

THE INSTITUTE OF BIOLOGY SRI LANKA

PROCEEDINGS OF THE 32nd ANNUAL SESSIONS



Theme:
*Ecotoxicology: A discipline of
growing importance*



Council of The Institute of Biology, Sri Lanka 2011-2012



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SRI LANKA**

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“Ecotoxicology: A discipline of growing importance”

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About Institute of Biology, Sri Lanka

The Institute of Biology is a leading professional body of biologists in Sri Lanka. Its current membership is over 400. The institute was formulated in a small way by a group of Sri Lankan biologists led by Prof. B. A. Abeywickrama (Emeritus Professor of Botany University of Colombo in 1981). It became an incorporated organization by Act of Parliament No 22 in 1984.

The objectives of the institute are:

1. To promote and advance the science of biology and its applications in Sri Lanka
2. To advise the government, and give counsel to public corporations, local bodies and other institutions on all matters connected with the application of biology to the progress and development of the country.
3. To promote acquisition, dissemination and interchange of biological knowledge by providing a forum for the presentation of original communications and discussions and maintaining libraries which publish matters of interest to the profession of biology.
4. To promote education in biology at all levels.
5. To promote, encourage and foster original research in biology.
6. To ensure the maintenance of high standards in the professional activities and the general conduct of its members.
7. To establish liaison with other scientific organizations
8. To establish and enhance the status of the profession of biology in Sri Lanka

Membership

The institute has around 400 members, working in industry, research, education and healthcare. The institute also awards Fellowships and Charter of Biology for members. There are 7 categories of membership and members are encouraged to transfer to other grades in due course. Eligibility for each category depends upon a combination of professional experience and academic qualifications. Fellows are entitled to use the designatory letters F.I.Biol (Sri Lanka) while the members are eligible to use M.I.Biol (Sri Lanka), associate members, A.I.Biol (Sri Lanka) and licentiates L. I. Biol (Sri Lanka).

The designation 'Chartered Biologist' endorses the high standards expected of biologists and is for international recognition as a hallmark of professional competence and ethical conduct.

Activities

The institute organizes workshops/seminars on current topics related to biology on a regular basis. It also plays an important role in biology education to a wider spectrum of participants ranging from those in the industry, those seeking self-employment, school children and general public. Details of events are posted on the IOB website. The institute website also gives information and advice on choosing a career in the biosciences for school children. The information provided on the web also keeps teachers informed on current events in the field of biology. The Biology Olympiad Competition organized solely by the Institute of Biology is a hallmark even in the country which provide opportunities to students in the country to become champions in biology both locally and internationally. The annual session provides a forum for both senior and junior biologists to present their research findings for a complex audience of scientists, policy makers and implementers. It is continuing for the 31st time this year.

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PRESIDENTIAL ADDRESS

Ecotoxicology: Why is it a discipline of growing importance?

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Ecotoxicology is the study of the toxic effects of chemicals on biological organisms, at the level of the organism, population, community or ecosystem. It is a multidisciplinary field, which integrates broad areas of toxicology, ecology and environmental chemistry. Its ultimate goal is to predict adverse effects of environmental contaminants and devise efficient and effective means of action to prevent or rectify any identified detrimental effects. In instances where ecosystems have already become severely degraded due to environmental pollution, ecotoxicology will allow us to take the best course of action to restore the system to a healthy state.

The beginnings of ecotoxicology

It is not wrong to say that *Silent Spring* by Rachel Carson published in June 1962 had the greatest impact on the attitude of humans towards environmental contamination. It was the single most influential book that caused an awakening to the reality that substances synthesized and used for our benefit may cause considerable harm to non-target organisms and the environment. Six years after its publication, a sinister effect of pesticide use was documented: the accumulation of DDE, a compound produced when DDT is degraded, were causing reproductive failure in several predatory birds, including Peregrine falcons, Brown pelicans, Osprey and Bald eagles. Not only was this compound toxic to developing embryos, it also caused eggs to have abnormally thin shells that easily cracked under the weight of the adult bird during incubation. This resulted in the ban of DDT in 1972. It was only much later that the true impact of Rachel Carson's book was realized, and in the 1990s it was named 'one of the 25 greatest science books of all time' by the editors of the Discover Magazine. According to Rodrick (1992) this book created fears in humans about synthetic chemicals in the environment and, among other things, fostered interest in the science of toxicology.

Ecotoxicology could be considered a science of the twentieth century, which had its early development in the late sixties. The term ecotoxicology was first used by Truhaut in 1969 (Truhaut 1977). He defined ecotoxicology as "a branch of toxicology concerned with the study of toxic effects caused by natural or synthetic pollutants to the constituents of the ecosystem, animals, vegetables and microbes, in an integral context". This differed significantly from the 'classical toxicology' which was mainly concerned with the effects of contaminants at subcellular to organism levels, with a heavy emphasis on humans. Prior to the 1960s, little attention was paid to the impacts on populations and ecosystems. The radical shift from toxicology to ecotoxicology resulted from the recognition that the passage of chemical constituents in organisms through food chains may have consequential impacts at levels beyond the organisms that are directly affected.

This field has now progressed far beyond these simple beginnings, with the past few decades becoming exceptionally exciting for the ecotoxicologist. Increasing quantities of deleterious substances are being relentlessly discharged into natural ecosystems, while there has been a transformation of fundamental sciences such as ecology and toxicology into more complex and applied disciplines. We are now in a better position to understand the mechanisms and pathways of contaminants and therefore to predict their impacts on the environment. Consequently, recent years have seen dramatic advances in our recognition of the impacts on species and ecosystems.

Evidence of adverse impacts of pollutants on species and ecosystems

A contaminant is described as a biological, chemical, physical, or radiological substance which is not usually present in the environment and which, in sufficient concentrations, can adversely affect living organisms. Such contaminants may include deleterious gases, agrochemicals, heavy metals, radioactive compounds and pharmaceuticals which are being discharged into the environment in increasing quantities principally through anthropogenic activities. These environmental contaminants enter organisms primarily via inhalation, ingestion or absorption through the nose, mouth or skin. Once entered, the substance can elicit both direct and indirect toxic effects in the exposed organism. Some contaminants are toxic in any quantity, while others exert toxic effects only when undesirable levels are reached. The manifested effects can also be described in terms of their magnitude and duration. A direct lethal effect is known as an acute effect while a prolonged effect is termed chronic. From the late 1960s scientists have been investigating the modes of action, levels of accumulation and the manifested toxic effects of various contaminants in different test organisms and, consequently, a wealth of information has been generated. Let us look at some of the documented findings of other ecotoxicologists and of my work with other co-workers in the Department of Zoology, University of Colombo.

At the level of the organism, enhanced mortality is one of the most commonly observed direct effects, which has been demonstrated for many species exposed to ecologically relevant levels of aquatic toxicants. Some examples are the high levels of mortality in fish and amphibians exposed to pesticides and heavy metals (Lefcort *et al.* 1998; Elskus 2007; Wijesinghe and Ratnasooriya 2010; Ranatunga *et al.* 2012). Such impacts have been also shown, to a lesser extent, in higher taxonomic groups. In 1989, the Environmental Protection Agency of the USA estimated that carbofuran alone kills 1-2 million birds in that country every year (Schauber *et al.* 1997). In 1995, it was reported that the pesticide monocrotophos, sprayed to kill grasshoppers, was responsible for the deaths of at least 20,000 Swainson's Hawks in Argentina (Hooper *et al.* 2002). They state that birds swallowed pesticide granules directly mistaking it for seed, and indirectly, by consuming contaminated voles and deer mice.

Apart from direct mortality, scores of studies have established that contaminants bring about sublethal effects. Among them are growth retardation, delayed development and metamorphosis, and behavioural changes with notable declines in activity. Deformities have been also induced by contaminants. An example would be the loss of limbs and spinal cord deformities in frogs induced by pesticides (Jayawardena *et al.* 2011). Additionally histopathological alterations have been reported to occur in critical organs such as the liver, gills and kidney. For instance, Bandara

et al. (2008) have shown degenerative changes in the liver and muscles of the Asian common toad exposed to field levels of pesticides. The hematological profile of a species is a good indicator of the levels of environmental stress because blood parameters are altered with exposure to pollutants as revealed in many a study (e.g. Parma *et al.* 2007). Immuno-suppression has also been noted in certain species (Halloran *et al.* 1997). Others have demonstrated genotoxic effects (Reicher *et al.* 1999) which are triggered by exposure to environmental contaminants and include structural changes to the DNA, which if not repaired, can lead to the appearance mutations.

What is of concern is that, while sublethal damage may not always cause direct mortality, there is a strong possibility that reduced survival may occur in the long term. Some of the undesirable sublethal effects may result in decreased life span and reduced breeding success. Lethargy coupled with smaller sizes of exposed individuals reduces breeding success, makes them more susceptible to predation or reduces their competitive ability at securing food resources (Crossland 1998). Decreased nutrition causes growth retardation that in turn leads to reproductive failure. Inhibition of the innate immune defense system can also lead to greater susceptibility to lethal pathogens. Davidson *et al.* (2007) has shown that carbaryl exposure increases susceptibility of the foothill yellow-legged frogs to the chytrid fungus which is widely associated with amphibian declines.

The magnitude of the manifested effects in organisms exposed to a contaminant will depend on several factors relating to the scale of the dose and the duration and pattern of exposure. It will also be very much dependent on the species, age, stage of development and the health status of the exposed animal.

The effects of contaminants on organisms will have connotations at the population level. The enhanced mortality and the diminution in fitness will lower rates of recruitment in the long run causing populations to decline in some cases to the point of local extinction. This in turn will lead to narrowing of species distributional ranges. Here in Sri Lanka, we are constantly made aware of the increasing numbers of species that have become nationally threatened; pollution has been identified as one of the major contributors. Groups particularly at risk are the bees, butterflies, fish and herpetofauna. It is clearly apparent that many species that were once common in agro-ecosystems are now far less common or extremely rare.

What impacts could one expect at the community or ecosystem level? Any serious changes in species populations would inevitably cause imbalances and lead to malfunctioning of entire ecosystems. Soil contaminants have been shown to inhibit growth and seed germination of plants and because they are at the bottom of the pyramid, every other organism in the ecosystem would consequently be affected. Animals have gone to the brink of extinction because of the feeding associations that exist within communities. In some cases the predator is affected by a toxin resulting in a decline of predator populations, which then results in an unlimited expansion of the prey population, which in turn brings about destruction to the entire ecosystem. Sometimes prey are affected resulting in a decline in the predator population. Because of the differences in

tolerance levels some adaptable species may also stand to benefit and expand at the expense of the less tolerant specialist species. Local population extinctions will evidently bring about changes in species composition and shifts in food habits of species.

Some substances (e.g. organochlorines) possess two dangerous traits – persistence i.e. the ability to remain chemically active for a long time and the solubility in fat resulting in accumulation in fatty tissues within organisms over time. This is commonly referred to as bioaccumulation. Because of these two traits, contaminants can be transported along a food chain with the harmful substances generally becoming more concentrated at the higher trophic levels, a process known as biomagnification. Some exceptions have, however, been documented. A combined effect of bioaccumulation and biomagnification would probably explain why the invertebrate community in a pond was found to be significantly different in species diversity and abundance from the original community two years after the application of the insecticide fenvalerate (Relyea 2005). The effects of environmental toxicants therefore have dangerous ramifications that flow from organisms through to populations and communities and ultimately to ecosystems.

Increasing deterioration of the natural environment

All over the world, the ongoing discharge of pollutants in the form of industrial effluents and domestic sewage and effluents has caused considerable and irreversible degradation of our natural habitats which makes ecotoxicology a discipline of growing importance. This interest is particularly strong in aquatic systems where many non-target organisms are more frequently exposed to deleterious substances. Therefore let me, as example, consider the pollution of the freshwater ecosystems stemming from the intensified use of agrochemicals in Sri Lanka.

Pesticides were first introduced to Sri Lanka in the 1940s to control malaria, but since then, there has been a significant rise in the import and application of agrochemicals in the country. According to the Registrar of Pesticides the total quantity of pesticides imported to the country has doubled over the last decade. The insecticide formulations imported increased from 849 tonnes in 2001 to 1713 tonnes in 2011. The respective values for herbicide and fungicide formulations are 2056 and 553 in 2001 and 5031 and 949 in 2011. According to a World Bank report, farmers in Sri Lanka use 284.3 kg of fertilizer per hectare of arable land which is far greater than that in other countries of the Asian region (India – 153.5 kg.; Bangladesh – 164.5 kg; Indonesia – 189.1 kg). The development of the agricultural sector over the years, particularly in the dry zone with the Accelerated Mahaweli Project, has no doubt intensified the use of agrochemicals. Agrochemicals and their residues enter waterways directly, or as surface runoff contributing heavily to the problem of water pollution in these areas. Despite the seriousness of the situation, only a handful of studies have attempted to record levels of pesticide residue in water. A study in Polonnaruwa, revealed the presence of Propanil (1.02 - 1.18 mg l⁻¹) and Chlorpyrifos (1.45 - 6.77 mg l⁻¹) in water samples collected from the Meegalawa Wewa, where irrigated agriculture was practiced. More information exists, nevertheless, with regard to nutrient enrichment in both surface and ground water sources in agro-ecosystems. Ground water, in intensively cultivated areas, typically have nitrate concentrations in the range of 10-15 mg l⁻¹ compared to 0.2 mg l⁻¹ within the non-cultivated lands. A study of 58 irrigation reservoirs across

the island revealed that all of them were in a state of eutrophication in both paddy growing seasons, having total phosphorus levels of more than $30 \mu\text{g l}^{-1}$ (Yatigamma *et al.* 2007). In fact, 79% of the wells in the dry zone had nitrate concentrations which exceed levels recommended by the World Health Organisation (WHO) for safe drinking water. Leaching of agrochemicals from intensively cultivated soil is also responsible for elevated concentrations of chloride, nitrate and potassium observed in many irrigation wells in the Kalpitiya peninsula (Lawrence and Kurupparachchi 1986). Consequences of excessive nutrient pollution i.e. eutrophication has been reported from many areas across the country. Eutrophication is also a matter of concern in the Kotmale reservoir (Piyasiri 2000). Phosphate enrichment has been reported from Nuwara wewa and to a lesser extent in Tissa Wewa in the Anuradhapura district. Nutrient enrichment is occurring in the Kandy Lake and Lake Gregory in the hill country. In all these cases increased nutrient loads in water have been largely attributed to the excessive use of fertilizers in the catchment areas.

The future challenges for ecotoxicologists

The merging of the two disciplines ecology with toxicology has resulted in the relatively young field of ecotoxicology. Despite the wealth of evidence generated so far, we have only yet begun to address the diversity of problems and questions concerning the effects of pollutants on individuals, species and ecosystems. Some of the areas that pose challenges in the ecotoxicological arena are given below.

One of the most common methods of assessing effects of various pollutants at the basic level of the individual is through empirical trials. Standard toxicity tests are carried out exposing selected study species to different concentrations of the pollutant to observe its effects. There are, however, several limitations in the selection of taxa/species for conducting such laboratory-based toxicity tests. Some taxa (e.g. amphibians and fish) have been found to be ideal indicators of the levels of pollutants in water bodies because of their high sensitivity to environmental stressors. Additionally, other characters that make species suitable for such tests would be short generation time, ease of rearing and handling. Apart from practical constraints, there are ethical issues that prevent the use of animals from higher taxa (birds and mammals). We must, however, devise practical and ethically acceptable means of investigating or making realistic predictions of the adverse impacts on these species as well. It should also be borne in mind, before generalizing, that the toxicity of a given substance may be highly variable between taxonomic groups and even between related species.

There are also major problems in extrapolating the findings generated from empirical trials to natural scenarios. One of the basic problems, particularly in countries such as Sri Lanka, is the lack of quantitative data on the levels of pesticides found in the water bodies within the country. Exposure trials will become more realistic only when field levels of the tested pollutants are used. In the case of agrochemicals in particular, it has been the view of some toxicologists that research should always focus on formulated products entering the natural environment, instead of assessing effects of the individual active ingredients. This stems from findings that commercial products elicit effects that are different to those induced by the active ingredients. A

case in point is the northern leopard frog which suffered reduced larval growth and deformities when exposed to the formulation round up but not when exposed to the active ingredient glyphosate (Howe *et al.* 2004). We need also to take into consideration that, in the field, more often than not, pollutants occur as mixtures, making it difficult to identify abnormal conditions and relate them to likely causes. Effects are, at times, accelerated because of synergism among environmental pollutants where the combined effect of two or more pollutants is greater than that caused by the individual pollutants acting alone. Also, some pollutants may react with natural elements in the environment forming substances that have a greater potency than the primary compound. Another confounding problem is the alteration of toxic effects by environmental factors such as solar radiation, sediment loads and pH. These factors may facilitate the conversion of the active ingredients to more potent or less potent substances which in turn would alter the magnitude of the manifested effects.

It is because of these complications that systematic field studies are limited both here and elsewhere in the world. Under the prevailing conditions, therefore, standard toxicity trials using realistic exposure levels would, without doubt, provide a strong basis for predicting the toxicity of various contaminants on non-target organisms.

One of the areas where information is most lacking is how the effects of pollutants on individuals affect population dynamics. For example, we must know, or at least be able to predict, what concentrations of a particular pollutant cause levels of mortality that would in turn affect population recruitment and growth. To make extrapolations from individuals to species, we need to also know more about the factors that bring about natural mortality.

In conclusion, it is my belief that scientists involved in ecotoxicological research fervently hope that their findings would lead to a better understanding of the impacts of contaminants on organisms, species and ecosystems. Hopefully this should in turn indicate possible ways of efficiently managing and conserving natural ecosystems and providing a safe and healthy environment for our present and future generations.

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FELICITATION

Professor Sarath Wimalabandara Kotagama

Citation presented by

Dr. N. Pallewatta

Department of Zoology, University of Colombo

It is my great pleasure to present the citation of Professor Sarath Wimalabandara Kotagama, Professor of Environmental Science in the Department of Zoology, University of Colombo, who is one of the most influential environmentalists and ornithologists of our time. I am indeed honoured and privileged to read your felicitation, sir, in this gathering of your students, colleagues and friends.

Born in 1950 in Bandarawela, Professor Kotagama received his primary and secondary education at St. Thomas' Preparatory School, Bandarawela, and then at St. Thomas' College, Gurutalawa, after which he moved to Colombo to complete his school education at St. Thomas' College, Mount Lavinia. He gained admission to the University of Colombo and graduated with Honors in Zoology in 1974. In 1977 he proceeded to the University of Aberdeen, Scotland, for his post graduate studies, and in 1982 was awarded the Degree of Doctor of Philosophy. After his return he served as a lecturer in the Department of Zoology, University of Colombo, in the Faculty of Natural Sciences. He left this position in 1985 and joined the academic staff of the Open University of Sri Lanka where he also served as the Head of the Department of Zoology. In 1997 he was recruited to the Department of Zoology, University of Colombo, as the Professor of Environmental Science.

Over the years, Prof. Kotagama has made a tremendous contribution towards uplifting the status of environmental education in Sri Lanka. He introduced and coordinated many courses in the fields of Environmental Science, Wildlife Management, Biodiversity Conservation and Ornithology. He acted as the Course Coordinator for the Special Diploma in Wildlife Conservation and Management offered to the staff of the Department of Wildlife Conservation. He also served as the Coordinator of the Masters programme in Environmental Science offered by the Department of Zoology, University of Colombo. He imparted his expertise in the fields of Conservation Biology and Environmental Science to other universities, both local and foreign. His strong focus on field exposure provided hands on training to many an undergraduate, which resulted in increasing their enthusiasm and so instilling in them a keen interest in the natural environment. I still remember the many field excursions he used to take us on, to places that we had never been before. Many of his students are here today and I am sure they will all attest to the fact that these memorable experiences increased our passion for nature conservation.

His postgraduate research at the University of Aberdeen, Scotland, was on the behaviour and feeding ecology of the Rose-ringed parakeet (*Psittacula krameril*) in Polonaruwa, which I believe was one of the earliest signs of his ardent interest in birds. Over the years his work has focused on the biology, behavior and conservation of birds of Sri Lanka. He has supervised a number of postgraduate students which includes 3 doctorates and 12 masters degrees. He has also guided and supervised many foreign students. His keen interest in birds resulted in research publications in peer reviewed local and international journals. He has authored and edited a large array of books on birds which have proved to be useful to school children, undergraduates, researchers, academics and the general public. It is seldom that you come across a bird watcher in Sri Lanka not being in possession of one of the many illustrated bird guides he has produced. For the convenience of Sri Lankans, these books have been translated into the Sinhala and Tamil languages. His work, over a period spanning four decades, has generated a wealth of scientific knowledge on the birds of our country, and has highlighted the need for their conservation. Accordingly many bird conservation programmes across the island have been initiated and are spearheaded by him.

One of his most significant achievements in the field of ornithology was the establishment of the Field Ornithology Group of Sri Lanka (FOGSL) in the year 1976. It is a non-profit organization affiliated to the Department of Zoology, University of Colombo. Through FOGSL he has succeeded in bringing together persons who are interested in the study and conservation of birds of Sri Lanka. This has generated much interest among laypersons and students in the study and conservation of birds. He has initiated, directed and carried out island-wide field studies on various aspects of bird biology, and established links with international bird conservation groups and programmes. Special mention should be made of the contribution of FOGSL to the international bird ringing programme. The programme activities carried out annually in Bundala and Jaffna are under the personal supervision of Professor Kotagama. This programme has provided training to scores of students on handling and ringing birds. His yeomen service to this field alone has earned him the title of “Father of Ornithology” in Sri Lanka.

Apart from his interest in birds, over the past forty years, he has worked on many other wildlife projects. One of the initial projects undertaken by him was the control the wildboar at the Kantale sugar Plantation. Owing to his experience in the fields of Wildlife Management, Environmental Science and Ornithology, his expertise and services are sought by government, universities and many other institutions, and by individuals. Some of the notable positions he has held are: Director of the Department of Wildlife Conservation over a brief period in 1989 -1990; Local Team Leader/Protected Area Management Specialist in the Protected Area Management and Wildlife Conservation Project (ADB) of the Ministry of the Environment and Natural Resources (2002-2006); Biodiversity Consultant at the Ministry of Transport, Environment and Women’s Affairs (1994 to 1995); and Wetland Consultant for the Wetland Conservation Project (1992).

He has served in numerous statutory committees and councils. I will mention a few of them. He was a member of the Fauna and Flora Advisory Committee of the Department of Wildlife Conservation and the Environment Council of the Central Environmental Authority. He also

served on the Advisory Committee of the National Museum. He was the Chairman of the Environment Council of the Central Environmental Authority, the National Biodiversity Experts Committee, and the Biodiversity and Environment Research Committee of the National Science Foundation. Today, he holds the prestigious position of the General President of the Sri Lanka Association for the Advancement of Science.

Through his involvement in these boards he has contributed to formulating reports, management plans, and policies that are of significance for biodiversity conservation in the country. For instance, he formulated the National Wildlife Policy of Sri Lanka in 1990 and authored the National Wetland Conservation Policy in 1997. He has represented the country at many international fora including at conferences of the parties of the Convention on Biological Diversity. He has many international affiliations through which he has been able to bring in foreign collaboration. He was the Secretary General, Pan Asian Ornithological Congress from 1996 to 2000 and was the Vice President, Bird Life Asia Council, Bird Life International, Cambridge, in 2000. He has served as the National Coordinator for many programmes such as the Asian Wetland Survey and the Asian red data programme.

Professor Kotagama has been the recipient of many awards and accolades during his illustrious career. In recognition of his outstanding services he has rendered to his motherland, he was presented with the President's Environment Award in 2004 and the Desha Manya Parisara vedi Samaja Daja Deshamanya in 2007 by the Government of Sri Lanka, Nijabima Harasara award 2011 by the Uva Provincial Council, and the National Exemplary Citizens Award in 2011. In view of the exemplary service he rendered to uplift national science education, he was awarded the "Sabaragamuwa Sarasavi Keerthi Sri" in 2005 by the University of Sabaragamuwa. The Sri Lanka Association for the Advancement of Science presented him with the Environment Committee Award in 1993 for his outstanding contribution towards environmental protection and in 2003 a Special Achievement Felicitation Award for taking science education to the people. For his research publications, he received Presidential Awards in the years 2003, 2004 and 2005. He has also received a prestigious international award in 2003 i.e. the Distinguished Achievements award for extraordinary service in contributing to environmental reform and education given by the Society for Conservation Biology.

Distinguished members of the Institute of Biology, it is very fitting and appropriate to recognize and felicitate a great Conservationist and Biologist of the highest caliber. I have therefore great pleasure in presenting to you Professor Sarath Wimalabandara Kotagama, for felicitation today by the Institute of Biology.

ABSTRACTS OF PAPERS

A preliminary study on the Ecosystem Services of four species of insectivorous bats in two natural caves, Wavulpena and Wavulgalge in Sri Lanka

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Insectivorous bats are considered to be the primary consumers of nocturnal insects. Yet, only a few studies have attempted to quantify or place an economic value on this service. The objective of the present paper is to examine the quantity and range of prey consumed by four species of insectivorous bats (*Rhinolophus rouxii*, *Hipposideros speoris*, *H. lankadiva* and *Miniopterus schreibersii*) in the two largest known bat populations (Wavulgalge and Wavulpena caves) in Sri Lanka. For calculating the weight of the prey consumed by bats, data from one of our previous studies, in these cave populations, was used. Our analysis showed that there was on average, a minimum of 30% increase in the weight of each individual after foraging. We calculated the weight of the prey consumed by an individual in a single night as follows;

$$W_p = N_b(W_b * 0.3)$$

(W_p = Weight of the prey consumed; N_b = Population size of bat species, W_b = Body weight of an individual bat of a given species). Accordingly the weight of the prey consumed by bats in both caves in a single night were: *R. rouxii* - 281.2kg, *H. speoris* - 35.4kg, *M. schreibersii* - 188.2kg and *H. lankadiva* - 163.5kg. This shows that the bat populations in the two caves consume over 1168kg (Wavulgalge: 282kg; Wavulpena cave: 886kg) of insects in a single night. The annual prey consumption of the two populations was 426,420kg. Bats consume a wide variety of insect prey from 15 Orders. The major Orders that constitute the diet of bats were Coleopterans, Dipterans and Lepidopterans which are harmful to human wellbeing either as agricultural pests or insects transmitting specific pathogens to humans, such as mosquitoes. While helping to sustain both natural and human dominated ecosystems as primary and secondary consumers, bats are extremely beneficial to human wellbeing, suppressing many harmful insects. Awareness programmes of these services can be effectively used as a potent tool in harnessing public support in the conservation of bats, many of which are an already threatened group of mammals, due to anthropogenic activities.

Key words: bats, insect suppression, Ecosystem Services, Wavulgalge cave, Wavulpena cave

I acknowledge with gratitude the placement and the facilities provided by Prof. Adam Holbrook, Associate Director of CPROST, Simon Fraser University, Canada during this study.

Documenting diurnal captive behavior of the endemic mouse-deer in Sri Lanka

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In Sri Lanka the mouse-deer of the family Tragulidae are represented by one genus *Moschiola* and two species, *M. meminna* (White-spotted chevrotain) and *M. kathygre* (Yellow-striped chevrotain) both endemic to the island. The former is confined to the dry zone whilst the latter is confined to the wet zone. The present study attempted to inventorize and study the behaviour of these two species using the captive population held at the National Zoological Gardens, Dehiwala. The two species are housed in a single enclosure of around 45 m² with 14 individuals of *M. kathygre* and 7 individuals of *M. meminna*. All except two of the *M. meminna* were adults and were from both sexes. The study was conducted during January – April 2011 from 0600 – 1800 hrs. Both scan sampling and focal animal sampling were used to prepare activity and time budgets, separately, for the two species.

The captive behavioural inventory of the endemic mouse-deer in Sri Lanka consisted of six broad behavioural categories: (i) Introductory - Nose-anal; (ii) Aggressive – chasing; (iii) Submissive - fleeing and slinking; (iv) Amicable - sleeping, suckling, feeding and licking, (v) Individual - foraging, resting, ruminating, self-grooming, alert, stretching, scratching, licking, urinating, sitting and defecating and (iv) Exploratory - sniffing and following. A greater proportion of individuals of both species exhibited a higher frequency of and allocated a greater amount of time on individual interactions throughout the day in comparison to other types of behavior for which peaks were noted. In general, only around 2 % of the animals on a given day showed introductory behavior probably due to the familiarity of the cage mates. Also, aggressive interactions were few and this too were restricted to the males. There was a clear absence of both aggressive and submissive interactions between 1400 – 1600 hrs. It was evident that the patterns of activity of the two species were very similar with only minor variations. For instance *M. meeminna* indulged in exploratory behaviour more frequently than *M. kathygre*. *M. meminna* also spent more time on active behaviour with two activity peaks in the morning and evening, whilst *M. kathygre* had only one peak activity time i.e. in the morning. Both species were restful during the afternoons during which the supplied concrete structures and shading plants were heavily used.

The present study reveals that animals are active during the day and that they are generally solitary animals. Social behaviour was apparent only during periods of lactation. It should be noted however, that the majority of the individuals at the zoo have been born and bred in captivity. Field observations of the mouse deer have shown that these animals are also activity at night. Therefore further studies are required to ascertain to what extent these behavioral patterns differ from those in the wild. Knowledge on aspects of behavior of wild individuals will be essential for the proper management of individuals held in captivity.

Keywords: Behavior, captivity, *Moschila meeminna*, *Moschiola kathygre*, mouse deer

Financial assistance from the University of Colombo is acknowledged.

**Some aspects of reproduction of the viperine sea snake
Thalassophina viperina (Schmidt, 1852) from Jaffna waters**

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The current understanding of the sea snake reproductive biology is limited. Detailed reproductive behaviour of sea snakes has been reported only for few species. The reproductive biology of the Sri Lankan marine snakes is poorly documented. *Thalassophina viperina* (Schmidt, 1852) is one of the common sea snakes reported from the Vadamarachchy coastal area. However reproductive information pertaining to this species is limited. Therefore a study was designed to investigate some aspects of reproduction of this species in the Jaffna coastal waters. The study was conducted between December 2011 and May 2012 on the coast of Vadamarachchy at ten landing centers as weekly bycatch sampling. Sea snakes were collected, brought to the laboratory and the followings such as the weight, total length, snout vent length, sex and maturity level were noted. Altogether 61 *T. viperina* were collected during our study period. Gravid females contained vitellogenic follicles in December and January and two individuals had fully formed embryos during May 2012. During the study period the number of follicles varied from 2 – 3 per female. The average clutch size was 3.85. The gravid females with ovarian follicles observed since December 2011 and embryos in May 2012 indicated the active reproductive season that falls between December and May and with a gestation period of around 6 months. The minimum length of a female bearing follicles was 69.4 cm. The total length of an embryo varied between 25.4 – 30.3 cm. Our study revealed a very strong viviparous condition – the umbilical cord attachment was in the embryo of *T.viperina*. The point of attachment was varied from 2.7 to 3.6 cm anterior to the anus of the embryo. This observation is reported for the first time in Sri Lankan marine snakes.

Keywords: Sea snakes, reproduction, viviparity, gravid, vitellogenic follicles, gestational period

Diversity and abundance of vector mosquitoes in Wanawasala area of Kelaniya in Sri Lanka and some factors affecting their abundance and distribution

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Present study was conducted to determine the diversity and abundance of vector mosquito species found in Wanawasala area, Kelaniya of Sri Lanka and to investigate some physicochemical, environmental and biological factors affecting the abundance of mosquito larvae in study area during the period from February to July, 2011.

The survey was carried out to cover a maximum of 30 outdoor sampling locations. Physical, chemical and biological parameters were recorded for each sampling sites and samples of mosquito larvae were collected with the standard mosquito scooper using dipping method, once a month. Meanwhile laboratory experiments were carried out to determine the effect of different levels of pH on larval survival of *Culex quinquefasciatus* mosquitoes.

Morphological identification of 2683 mosquito larvae collected during the study period was dominated by *Culex quinquefasciatus*(55.2%), *Cu.gelidus*(13.7%), *Armigeres* sp.(11.5%). Results revealed that rice field mud flats were the highest diverse habitat type found in Wanawasala area which included four *Culex* species and one *Armigeres* species (Shannon wiener diversity index/H' = 0.6648). Blocked drains were associated with significantly higher *Culex quinquefasciatus* larvae and over the time period their abundance was significantly increased compared to that of other mosquito species found in study area (P < 0.05). *Cu. gelidus* and *Cu .whitmorei* were restricted to habitats with live vegetation cover such as rice field habitats and marshy lands with higher water conductivity and turbidity. *Culex quinquefasciatus* larval habitats had low dissolved oxygen content and high biological oxygen demand with respect to the habitats of other mosquitoes. Their habitats were also positively associated with tubificid and chironomid larvae and negatively associated with vegetation cover. The larval abundance in Wanawasala area was positively associated with monthly average rainfall. According to laboratory experiment *Culex quinquefasciatus* larval survival was rapidly declined at pH level 9.4 in highly alkaline water and they cannot survive in pH of more than 11.0.

Keywords: Vector mosquitoes, diversity, abundance

**Analysis of the phylogenetic relationships of *Anopheles* species,
subgenus *Cellia* (Diptera: Culicidae) in Sri Lanka by using
ITS2-rDNA cistron**

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Acquiring information on molecular identities has become a fundamental approach for species differentiation and phylogenetic reconstruction of any taxa. Based on this knowledge effective disease control measures, such as abatement of malaria, could be devised. Sequence comparison of ITS2 region as a gene marker for molecular phylogeny is widely used because of high degree of variation even closely related species. The objective of this study was to conduct studies on genetic characterization of ITS2 region of rDNA cistron for species identification of some *Anopheles* species (subgenus *Cellia*) in Sri Lanka and to reconstruct the phylogenetic relationship among them. All Anopheline mosquitoes were identified to its species level by using standard mosquito identification keys. Those identifications were confirmed by the comparison of the ITS2 sequences obtained here and those in GenBank database. The phylogenetic relationships for these mosquitoes were estimated based on ITS2 sequence data by using Mega 4.0 software. *An. peditaeniatus* was chosen as the out group. The phylogenetic relationships inferred from UPGMA and maximum parsimony analysis revealed unique topology with one distinct monophyletic clade. Anophelines were grouped to three subclades: subclade 1 consisted of *An. culicifacies*, *An. varuna*, and *An. aconitus*. Subclade 2 consisted of *An. jamesii*, *An. maculatus*, *An. pseudojamesii*, and *An. karwari*. The other subclade 3 contained *An. subpictus* and *An. vagus*. The phylogenetic positions of the different malaria vectors that have the potential or already known to be active in malaria transmission are grouped into two main clusters. The molecular phylogeny obtained in this investigation proximately relates to that of the classical morphological taxonomy. Myzomyia series forms a distinctive cluster while Pyrethorophorous and Neocellia series formed a separate cluster. The relationship among Anophelines can be used to link with the grouping patterns used for morphological standpoint as well.

Justification: In mosquito scientific nomenclature, *Aedes* and *Anopheles* both genus names start with the letter "A". Therefore to avoid the discrepancy, the standard is to use *An* for *Anopheles* and *Ae* for *Aedes*.

Key Words: Anophelines, ITS2, Phylogeny

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Molecular confirmation of the presence of *E. coli* in shallow well water

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Infectious water-related diseases are a major cause of morbidity and mortality worldwide. Immense burden of disease caused by water-related pathogens pose a challenge to public health sectors. The presence of coliform bacteria, such as *E. coli*, in surface water is a *common indicator* of fecal contamination. Biochemical tests used to detect the presence of *E. coli* in environmental water are cumbersome, have lower specificity and sensitivity and require confirmation. Coliform bacteria in shallow well water samples isolated by membrane filter technique were examined by IMViC (indole, methyl red, Voges-Proskauer and citrate) tests to determine the presence of *E. coli*. DNA from bacterial colonies, tested positive for *E. coli* by IMViC tests was extracted by Guanidine thiocyanate-based purification method and PCR amplified using uidA primers specific for *E. coli* gene β -D glucuronidase to confirm the validity of the IMViC tests. Samples that gave positive results for indole and methyl red tests and negative for citrate and Voges-Proskauer tests also gave a characteristic DNA band of 380 bp length confirming the presence of *E. coli* and vice versa. The present study demonstrates that PCR is sensitive, relatively inexpensive and rapid compared to cumbersome biochemical tests and can be used to detect *E. coli* in water with high specificity.

Keywords: bacteriological analysis, IMViC tests, *E. coli*, Polymerase Chain Reaction, β -D glucuronidase gene

Effect of Macro & Micro Nutrients on Occurrences of Fusarium Wilt (*Fusarium oxysporum*) in Tomato (*Lycopersicon esculentum* Mill.)

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This research was conducted to test the influence of the macro and micronutrients on occurrences of *Fusarium* wilt. Plants were grown in *Fusarium* wilt infested soil; under Department of Agriculture (DOA) recommended macro nutrients and various combination of Ontario recommended dosages of secondary and micro fertilizer supplementations. There were seven treatments in this experiment and each treatment was replicated in three times and each replicate consisted of six plants. Other than the DOA recommendation, a group without fertilizer and five combinations of Ontario recommended dosage of secondary and micronutrients for tomato were also used as treatments. The treatments were arranged in a Randomized Complete Block Design. All cultural practices were carried out according to the DOA recommendations. The mean data was subjected to the statistical analysis using SAS package and mean separation (Duncan's Multiple Range test at 5% probability level) procedures. Micronutrient containing treatments showed significantly better results in tolerance of *Fusarium* wilt than the DOA recommendation. This could be due to the supplementation of all the essential macro micro nutrients; which is known to regulate metabolic activity associated with defense mechanisms of a plant. An adequate supply of Manganese and other micronutrients are playing a key role in enhancing most of the active defense mechanisms. Supplementations of macro secondary and micro nutrients promote healthier plants that are better equipped to survive and defend themselves against diseases. Study revealed that, application of secondary and micro nutrients other than the macro nutrients can be positively influence the tolerance of *Fusarium* wilt in tomato.

Keywords: *Fusarium* Wilt, Micro Nutrients, Macro Nutrients, Tomato, Tolerance

Floral biology and breeding system of *Osbeckia octandra* (L.) DC.

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Heen Bowitiya (*Osbeckia octandra*- Melastomataceae) is an endemic, medicine plant in Sri Lanka. Due to paucity of reproductive biological information, the present study aimed to investigate the floral biology of *O. octandra* in relation to pollination and to identify its breeding system. Floral level phenological changes; timing and duration of anther dehiscence, stigmatic receptivity; UV color attractants and movement of the style were investigated from 25 flowers in 10 individuals. Pilikuththuwa forest and abandoned lands. Effective pollinators were observed and identified among collected floral visitors. Pollen histochemistry was analyzed using Iodine, Sudan III and Ninhydrin solutions. Viability of pollen after anther dehiscence and under storage conditions (at 4 °C and 0 °C); and *in vitro* pollen germination was tested using sucrose series (0 - 25%). Breeding system was estimated by Pollen Ovule (P:O) ratio, Out Crossing Index (OCI) and controlled pollination experiments with minimum of 60 replicate flowers for each treatment.

Purple color petals and yellow anthers increased pollinator attraction. Yellow anthers were clearly visible under UV which enhanced attraction of pollinators. While inflorescences of *O. octandra* borne with mirror image flowers, the flowers were monomorphic enantiostyous and herkogamous. Monomorphic enantiostyly did not significantly affect natural pollination success (one way ANOVA, $p < 0.05$). While flexistyly at closing stage of flower facilitated autogamy, geitonogamy was facilitated by simultaneous occurrence of pistillate and staminate phase flowers within an individual.

Pollen of *O. octandra* were strachless, however, it contained lipids and amino acids. Stigma was papillate. The optimum sucrose concentration and required duration for the highest pollen germination was 15 % and 3 hrs respectively. Pollen collected at 8.30 a.m. showed the highest pollen germination. Pollen storage at 4 °C and 0 °C were not appropriate. The effective pollinators were bees (*Amegilla* spp; Hymenoptera). The OCI value (4) and P:O ratio (1951 .89+23.45) indicated flowers were partially self-compatible, very high demand for pollinators and its facultative xenogamy. The highest fruit set was obtained from natural pollination (66.67+3.40) and was not significantly different with controlled cross pollination (61.67+1.69). Artificial autogamy (3.33+1.69) was not significant different from geitonogamy (3 1.67+1.69). The *index of self-incompatibility* value of *O. octandra* (0.63) revealed that flowers were partially self incompatible. The findings of this study strengthened the knowledge of reproductive biology and breeding system of *O. octandra*.

Keywords: *Osbeckia octandra* / Floral biology / breeding system / pollination / pollen germination

Effect of microbial biofilms inoculated to selected forest trees at nursery stage

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In Sri Lanka, forest cover is declining at the rate of 1.5%, annually. In order to sustain the forest cover of 29%, reforestation efforts have been undertaken using native or exotic plant species. However, nursery plants face up to 50% failure in establishment in reforestation. Incapability of exotic species to establish in sufficient numbers on degraded lands has been thought to be due to lack of supporting soil microbial consortia. Therefore, attention has been paid to use native trees, which are however hindered by slow growth rate. Thus, the use of beneficial microbial inocula would facilitate the early survival and rapid growth of seedlings, through biological nitrogen fixation, soil moisture conservation, improved soil-plant nutrient cycling, secretion of growth hormones etc. An inoculum of beneficial microbes was developed in biofilm form, by using bacterial isolates from the rhizosphere of five native tree species namely, *Diospyros ebenum* (Ebony), *Berrya cordifolia* (Trincomalee wood), *Azadirachta indica* (Neem), *Cassia fistula* (Golden shower tree), *Chloroxylon swietenia* (Satinwood) and *Chukrasia tabularis* (Burmese almond wood). Isolated bacteria were screened for their ability of biological nitrogen fixation, organic acid production, solubilization of phosphate and plant growth promoting effect. Nine diazotrophic bacteria were selected and tested for biofilm formation ability with a fungus isolated from the rhizosphere of native tree species. Prepared biofilms were screened for plant growth promoting effect using an indicative plant. Selected best biofilm was applied to four major forest plantation tree species *Artocarpus heterophyllus* (Jack), *Tectonia grandis* (Teak), *Eucalyptus leucoxylon* (Eucalyptus) and *Swietenia macrophylla* (Mahogany). *A. heterophyllus* and *S. macrophylla*, showed improved plant growth, as reflected by increased plant height, leaf number and quick recovery of leaf flush after induced drought. It was also noted that it is important to test species-specific inocula, if their performances are to be further improved. Thus, there is a possibility of using biofilmed inocula to improve ecological sustainability of reforestation programs. Large-scale studies are however recommended.

Keywords: Microbial Biofilms / Reforestation/ Native tree species

Land-cover change detection using multi-temporal satellite images in Dambulla, North Central Province, Sri Lanka

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Dambulla is an emerging metro centre, which has been developed into an agro-based trade city centre. Tourism is also demanding since the city has preserved cultural and historical values. Economic growth of the city influenced changing land-cover in large scale over the past decade. This study attempts to evaluate the land-cover (for example vegetation) change in the Dambulla area during the last 25 years, using multi-temporal satellite images. Landsat images of the study area were acquired from the Global Land Cover Facility. Landsat MSS satellite image for year 1976, Landsat ETM+ for year 2001 and mosaic Landsat TM images for years 1987 & 1994, 1992 & 1994 and 1999 & 2001 have been utilised to quantify the land-cover changes from 1976 to 2001. ENVI[®] EX software package was used to detect the changes from 1976-1994, 1987-2001, and 1992-2001 using Normalized Difference Vegetation Index (NDVI). ArcGIS 9.3[®] and ArcGIS Explorer Online platforms were used to make land-cover maps of the study area. Results showed that vegetation was decreased by 9% in 1976-1994, 19% in 1987-2001 and 34% in 1992-2001. According to the land-cover map in 2000, the majority of changed land area in 1992-2001 was cultivated and urban developed areas. Landsat MSS image in 1976 showed that Kandalama tank was contracting in size. The results showed that the prominent forests are confined to the north-eastern part of the study area. Transition from scrublands to cultivated lands in between forests was also increasing. In conclusion, the vegetation change in between those three time steps has become doubled. Therefore, the present findings provide valuable baseline information for environmental managers to evaluate trends in land-use/land-cover change in order to improve sustainable land management.

Keywords: Land-cover, Change Detection, NDVI, Dambulla

Oral diuretic activity of Sri Lankan low grown Orange Pekoe grade black tea (*Camellia sinensis* L.) in rats

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In Sri Lankan ethnomedicine, black tea is claimed to be a diuretic. However, the grade of the tea is not specified although some studies have shown that bioactivity differs with the grade. Recently, we showed the diuretic potential of two types of broken grade black teas, namely Dust No.1 and Broken Orange Pekoe Fannings (B.O.P.F.). However, the diuretic potential of whole leaf grade of Sri Lankan black tea is not known. The aim of this study was to investigate the diuretic potential of Sri Lankan low grown orthodox whole leaf grade black tea (*Camellia sinensis* (L) O. Kuntz ; Family: Theaceae) using Orange Pekoe (O.P.) grade tea. Different doses of black tea brew (BTB) of O.P. grade tea [111 mg/kg (equivalent to 0.75 cups, N=7), 223 mg/kg (equivalent to 1.5 cups, N=15), 446 mg/kg (equivalent to 3 cups, N=15) and 1339 mg/kg (equivalent to 9 cups, N=12)] or water (control, N= 16) or Furosemide (reference drug, N=15) were orally administered to hydrated rats and their cumulative urine output was monitored at hourly for 6h. The results showed moderate (compared to Furosemide), dose dependent diuretic activity. The onset of the diuretic activity was very rapid (within 1h) with an equally rapid peak diuresis (1-2h), but with a short duration of action (up to 2h). EC₅₀ value for the diuretic action was 509.18 mg/kg. The diuretic action was accompanied with significant (P<0.05) increase in urinary Na⁺ (Natiuresis), K⁺ (Kaliuresis), pH, glomerular filtration rate (in terms of creatinine clearance), aldosterone secretion index, thiazide diuretic index, intra and extra cellular pH regulatory index and urinary alkali index, whilst the carbonic anhydrase inhibition index was significantly reduced. In contrast, urinary Cl⁻, and HCO₃⁻ levels, total dissolved solids, conductivity, density, and specific gravity remained unaltered. Overall, the results suggest that, Sri Lankan low grown O.P. grade black tea has moderate diuretic activity mediated via multiple mechanisms.

Keywords: *Camellia sinensis*, Orange Pekoe grade tea, diuresis, urine output

Acknowledgement: This investigation received financial support from the NSF under grant number NSF/Fellow/2011/01.

Pharmacognostical identification of Sri Lankan endemic medicinal plant *Canarium zeylanicum* Blume

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Canarium zeylanicum Blume (English: Ceylon elimi tree) of family Burseraceae is vernacularly known as “Mal Kekuna” and is an endemic medicinal plant whose stem bark and oleo-gum-resin is used in Sri Lankan Traditional Medicine to treat various ailments such as fractures, ulcers, inflammatory joint disorders etc. since ages. Nevertheless, no work has yet being carried out to establish its identity, quality and purity so far as its standardization is concerned. Therefore, present study was carried out to establish its identity by morphological, microscopical, physico-chemical, Thin Layer Chromatography, essential oil content and GCMS identification of essential oil as per WHO guidelines on quality control and standardization of medicinal plant materials. Distinct morphological characters observed were rough matured stem bark which is off white coloured, short fractured, imparting characteristic aromatic odor of family Burseraceae. Important microscopical structures were groups of thick walled sclerenchyma in the cortex region, thick walled narrow lumen fibers, volatile oil oozing cells from the cortex and groups of tangentially running phloem medullary rays. Determination of the physico-chemical parameters was triplicate and yielded moisture content of 11.80%, total ash content between 3.34-3.73%, acid insoluble ash content of 0.80-0.84%, extractable matter in water 21.04% and 22.0% in 70% ethanol. TLC finger print was developed that eluted 5 distinct spots with the Rf values 0.25, 0.31, 0.49, 0.62, 0.72, 0.89 consecutively coloured light pink, grayish blue, Prussian blue, light blue, grayish blue and grayish blue. Essential oil content estimated was 1-1.5% W/W on dry basis. GCMS studies were carried out with hexane fraction that eluted around 11 compounds such as; zingiberene, alpha-phellandrene, beta-phellandrene, alpha-pinene, beta-pinene, alpha-copaene, alpha-muurolene, alpha-cubebene, isodene, delta- cadinene. Therefore, the present research data would help in identification of *Canarium zeylanicum* stem bark.

Keywords: *Canarium zeylanicum* stem bark, microscopy, physico-chemical, TLC fingerprint & GC/MS

Acknowledgement: NSF funding RG/2011/NRB/04

Preliminary investigation method to determine the efficacy of *cryptolepis buchanani* and *glycyrrhiza glabra* plant extracts in wound healing.

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Many medicinal plants and plant parts are utilized in the preparation of Ayurvedic and indigenous medicines, which aid in healing of wounds. Scratch wound assay (wound healing assay WHA) could be used to identify potential sources that have wound healing properties. In this method monolayer of cells in tissue culture well plates are used for analysis. In this study WHA was carried out for different extracts of two plants. *Cryptolepis buchanani* and *Glycyrrhiza glabra* on monolayer of Madin-Darby Canine (MDC) Kidney cells and Hela human liver (Hep2) cells. Cells were grown in growth medium. Cells were distributed on 12-well tissue culture plates (SARSTEDT) with growth medium. After formation of monolayer of cells, a wound was made by mechanically scratching the cell layer. Then extracts or fractions of extracts of plant materials were introduced using dimethylsulfoxide (DMSO) as the carrier medium at a sample concentration of 0.1mg/mL. Migration of cells to heal the scratch wound was captured with the help of phase contrast microscope using imaging technology. Initial width of the scratch and the width of the scratch after treatment, at different time intervals were measured by using a stage micrometer. Healing of wounds was observed at different time intervals, 12 to 24 hours for Madin-Darby Canine Kidney cells and 24 to 72 hours for Hela human liver cells. The time taken for the scratch wound to heal is used to estimate the relative activities of the extracts. In the MDC assay, the hexane and dichloromethane extracts showed similar activities (>95% closure in 24 hours), while the ethyl acetate and MeOH extracts were inactive. In the Hep 2 assay, only the dichloromethane extract of *Glycyrrhiza glabra* was active (>95% closure in 49 hours),

