universe the former is based on the *continuous rotation* of the universe. The concept of the expansion of the universe is based on Hubble's Law, who interpreted the redshift, which is observed in the universe, as the evidence of the expansion of the universe. Hubble said that the velocity of the receding galaxies is directly proportional to the constant H and the distance of galaxy from the earth. His law states that the farther the galaxy the greater is the rate of expansion. The presentation of the UTR will in fact lead to a hot debate between a *rotating* universe and an *expanding* one and its success will ultimately emanate from its better ability to explain events and phenomena. The expanding universe has led to the Big Bang theory. But lots of issues still remain unresolved. Different scientists have different questions in mind that they think still remain unanswered or incompletely answered. For example, some of these have been enumerated as follows in an article, "The Hubble Law" by Dob B. DeYoung**:**

1. "*What is the true value of the Hubble constant?*
2. *Why is the solar neutrino flux less than half its expected value?*
3. *Why has extraterrestrial life not been detected in many other places in space?*
4. *What was the origin of the assumed Big Bang 'kernel' of mass-energy, and why did it 'explode'?*
5. *How did the first stars and galaxies spontaneously form?*
6. *Are there actual planets circling other stars?*
7. *Is the redshift of starlight actually due to universe expansion, or could there be another cause?*
8. *How far away are the quasars, and what actually are they?*
9. *Do galaxies evolve with time?*
10. *Where is the missing mass required by the Big Bang? This is also variously called hidden, dark, cold or exotic matter.*
11. *What is the origin of cosmic radiation?"*

The UTR will explain most of these in a fitting manner in due course of time. Hubble's law will have to be revised. v in the Law would then indicate not the velocity of the galaxy but the velocity of a particular zone of the rotating universe. The velocity measurement will also change. The new gamma factor will have to be taken into account, while calculating the velocity based on the redshift. Moreover, the velocity of our zone (about 420, 000 kms/sec) will be required to calculate the speeds of other zones. In the rotating universe, following considerations will play an important role:

First, there will be *no bar on the speed*; many areas in the universe will be found rotating with thousands of times the speed of the light. The outer zones will be speeding with much greater speed than the inner zones.

Second, *blue shifts* will also be important, as there may be some regions, which may be lying in slower zones than ours. There are many evidences of blue shift. For example, Andromeda galaxy is in our 'nearby' local group of about 30 galaxies. Its light shows a slight blue shift. It is to be seen whether it is due to the gravitational attraction between this galaxy and our galaxy, or it may be due to its lying in a slower zone. With more extensive mapping of the universe in future, predictably the innermost zones may be found containing nothing but Hydrogen, without any stars and

galaxies. The chances of observing blue shifts in substantial numbers may therefore remain scarce.

There is a possibility of reaching the conclusion that the variety of radiation detected from time to time are due to their arising from different zones rotating with different velocities. The greater-frequency-radiation (gamma rays, ultraviolet rays, cosmic rays etc.) may be coming from the slower zones, the higher frequency being due to their having gained in energy while crossing the faster zones. The lower-frequency-waves (Infrared, microwave, radiowave etc.) may be coming from faster zones, as their energy will decrease as they enter the slower zones.

Third, galaxy formation will be seen in the view of *different time-scales*, as the time will show distinct changes from one zone of the universe to others. Some galaxies may in fact be *younger* than ours, and some *older,* relative to our time scales. *Those nearer the axis of the universe will age much faster than do those away from the axis*. The rate of the decay of stars may vary.

Fourth, the energy component of each particle will be greater in zones distant from the axis. The nuclear reactions will therefore take different forms, as greater amount of energy will be produced. Quasars may be such distant objects.

Quasars are very bright centres of some very distant galaxies, where some sort of energetic action is assumed to be occurring. It is thought that the falling of matter into the super-massive black hole can result in very hot regions where huge energies are released, powering the quasar. The visible emission only occurs very near the centre of the galaxy. But huge regions of radio emission, produced by the quasar, can stretch out to large distances outside the galaxy. It is argued that the electrons near the centre of the quasar can be accelerated to speeds *near the speed of light*. In the presence a magnetic field, (which is present in these same regions), the electrons move along helical paths (paths that look like a stretched out slinky). As a result, they emit radio waves, called *synchrotron radiation*, since these waves are observed on Earth when physicists send high-energy electrons around in circles using magnetic fields, in particle accelerators called synchrotrons. It appears that galaxies may act as quasars only during the early stages of their lives.

Quasars have become controversial on account of the *extraordinary redshift* they show. The present day understanding of the quasars shows that (I) they are not necessarily star-like and have complex structures, (2) though many of them are radio sources, all of them are not, and (3) the *high red-shift* is the continuing hallmark of the quasars. Till now, the highest red-shift available is *3.78*. On the basis of the understanding of the Doppler shift, any red-shift *over that of 1.00 means a faster than light-speed* velocity of the source, A value of 2.00 would mean a relative speed of double the light speed. This would clearly mean that they are moving at *much higher speeds than the light*. But again, Einstein's ghost scared the cosmologists who started finding out alternative explanations for this high redshift. Obviously, these attempts have not been convincing. These have led to still bigger complications. The controversy is summed up in "*The Universe of Motion"* by Dewey B. Larson. He says:

"While the high redshift problem was circumvented in conventional astronomical thought by this sleight-of-hand performance with the relativity mathematics, the accompanying distance-energy problem has been more recalcitrant, and has resisted all attempts to resolve it, or to evade it. Reference was made to this problem in……If the quasars are at cosmological distances—that is, the distances corresponding to the redshifts on the assumption that they are ordinary recession redshifts—then the amount of energy that they are emitting is far too great to be explained by any known energy generation process, or even any plausible speculative process. On the other hand, if the energies are reduced to credible levels by assuming that the quasars are less distant, then conventional science has no explanation for the large redshifts…….Obviously something has to give. One or the other of these two limiting assumptions has to be abandoned. Either there are hitherto undiscovered processes that generate vastly more energy than any process now known, or there are hitherto unknown factors that increase the quasar redshifts far beyond the normal recession values."

The UTR will explain this by stressing that none of these two factors need be abandoned. The UTR will lead to the assumption that very distant bodies lying near the periphery of the universe will have much lesser effective age than our galaxy has, despite the fact that they may have been created almost simultaneously. This is because they are speeding with a velocity much greater than that of light and also than that of ours. This will produce high red-shift. The energy content in those galaxies will also be greater for the same amount of matter. This is because, according to this theory, $E=mc^2$ indicates the kinetic energy content of the particles in a particular zone, depending upon its velocity. c here is in fact the speed of the zone. *The matter in the faster zones will therefore have much greater energy content than that in our region*. This may ultimately answer not only the presence of quasars but also their specific nature—their high redshift and excessive energy.

The *Microwave Background Radiation* is uniform heat radiation found everywhere in space. The Big Bang theory states it is *the light from the Big Bang red-shifted to a fantastic extent*. But the UTR has an alternative explanation. This fantastic shift may be due to the *light*

coming from very distant regions of the universe, which are rotating at very *huge rates*. As the light travels to comparatively very small velocity zones, it loses energy giving it the huge shift towards low frequency.

Another source of controversy in recent years has been the source of *gamma rays*. More recent observations indicate that gamma ray sources are not in our galaxy but lie at far distances *outside* our galaxy. This is now argued that they must be coming from high-energy sources or from the merger of two balckholes or two neutron stars, because such enormous amount of energy suggests a gravitational source. The UTR offers another possible alternative, which may be explored. These may be coming from areas in the slower zones and may have gained in energy after having entered the faster zones. Alternatively, they may be coming from faster zones, where the energy-contents of the particle are higher. The first possibility however seems to be more plausible.

One of the most fundamental principles of the modern cosmology is that the universe looks *isotropic*. We can assume that though the universe has a periphery like that of the surface of the earth, we cannot see that periphery or beyond that. The universe will always look the same howsoever distant we see. This is because *the rotation of the universe rotates everything in it* including the light waves. Light waves coming from very distant portions of the universe will rotate before reaching us. It is also possible that light waves coming from distant areas may in fact be the echoing effect of other stars, which we can also see directly.

Let us assume an animal (or an instrument), which can only "see" through sound waves. It is not able to detect light at all. Now, it can only detect the sources of waves only from within the atmosphere of the earth. For it, earth will be *infinitely vast*. It can detect the sound from the same source coming from different directions. Due to the change in the properties of the sound coming from different directions, it can infer it to be coming from different sources. It cannot see the periphery or beyond the periphery of the atmosphere, and would see almost a similar picture on all sides. To detect the objects of the universe, we have or can have only waves, which cannot cross the Periphery of the Universe. Due to the rotating effect, the light waves from a very distant source may curve back after reaching the outermost areas of the universe and then reach the observer on earth. But if the human detectors cannot see beyond the universe, it does not mean that anything does not exist beyond it.

Origin of the Universe

How did the Universe originate and what will its fate be? These are questions that have always and will always haunt the philosophers and scientists. Physicists have been trying to find the answer. Scores of models have been presented. Most of them are based on General theory of Relativity. Despite its successes, the Standard Model has plenty of known problems. In the June 2003 issue of Scientific American, in an article, captioned, "*The Dawn of Physics beyond the Standard Model,"* Gordon Kane has listed ten theoretical problems:

"1. It (the standard model) implies a tremendous concentration of energy, even in the emptiest regions of space. This so-called vacuum energy would have either quickly curled up the universe long ago or expanded it to a much greater size.

2. The expansion of the universe is accelerating, and this cannot be explained by the standard model.

3. There is reason to believe that in the first fraction of a second of the Big Bang, the universe went through a period of extremely rapid expansion called inflation. The fields responsible for inflation cannot be those of the Standard Model.

4. If the universe began as a huge burst of energy, it should have evolved into equal parts of matter and anti-matter. This did not happen. The universe is matter. The Standard Model cannot explain this.

5. About a quarter of the universe is invisible cold dark matter that cannot be particles of the Standard Model.

6. In the Standard Model, interactions with the Higgs field cause particles to have mass. The Standard Model cannot explain the form these interactions must take.

7. Quantum corrections apparently make the Higgs boson mass huge, which would make all particle masses huge, which is obviously not the case.

8. The Standard Model cannot include gravity, because it does not have the same structure as the other three forces.

9. The values of the masses of particles cannot be explained by the Standard Model.

10. There are 3 generations of particles. The Standard Model cannot explain why there is more than 1 generation."

Recently, Quantum mechanics has been used to explain some of the unanswered questions. But almost all the scientists agree that the universe began at the Big Bang. Describing the beginning of the modern theory of the origin of the universe, Hawking says:

"At that time, which we call the Big Bang, the density of the universe and the curvature of space-time would have been infinite. Because mathematicians cannot really handle infinite numbers, this means that the general theory of relativity on which Freedman's solutions are based predicts that there is a point in the universe where the theory itself breaks down. Such a point is an example of what the mathematicians call singularity. In fact, till now, our theories of science are formulated on assumption that space-time is smooth and nearly flat, so they break down at the Big Bang singularity, where the curvature of space-time is infinite. This means that even if there were events before the Big Bang, one could not use them to determine what would happen afterward, because predictability would break down at the Big Bang."

The inflation theory states that the initial expansion was very fast. But scientists have raised several objections to this theory. They have argued that, to expand this fast, objects must have been moving *faster* than the speed of light. This objection has been answered by the argument that although objects in space cannot travel faster than the speed of light, *space* itself can expand this fast, carrying the objects with it. This is a strange argument though. Space is no empty space; it contains various fields and may contain Dark Matter. Secondly, there is no proven mechanism for creating this massive expansion. Einstein's corrective force and a concept called *false vacuum* have been presented to explain the effect. It is argued that, when the Big Bang took place, there was only one type of super-force. As the universe grew, this split into the four forces we have today. The energy released during this split is said to have been responsible for inflation. But the truth remains that this cannot be proved; for until we can perform experiments at *1028°K* there is no way in which to prove the theory as either correct or incorrect. Finally, there is no observational evidence of inflation. The evidence flatly contradicts its claim that the universe is a closed one.

Now, let us try to visualise what would be the picture of the beginning of the universe after the acceptance of the UTR. I have to admit at the outset that I have not yet worked on this aspect in detail, as it will require a substantial time to work out all the details, but certain things are evident:

First, the UTR declares motion as the most fundamental property of the universe. If motion is not there, the matter can have *no property* and there can be *no laws* in force. The theory says that the universe is rotating as a whole (Uniglobe). It is this rotation that has provided all the properties to the matter. So, it would be in the fitness of things to say there was a time *when the universe did not rotate* on its axis. The matter then was spread in a huge space in the form of a *haze*. There was absolutely no movement; the matter did not have any mass acted upon by forces; there were no forces and no form of energy including temperature. Everything, including space was *devoid of property*. Time did not exist. In short, the universe was nothing but an *inanimate ocean of inanimate or dead material,* which may have been the debris of an earlier universe. Thus, while the accepted theories of the origin of the universe visualise the universe as beginning from a singularity having infinities and breakdown of laws, the UTR would visualise the origin of universe *from a huge space filled with inert material, where there was no law in action*. The modern theory is untenable because it is highly *unlikely that laws could have originated from a situation where the laws had broken down*. The origin of laws from an earlier event witnessing the breakdown of all laws of Physics also disturbs the law of causality. *Causality* and *determinism* have been the cornerstones of classical Physics as well as the Theories of Relativity, and was vehemently defended by Einstein and other physicists including the most vociferous opponents of the Copenhagen Interpretation. The truth is that the origin of law from a situation of lawlessness is something that cannot be acceptable. On the other hand, the UTR would visualise the origin of laws not from a situation where there was *lawlessness* but from a situation where there were *no laws in force* yet, because the matter in the universe was not yet in position to understand and follow the laws.

Second, the first step in the origin of the universe would be the beginning of the rotation of the universe on its axis. It will be discussed later how this rotation started. But it is clear that it got underway owing to the supply of energy from *outside* the universe. As soon as the rotation began, the universe would have awakened from the slumber or got revived from death. The material present in the universe started moving, and with the movement the properties and forces started appearing. The material particles started running towards the periphery of the universe where the supply of energy was coming from, and the gravitational attraction and the kinetic energy created by the motion started attracting them towards one another. Every single particle in the universe and space started rotating. Time started to move. So the *beginning of space-time as a functional entity took place not at the Big Bang but at the beginning of the rotation of the Uniglobe.*

Third, with the beginning of the rotation of the universe, the gravitational attraction between the finest components of the haze led to the *coalescing of material*. One of the likely courses of development would be like this: The massive amount of kinetic energy associated with particles would eventually lead to the formation of nuclei. As the mass-energy of the

material in the outer zone will be much greater, the matter from the inner zones will first get attracted towards the outer zones, and a *ring like universe* may develop. Then the attraction between the matter would lead to condensing of the matter at the centre. The structure that formed in the centre would be a *spongy* mass with central region containing hydrogen atoms. The temperature within the condensed mass would continue to rise. As the density increased further, the temperature increased even more. When the matter condensed substantially, the pressure in it became too big to keep it as one single mass. It exploded with a Big Bang, and with the explosion the material ran in all directions towards the periphery with *extremely high* (much much higher than the speed of light), though different, speeds. Soon the materials started concentrating in different areas that gave rise to different components of the universe. The universe continued to rotate and the gravitational forces between the masses led to the development of various *rotating frames*, which we now know as planets, stars, galaxies, clusters, superclusters, Megagalaxy, etc.

Fourth, it has to be studied whether the atomic particles that exist today were created in the pre-bang phase or the post-bang phase. It is more likely that the atomic particles had already formed, and even the elements had appeared in the pre-bang phase. To visualise what happened at what stage, the most important point that has to be noted is that the rotation of the universe would impart different speeds to the different areas. Those near the axis will have smaller speed and those farther away will have a greater speed. Therefore, the energy will be the maximum in the outer zones and will smoothly taper down towards the inner zones. What effect will these differences in energy create would be an important consideration in finding out the sequence of events.

Fifth, it has to be studied what form of radiation would have been produced in the whole process. While the big condensed mass at the centre would be rotating with huge speeds, the rest of the space might have been filled with radiation. Can microwave background radiation be that radiation?

It is clear from the above that Big Bang will be a hugely different event from the Big Bang of the current theory. To differentiate the two we will call the Big Bang in the UTR as the BIG BURST from hereon. The most important distinguishing features between them are as follows:

First, Big Bang started from Singularity having *infinite density, zero size and infinite* temperature. Big burst would be a much *subtler* event starting at a massive density and massive heat. But *neither it would start from a singularity nor start from a state of infinite heat and zero size.*

Second, in standard theories, the space-time is assumed to have *begun* at the Big Bang, but not in the case of the Big Burst of the UTR, which had a phase where time had already begun. In the UTR, Big Burst is not the starting event but an *intermediate* one.

Third, the Big Bang started from a stage where *laws were broken*, but Big Burst would start from a previous event, where there were *laws already existing*. Causality will therefore be better maintained.

Fourth, in the Big Bang, it is hard to imagine how density fluctuations began giving rise to galaxies and stars. The use of quantum mechanics to describe the earliest events is nothing but an attempt destined for failure. The uncertainties of quantum mechanics have been *assumed to be the cause behind density fluctuations on the ground that the universe was of an extremely minute size*, where quantum mechanics could work. This is an absurd idea because quantum mechanics is related not just to the *size* of microscopic structures, but also the *properties* of the subatomic particles. Subatomic particles are not only of extremely mall size but also of a *minute mass*. The universe at the start of the Big Bang, on the other hand, had *infinite mass*. The heat content in the initial phase was *extremely high* compared to that in the atom. Furthermore, there is a *special* relationship between the particles acting within the atom, and between the particles and other atoms surrounding them. Obviously, such relationship was non-existent at the beginning of the universe. The problem of density fluctuations does not arise in the Big Burst, because density fluctuations would have already appeared in the condensed mass, which could in fact have been a *spongy* structure.

Fifthly, The uniform microscopic radiation can also perhaps be better explained in the UTR.

Sixthly, while Big Bang was an explosion, not in, but *of space*, there can be two possibilities in the Big Burst. As the UTR assumes the beginning of the creation of the universe with the beginning of the rotation of the universe, the extraordinary speed of the rotation would cause contraction of space, but the gravitational pull among the particles would cause them to get denser. Obviously, there will be a free space or vacuum (with no matter except the particles of different forces) outside the concentrated mass at the centre. The Big Burst can either be a burst into space, or that *into the space as well as of* it. These possibilities have to be discussed in arriving at the Final Model of the Origin of the Universe.

The vast difference between two theories can therefore be appreciated. In the Big Bang theory, there is no answer to where the infinite mass and energy of the singularity came from, and *what was there prior to the Singularity*. It leads to the compulsion of the continuous creation of space, for the Big Bang was an *expansion of space, which is still continuing, and can*

continue forever. Where from this space is coming, there is no answer. In the Universal Theory of Relativity, the origin of universe would not begin at infinities, but from a position having *absence of any matter with properties*. The need of the Creator is there in both theories, but the *Big Bang starts at an event where there had already been created a huge energy-mass, while the UTR starts with the creation of energy-mass itself***.** The role of God will be discussed later.

Thus it can be seen that the origin of the universe in the UTR has three main stages which are akin to the stages of human development. First stage can be called a *Prenatal or Foetal Stage*. In this stage, the foetus of the universe started to form at the centre of the Universe. Once the foetus got fully developed, began the second stage: the *Natal Stage or the Stage of Delivery, the Big Burst***.** The matter gathered in different areas in several rotating frames of universe, like planets, stars, galaxies, clusters and superclusters. Then started the *Postnatal Stage,* in which the development of the Universe continued with eventually the creation of the complex chemical structures and living beings.

There are many problems at the structural level also, which the standard model of the origin of the universe cannot fully explain. The universe is made up of billion of galaxies, some of which are smaller and some greater than ours is. However, what amazes cosmologists is that most of the universe is devoid of any luminous matter, and is formed of gigantic empty spaces. It is hard to find how these *gigantic voids* were formed and whether these voids are empty. One thought is that the universe may contain just one gigantic void in which large superclusters and clusters are floating. The other possibility is that superclusters form one gigantic chain within one gigantic void so that it is possible to traverse through one chain to the other. The third possibility is that galaxies cluster to form sheets separating vast regions of empty space just as soap filaments and bubbles formed out of them. These structural features are also not easily explainable by the Big Bang models. If the universe started from a highly dense singularity, what caused these voids to appear? At the same time there are structures like Great Wall, which is a gigantic structure of up to at least 100-200 Mpc scales. The truth is that these structures and more generally the formation of galaxies have been puzzling scientists, because it is difficult to imagine these on the basis of the Big Bang models. Let us reproduce here some of these concerns:

> *"My view is that there is something fundamentally wrong in our approach to understanding such large-scale structure—some key piece of the puzzle that we're missing." (Waldrop, M. Mitchell;* Astronomers Go Up Against the Great Wall, Science*, 246:885, 1989)*
>
> *"The problem of explaining the existence of galaxies has proved to be one of the thorniest in cosmology. By all rights, they just shouldn't be there, yet there they sit. It's hard to convey the depth of frustration that this simple fact induces among scientists." (Trefil, The Dark Side of the Universe, p. 55)*
>
> *"We cannot even show convincingly how galaxies, stars, planets, and life arose in the present universe." (Michael Rowan-Robinson, "Review of the Accidental Universe," New Scientist, Vol. 97, 20 January 1983, p. 18)*
>
> *"A completely satisfactory theory of galaxy-formation remains to be formulated." (Joseph Silk, The Big Bang San Francisco: W. H. Freeman and Co., 1980 p. 22)*
>
> *"The theory of the formation of galaxies is one of the great outstanding problems of astrophysics, a problem that today seems far from solution." (Steven Weinberg, The First Three Minutes, New York: Bantam Books, Inc., 1977, p. 68)*
>
> *"Fifty cosmologists attended a conference on galaxy formation. After summarising much observational data, two of the most respected authorities optimistically estimated the probability that any existing theory on galaxy formation is correct is about 1 out of 100. (P. J. E. Peebles and Joseph Silk, "A Cosmic Book,"* Nature*, Vol. 335, 13 October 1988, pp. 601–606)*
>
> *"In its simplest form, the Big Bang scenario doesn't look like a good way to make galaxies. It allows too little time for the force of gravity by itself to gather ordinary matter—neutrons, protons and electrons—into the patterns of galaxies seen today. Yet the theory survives for want of a better idea." (Peterson, Seeding the Universe, p. 184)*
>
> *"The discovery of the Great Wall of galaxies and the filamentary clumping of galactic matter has greatly surprised traditional astronomers who think that galactic matter should be uniformly distributed—according to their theories, at least. Until these discoveries, almost everyone was betting their house on a*

uniform distribution of galaxies throughout the universe. In fact, the exact opposite has proved to be the case: galaxies, clusters of galaxies, and even superclusters (clusters of clusters) are distributed in gigantic filamentary and sheet-like patterns….

"Cosmologists have tried shoehorning these discoveries into their existing theoretical structures by hypothesising different kinds of dark matter or by asserting that the Big Bang contained irregularities, which resulted in clumping of galaxies and clusters. However, all these attempts to account for the Great Wall and other structures run into other problems. For example, postulating irregularities in the Big Bang fails to explain the observed uniformity of the universe's microwave background radiation…

"Some cosmologists are trying to piece together models containing both cold dark matter, which may explain the stability of galaxies, and hot dark matter (neutrinos), which may explain the larger-scale structures. However, this approach seems inelegant to many theorists, who are uncomfortable hypothesising agents for which there is no observational or experimental evidence." (New Science Paradigms, The Great wall)

We know now that stars group into galaxies. Some 100 billion of galaxies are observable in the universe. They form huge clusters journeying through space. Galactic superclusters may contain thousands of galaxies and may stretch hundreds of millions of light years across. Superclusters are arranged in *filamentary* and sheet-like structures, separated by gigantic voids of apparently empty space. Fifteen or sixteen smaller galaxies along with Milky Way and Andromeda form the Local Group cluster of galaxies. Near Local Group, there is huge Virgo Cluster. These clusters and clusters of clusters are moving. The Milky Way and Andromeda are moving toward each other, the Local Group is moving toward the middle of the Virgo cluster; and the Virgo cluster and a neighbouring supercluster are speeding toward a mysterious destination called *"The Great Attractor"*. Moreover, using shape-finders some scientists have been able to show that for a wide range of model universes, clusters of galaxies align themselves to form one-dimensional filaments. Indeed they predict that *the larger the size of a cluster the more likely it is to be filamentary in nature*. This filamentary nature will also be better explained by the rotation of the Uniglobe. Commenting on these structures and their movement, a report on the web-page of *New Science Paradigms* says:

"These structures and their movements cannot be explained as part of the general expansion of the universe. Conventional astrophysics theorises that they must be guided by gravitational forces. But astronomers have not detected enough matter to account for the tremendous gravitational pull needed to explain the motions of stars in galaxy arms, galaxies and larger structures. For years now, astronomers have been haunted by a sense that the universe is controlled by forces they don't fully understand. Recent observations provide a striking confirmation ….

"Astronomers are up against the wall—the Great Wall of galaxy clusters. The Great Wall is the largest known structure in the universe: a 15 million-light-year thick sheet of galaxies, $500x10^6$ light years long by $200x10^6$ ly wide—and it may extend farther, into areas blocked from observation by the spiral arms of our own galaxy. The Great Wall is about $200\text{-}300x10^6$ ly from earth. It limits vast voids of nearly empty space containing almost no galaxies at all—only some vast, diffuse clouds of hydrogen. ……Both the Great Wall and the adjacent voids are far too large for classical gravity-based astrophysical theories to explain. All theories currently popular among traditional astronomers have great difficulty accounting for such enormous structures. One important observable, the 2.7 degree K cosmic background radiation—which is usually described as the afterglow of the Big Bang—argues for a very smooth, uniform distribution of galaxies. According to conventional astrophysics, the Great Wall is definitely anomalous."

In the UTR, these voids and huge structures will be easier to explain. The big mass formed in the centre after the rotation of the universe began was not a singularity as singularities are banned in this theory. As the big mass in the centre coalesced from a haze of matter due to newly acquired gravitational attraction as the result of the rotation, which was at different speeds in different regions, the *density fluctuations* in the big mass would be obvious. And as the big burst was not just the burst of the space as claimed by the Big Bang, but the burst *in* the space, the formation of voids can be understood. The great filaments and voids can be explained only by the second postulate of the theory that says that the universe *as a whole* (Uniglobe) is rotating on its axis. The presence of great voids with nothing but Hydrogen is an important pointer to the truth of the theory. According to the theory, the regions near the axis will be rotating with very small speeds compared to the outer regions. In these spaces, the energy content of the particles will be greatly lower than in other areas.

The strong nuclear force will therefore be not strong enough to bind the protons among themselves or with neutrons. The hydrogen alone will therefore be formed. With the big burst, the hydrogen may spread in other areas of voids but the greatest concentration should remain in the regions close to the axis.

Fate of the Universe

Physicists have predicted the fate of the universe in different ways. There are many models. In the *Open Universe* theory, the universe will continue to *expand forever*. In the *Closed Universe* theory, the universe will eventually start *contracting* and will again end at the singularity after which another phase of expansion may begin. (There are researchers who claim that this is not inevitable.) In the *Flat Universe* theory, the expansion will just remain at the *critical rate*. In all the theories, the universe will have a deadly end. In the UTR, another possibility looms large on the universe. As soon as the universe stops rotating, all the properties of the matter and space will be met with an *immediate death*. The absence of the force of gravitation would cause the matter to again become like a haze. All the movements will stop *abruptly*. Time will cease to exist. Space will again become dead. There will be *one time death of the universe as a whole,* which can be called the *Final Catastrophe*. In the current theories of the universe, the death of the different portions of the universe will not be simultaneous. There may be a gap of millions of years between the death of different stars. In the UTR, the death of stars or individuals can go on within the universe while the universe as a whole survives, but a time may come when the rotation of the universe as a whole stops, which will cause the death of the universe as a whole. In fact, it can be realised that *in the current theories of the universe, there is no birth or death of the universe* as such and it deals only with *creation and death of the parts* of the universe. Th UTR on the other hand talks of the *birth and the death of the universe as a whole*. As has been described earlier, the stages of the universe are similar to those of human development. First, there was a prenatal stage, then natal and then postnatal. The universe has already grown quite old, and it may die a sudden death anytime.

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References

1. Javed Jamil. Philosophical implications of Universal Theory of Relativity: Role of God in the Universe, Time, Determinism, Quantum Mechanics. Nature and Science. 2006;4(1):1-22.
2. Javed Jamil. Rediscovering the Universe: the Beginning of the Final Revolution. Nature and Science. 2006;4(2):25-30.

Soybean Yield Forecast Application Based on HOPFIELD ANN Model

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Abstract: This article establishes the estimate's mathematics model of the soybean's yield, using the artificial nerve network's knowledge, and by the model we can increase accuracy of the Soybean Yield Forecast. [The Journal of American Science. 2006;2(3):85-89].

Keywords: ANN; Soybean ; Hopfield ; Yield Forecast

1. Foreword

By setting up simulation model , we can get some relevant conclusions and realize predict function in order to assist people making decision [1]. In general situation, it is very difficult to set up relative and accurate mathematics model reflect objective systems, it is even impossible sometimes, but there are certain relations between various kinds of factors[3] [4]. If we can find the mathematics model which reflect the realistic input and output system, it has very important significance in Yield Forecasting.

Assume the system mathematics model as follow:

$$\hat{y} = \hat{f}(x_1, x_{2,} \cdots x_n)$$

Set up the mathematics model:

$$y = f(x_1, x_2, \cdots x_n)$$

If $Q = |y - \hat{y}| = \left|\hat{f}(x_1, x_2, \cdots x_n) - f(x_1, x_2 \cdots x_n)\right| \to 0$

Then we think that the model $y = f(x_1, x_2, \cdots x_n)$ is successful, and we can use it in Forecasting. In numerous artificial nerve network models, Hopfield nerve network is widely used, this text apply this model to soybean predict field, In order to improve utilization ratio of fertilizer, impel the soybean excellent quality, high yield , reach the unity of economy, the ecology and social benefit.

2. Brief introduction of the Hopfield Nerve network

Hopfield Nerve network Mathematics model as follow[2]:

$$\begin{cases} C\dfrac{du_i}{dt} = -\dfrac{u_i}{R} + I_i + \sum_{j=1}^{N} T_{ij} V_j \\ V_i = g(u_i) \quad i = 1,2 \cdots, N \end{cases}$$

T_{ij} —join Proportion Number value between neuron member i and j

$g(u_i)$ — monotony Increase progressively Continuous function, and $T_{ij} = T_{ji}$

u_i—Inputting value of i, V_i—Exporting value of i.

Simplify models.

$$\begin{cases} \dfrac{du_i}{dt} = \sum_{j=1}^{N} T_{ij} V_j + I_i \\ V_i = g(u_i) \quad i = 1,2\cdots, N \end{cases}$$

define systematical energy function is:

$$E = -\frac{1}{2}\sum_{i=1}^{N}\sum_{j=1}^{N} T_{ij} V_i V_j - \sum_{i=1}^{N} V_i I_i$$

We can prove dE/dt≤0, only when dVi/dt=0 that dE/dt=0 (i=1,2,⋯,N), that is to say that the system's Stable and Balanced point is extreme small point of Energy Function E, so the operation course on this network is a course seeking excellent the extreme small point in fact. Goal function is the energy function of this network system[5].

3. Setting-up The predicted and Simulation model of soybean's Yield

As mentioned above, it is the more difficult thing to set up its intact prediction mathematics model for the realistic system. So is the relation of Yield with the balance of soybean applies fertilizer, because six indexes which can influence the balance of the soybean to apply fertilizer. As there is a relation between a great deal of factors (the independent variable) and the yield of soybean. Set mathematics model systematically as:

$$\hat{y} = \hat{f}(x_1, x_{2,} \cdots x_n)$$

x_1 ， $x_2 \ldots x_n$——Influence factors of the output of soybean，Such as：Soil organic matter、The ammonium form nitrogen、The nitre form nitrogen、Quick-acting phosphorus、Quick-acting potassium、pH value etc，which can instruct the balance of soybean to apply fertilizer。

We can make use of following model to fit, and get[6]

$$y = \beta_0 + \sum_{j=1}^{N} \beta_j x_j + \sum_{j=1}^{N}\sum_{i\le j} \beta_{ij} x_i x_j + \sum_{j=1}^{N}\sum_{i\le j}\sum_{k\le i} \beta_{ijk} x_i x_j x_k + \cdots$$

In above formula, $\beta_0, \beta_j, \beta_{ij}, \beta_{ijk}, \cdots$, parameters to be estimate; Under normal conditions, it is enough to use two steps to fit, after every parameter appeared in the estimation that we can predicted the function.

Assume P is the sample number, $\hat{y}_p (p = 1,2\cdots, p)$ is variable, $x_{pj}(\mathrm{j} = 1,2,\cdots,\mathrm{N})$ is number

$\mathrm{Q} = \frac{1}{2}\sum_{\mathrm{p}=1}^{\mathrm{P}} (\mathrm{y_p} - \hat{\mathrm{y}}_\mathrm{p})^2$ reach extremely small.

y_p——The output of the fitting model；Define the energy function :

$$E = \mathrm{Q} = \frac{1}{2}\sum_{\mathrm{p}=1}^{\mathrm{P}} (\mathrm{y_p} - \hat{\mathrm{y}}_\mathrm{p})^2$$

$$= \frac{1}{2}\sum_{p=1}^{p} (\beta_0 + \sum_{j=1}^{N} \beta_j x_{pj} + \sum_{j=1}^{n}\sum_{i\le j} \beta_{ij} x_{pi} x_{pj} + \sum_{j=1}^{N}\sum_{i\le j}\sum_{k\le i} \beta_{ijk} x_{pi} x_{pj} x_{pk} + \cdots - \hat{y}_p)^2$$

take two steps, and get

$$E = \frac{1}{2}\sum_{p=1}^{p}(\beta_0 + \sum_{j=1}^{N}\beta_j x_{pj} + \sum_{j=1}^{n}\sum_{i\le j}\beta_{ij}x_{pi}x_{pj} - \hat{y}_p)^2$$

so we know

$$\frac{\partial E}{\partial \beta_0} = \sum_{p=1}^{p}(\beta_0 + \sum_{j=1}^{N}\beta_j x_{pj} + \sum_{j=1}^{N}\sum_{i\le j}\beta_{ij}x_{pi}x_{pj} - \hat{y}_p)$$

$$= P\beta_0 + \sum_{j=1}^{N}\beta_j(\sum_{p=1}^{P}x_{pj}) + \sum_{j=1}^{N}\sum_{i\le j}\beta_{ij}\cdot(\sum_{P=1}^{p}x_{pi}x_{pj}) - \sum_{p=1}^{P}\hat{y}_p$$

$$\frac{\partial E}{\partial \beta_j} = \sum_{p=1}^{p}(\beta_0 + \sum_{j=1}^{N}\beta_j x_{pj} + \sum_{j=1}^{N}\sum_{i\le j}\beta_{ij}x_{pi}x_{pj} - \hat{y}_p)x_{pj}$$

$$= \beta_0\sum_{p=1}^{P}x_{pj} + \sum_{j=1}^{N}\beta_j(\sum_{p=1}^{P}x^2{}_{pj}) + \sum_{j=1}^{N}\sum_{i\le j}\beta_{ij}\cdot(\sum_{P=1}^{p}x_{pi}x^2{}_{pj}) - \sum_{p=1}^{P}\hat{y}_p x_{pj}$$

$$\frac{\partial E}{\partial \beta_{ij}} = \sum_{p=1}^{p}(\beta_0 + \sum_{j=1}^{N}\beta_j x_{pj} + \sum_{j=1}^{N}\sum_{i\le j}\beta_{ij}x_{pi}x_{pj} - \hat{y}_p)x_{pj}(x_{pi}x_{pj})$$

$$= \beta_0\sum_{p=1}^{P}x_{pi}x_{pj} + \sum_{j=1}^{N}\beta_j(\sum_{p=1}^{P}x_{pi}x^2{}_{pj}) + \sum_{j=1}^{N}\sum_{i\le j}\beta_{ij}\cdot(\sum_{P=1}^{p}x^2{}_{pi}x^2{}_{pj}) - \sum_{p=1}^{P}\hat{y}_p x_{pi}x_{pj}$$

$$\frac{d\beta_0}{dt} = -\frac{\partial E}{\partial \beta_0}, \frac{d\beta_j}{dt} = -\frac{\partial E}{\partial \beta_j}, \frac{d\beta_{ij}}{dt} = \frac{\partial E}{\partial \beta_{ij}}, \text{ and order } g(x) = \begin{cases} x & x \ge 0 \\ 0 & x < 0 \end{cases}$$

Hopfield nerve network necessary parameters are：

$$I_0 = \sum_{p=1}^{P}\hat{y}_p, I_j = \sum_{p=1}^{P}\hat{y}_p x_{pj}\ ,\ \ I_{ij} = \sum_{p=1}^{P}\hat{y}_p x_{pi}x_{pj}\ ,\ \ T_{00} = -P\ ,\ \ T_{ij} = -\sum_{p=1}^{P}x_{pj}^2\ ,\ \ T_{ij\cdot ij} = -\sum_{p=1}^{P}x_{pj}^2 x_{pj}^2$$

$$T_{0.j} = T_{j.0} = -\sum_{p=1}^{P}x_{pj}\ ,\ \ T_{0.ij} = T_{ij.0} = -\sum_{p=1}^{P}x_{pj}x_{pi}\ ,\ \ T_{j.ij} = T_{ij.j} = -\sum_{p=1}^{P}x_{pi}x_{pj}^2$$

To the high-order situation, we can get:

$$I_0 = \sum_{p=1}^{P}\hat{y}_p, I_{ij\cdots k} = \sum_{p=1}^{P}(\hat{y}_p x_{pi}x_{pj}\cdots x_{pk}), T_{00} = -P, T_{(ij\cdots k)\bullet(ij\cdots k)} = -\sum_{p=1}^{P}(x_{pi}^2 x_{pj}^2\cdots x_{pk}^2)$$

$$T_{0\cdot(ij\cdots k)} = T_{(ij\cdots k)\cdot 0} = -\sum_{p=1}^{P}(x_{pi}x_{pk}\cdots x_{pk}), T_{i\cdot(ij\cdots k)} = T_{(ij\cdots k)\cdot i} = -\sum_{p=1}^{P}(x^2{}_{pi}x_{pk}\cdots x_{pk})$$

Among them, p=1,2,…,P； j=1,2,…N; k≤…≤i≤j.

Utilize above-mentioned parameter values we can construct soybean apply fertilizer Hopfield nerve network which predicts yield conveniently, This network reach a stable equilibrium of states, exports value which fit the parameter values of curved surface are what we need estimate promptly. The state of stable equilibrium at this

moment, is the state of the soybean of relatively high yield[7] [8].

4. The experiment of the computer

Choose a group of data as Form 1 shows are single factors, adopt 1 step to fit the relations of *yi* and *xi*, *Yi* is the average yield of soybean, *xi* is the amount of application of nitrogen, i.e $y = a + bx$, and i =1,2, …., 12, use ahead derived result, we can construct necessary every parameter respectively for network nerve Hopfield:

$$T_{aa} = -12, T_{bb} = -390, T_{ab} = T_{ba} = -78, I_a = 2074.3, I_b = 14242.8$$

This system dynamic equation is:

$$\begin{cases} \dfrac{du_a}{dt} = -12u_a - 78u_b + 2074.3 \\ \dfrac{du_b}{dt} = -78u_a - 390u_b + 14242.8 \\ a = u_a \\ b = u_b \end{cases}$$

use Euler method to solve, take initial value u_a=0, u_b=0, step h=1.0E-7, Change and take the place of a=2.5822887, b=5.0504757, to accelerate simulation operation disappear speed by, we change step h=1.0E-6, in the end we get steady result

a=138.26, b=5.322

So, the fitting equation received is: y=138.26+5.322x

Form 1. Experimental data

i	1	2	3	4	5	6	7	8	9	10	11	12
x_i	1	2	3	4	5	6	7	8	9	10	11	12
Y_i	143.5	148.9	154.2	159.5	164.8	170.2	175.5	180.8	186.2	191.5	196.8	202.1

Utilize least square method, Get equation:

y=139.26+5.822x

Form 2: It is these two kinds of methods that export the comparison of the result.

Using least square method get *y*=139.26+5.822x. Form 2 is export results to compare.

Form 2. Export results to compare

The method	Absolute error Total	Average absolute error	Square Error	Standard deviation error
Nerve network	2.853	0.319	0.258	0.628
Least square method	3.05	0.33	0.228	0.567

5. Conclusion

This paper has put forward a model to predict the yields of soybean with Hopfield nerve network raise the predicting accuracies of soybean yield, improve utilization ratio of fertilizer also, achieve the goal of increasing production[9]。

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References

1. Ma Feng-shi. Probability statistics. Higher education Publishing house, 1989.
2. Jiao Li-cheng. The network system theory of nerve. The electronic University of Science and Technology Publishing house, 1990.
3. Eugene Chamik, Introduction to Artificial Intelligence, Addison-Wesley Publishing Company Inc. 1985:12-44.
4. Subrahmanian. S. Jajodia. Data base system of multimedia. 1998.8：35-46.
5. Deck S H , Mokow C T, Heinemann P H, et al. Sommer Com parison of a neural network and traditional classifier for machine vision inspection [J]. Applied Engineering in Agriculture, 1995, 11.
6. Zhang Xin, Cai Wei-guang utilize ANN realize forecast modeling. 1997.
7. Haykin S. Neural Networks: A Comprehensive Foundation[M]. Macmillan College Publishing Company, Inc, New York, NY, U SA. 1994.
8. Zhang Ning, Zheng Junli. An Artificial Neural-Based A/D Converter Using Asymmetric Hopfield Network ICCAS-91. Shenzhen, China. 1999.
9. Muller B, Reinhardt J. Neural Networks An Introduction. Spinger-Verlag, 1990.

The Condition of Enhancement Effect of Surface Plasmons Excited in Near-Field Optical Structures

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Abstract: In this article, the condition of enhancement effect of Surface Plasmons (SPs) excited in near-field optical structures is demonstrated. If the system possesses smaller loss tangent metal-dielectric, the enhanced-filed intensity of SP is observed being increased. From the results of the analysis of Fresnel equations for a multilayer substrate in near-field optical structures, the higher dielectric coefficient for the prism will reduce the resonant angle, but, if reversely increases dielectric coefficient for intermediate layer, the resonant angle will be increased instead. The SP resonance condition is thus correlated with the loss tangent of the material and the enhanced filed intensity of SP of the system. [The Journal of American Science. 2006;2(3):90-92].

Keywords: surface plasmons; near-field optical structure; SP resonance; Fresnel equation

Introduction

Otto[1] first proposed the optical excitation of SPs by the method of attenuated total reflection (ATR). The SP is basically the transverse magnetic (TM) wave traveling along the metal-dielectric interface. The resonance modes are guided by electromagnetic field at the interface. SP has been used in various applications,[2-13] such as light modulators,[2-5] chemical sensors[6,7] photosignal Schottky diodes,[8,9] spectrometers[10] and so on. Most of the applications of SPs depend on the resonating behavior of SPs in multilayer system. The SP excited at the Sb/SiN interface can enhance the field intensity was first proposed by Tsai et al.[17] . However, the exciting mechanism of SPs has not been clearly understood yet. Basically, the excitation of SPs by light is denoted as a Surface Plasmon Resonance (SPR) for planar surfaces for nanometer-sized metallic structures. By implementing a mask layer to comprise a metal nanocluster-embedded dielectric film, such as Au film, AgO_x-type[18] film, are examples for the studies of generating optical-near field. In this report, the condition of the enhancement field density near metal film on SPs excited in near-field optical structure is demonstrated.

Theory

Consider a super-resolution near-field structure, as shown on Figure 1, in which the dielectric of medium 2 (ε_2) is equal to medium 4 (ε_4). Since the dielectric constant of medium 1 (ε_1) is higher than the dielectric constant (ε_2) or (ε_4), SP can be excited at the 2-3 interface or the 3-4 interface.

By using the Fresnel equations for a four-layer structure and the pole approximate expansion, the transmission coefficient can be expressed as following:

$$T_4 = 1 - \left| r_{12} \frac{(q - q_{23})(q - q_{34}) - \Delta q_{41}}{(q - q_{23})(q - q_{34}) - \Delta q_{42}} \right|^2 \qquad (1)$$

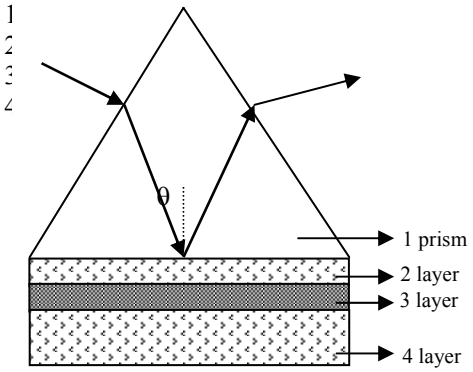

Figure 1. A diagram of the super-resolution near-field structure ($\varepsilon_1 > \varepsilon_4$). 1 indicates prism; layers 2 to 4 are the dielectric mediums; layer 4 is a metal medium

Calculation and discussion

In this study, an incident wavelength (λ) of the He-Ne laser (632.8 nm) and the dielectric constant of antimony (–22.36+35.21i) are assumed. Figure 2 depicts the plot of the transmission coefficient versus incident angle with four different prisms in the Super-RENS

(m=4). In Figure 2, the parameters used are ε_2 =4.84, ε_3 =–22.36+35.21i, ε_4 =4.84, d_2 =170 nm, d_3 =25 nm, ε_1 =5.00 (pluses), 6.00 (solid line), 7.00 (dashed line), and 8.00 (dotted line), respectively. From Figure 2, each curve has a maximum value and, by proper selection of the parameters, the reflectivity maximum can be made to approach zero. In addition, the resonant angle decreases when the dielectric constant of the prism increases. Figure 3 depicts the transmission coefficient versus incident angle with four different dielectric constants of the intermediate layer in the Super-RENS (m=4). The parameters used are ε_1 =8.27, ε_3 =–22.36+35.21i, d_2 =170 nm, d_3 =25 nm, $\varepsilon_2 = \varepsilon_4$ =2.84 (pluses), 3.84 (solid line), 4.84 (dashed line), and 5.84 (dotted line), respectively. The resonant angle increases when the dielectric constant of the intermediate layer increases.

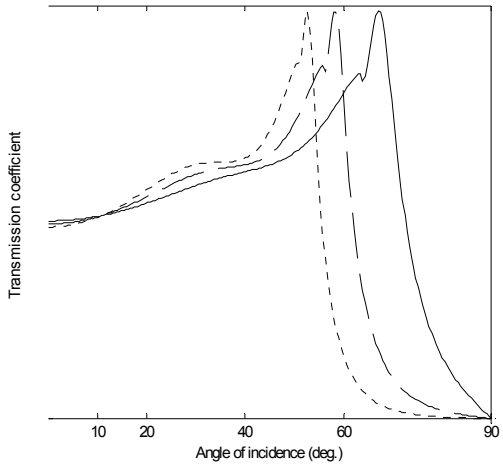

Figure 2. The plot of the transmission coefficient versus incident angle with four different prisms. The parameters used are ε_2 =4.84, ε_3 =–22.36+35.21i, ε_4 =4.84, d_2 =170 nm, d_3 =25 nm, ε_1 =5.00 (pluses), 6.00 (solid line), 7.00 (dashed line), and 8.00 (dotted line), respectively.

The plot of the transmission coefficient versus incident angle with four different dielectric constants of the metal film in the Super-RENS (m=4) is shown in Figure 4. The parameters used are ε_1 =8.27, ε_2 =4.84, d_2 =170 nm, d_3 =35 nm, ε_3 =–22.36+5.21i (dotted line), –22.36+15.21i (solid line), –22.36+25.21i (dashed line), and –22.36+35.21i (pluses), respectively. From Figure 4, the resonant angle decreases and the resonant half width increases when the imaginary part of the metal dielectric constant increases. In other words, the enhanced filed intensity is larger for a system using a metal with a smaller value of loss tangent. In Figure 5, it is shown the plot of the reflectivity versus incident angle with four different thicknesses of the metal film in the Super-RENS (m=4). The parameters used are ε_1 =8.27, ε_2 =4.84, ε_3 =–22.36+35.21i, d_2 =170 nm, d_3 =5 nm (dotted line), 15 nm (solid line), 25 nm (dashed line), and 35 nm (pluses), respectively.

From Figure 5, the reflectivity maximum can be made to approach one by proper selection of parameters. This is the optimum resonant condition for fabricating the Super-RENS.

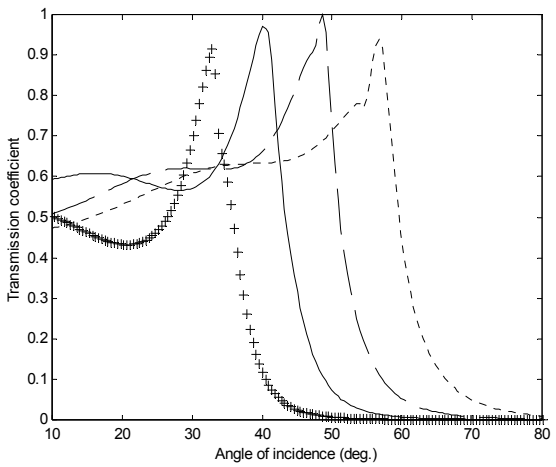

Figure 3. The plot of the transmission coefficient versus incident angle with four different dielectric constants of the intermediate layer. The parameters used are ε_1 =8.27, ε_3 =–22.36+35.21i, d_2 =170 nm, d_3 =25 nm, $\varepsilon_2 = \varepsilon_4$ =2.84 (pluses), 3.84 (solid line), 4.84 (dashed line), and 5.84 (dotted line), respectively.

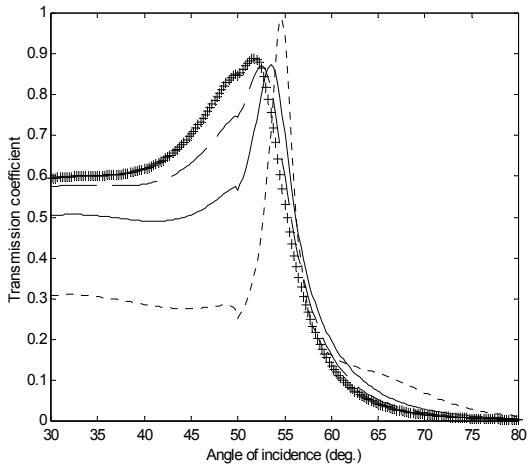

Figure 4. The plot of the transmission coefficient versus incident angle with four different dielectric constants of the metal film. The parameters used are ε_1 =8.27, ε_2 =4.84, d_2 =170 nm, d_3 =35 nm, ε_3 =–22.36 +5.21i (dotted line), –22.36+15.21i (solid line), –22.36+25.21i (dashed line), and –22.36+35.21i (pluses), respectively.

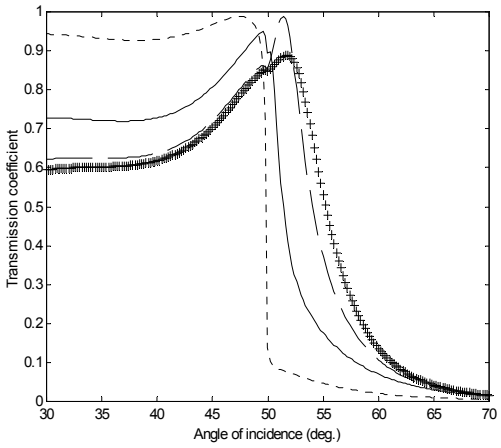

Figure 5. The plot of the transmission coefficient versus incident angle with four different thicknesses of the metal film. The parameters used are ε_1 =8.27, ε_2 =4.84, ε_3 =–22.36+35.21i, d_2 =170 nm, d_3 =5 nm (dotted line), 15 nm (solid line), 25 nm (dashed line), and 35 nm (pluses), respectively.

Conclusion

Conclusively, the enhanced filed intensity increases for a system using a metal film that has smaller loss tangent. The resonant angle decreases when the dielectric constant of the prism increases and the dielectric constant of the intermediate layer decreases and the optimum resonant condition in Super-RENS being made by proper selection of parameters.

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References

1. A. Otto: Z. Phys. **216** (1968) 398.
2. C. M. Lee, C. H. Liao, L. B. Chang and T. L. Chiao: Jpn. J. Appl. Phys. **35** (1996) 5448.
3. D. L. Chiao and C. M. Lee: J. Appl. Phys. **65** (1989) 3344.
4. J. S. Schildkraut: Appl. Opt. **27** (1988) 4587.
5. G. T. Sincerbox and J. C. Gordon: Appl. Opt. **20** (1981) 1491.
6. K. Matsubara, S. Kawata and S. Minami: Opt. Lett. **15** (1990) 7.
7. K. Matsubara, S. Kawata and S. Minami: Appl. Opt. **27** (1988) 1160.
8. I. R. Tamm, P. Dawson, A. Sellai, M.A.Pate, R. Grey and G. Hill: J. Appl. Phys. **74** (1993) 7481.
9. C. Daboo, M. J. Baird, H. P. Hughes, N. Apsley and M. T. Emeny: Thin Solid Films **201** (1991) 9.
10. E. Fontana, R. H. Pantell and M.Moslehi: Appl. Opt. **27** (1988) 3334.
11. E. Betzig and J. Trautman: Science **257** (1992) 189.
12. E. Betzig and J. Trautman, and R. wolfe: Appl. Phys. Lett. **61** (1992) 142.
13. R. T. Deck, D. Sarid, Grieg A. Olson and J. M. Elson: Appl. Opt. **22** (1983) 3397.
14. H. Kitajima, K. Hieda and Y. Suematsu: Appl. Opt. **19** (1980) 3106.
15. G. I. Stegeman, J. J. Burke and D. G. Hall: Appl. Phys. Lett. **41** (1982) 906.
16. J. Tominaga, T. Nakano, and N. Atoda: Appl. Phys. Lett. **73** (1998) 2078.
17. D. P. Tsai and W. C. Lin: Appl. Phys. Lett. **77** (2000) 1413.
18. Y. C. Hera, Y.C. Lan, W.C. Hsu and S.Y. Tsai: Appl. Phys. Lett. **83** (2003) 2138.

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