# Development of a Fruit Nectar from Ambarella (*Spondias dulcis*): A Value Added Product from an Underutilized Fruit Crop in Sri Lanka

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## Introduction

The underutilized fruit crops are the plant species that are traditionally used for their food, fiber, fodder, oil or medicinal properties. Wide range of underutilized fruit species are grown in tropical and subtropical regions and some of them are utilized for food purposes; as fresh fruits, curries, salads and as homely prepared processed foods (Pushpakumara *et al*., 2000).

Ambarella (*Spondias dulcis*) is one of the underutilized and seasonal fruit crops in Sri Lanka. Ambarella is widely utilized as a curry and chutney in Sri Lanka, however, the majority of the fruit production is wasted without utilizing during the seasonal periods. Nectar is a popular and widely consuming beverage manufactured basically from fruits worldwide. These fruits contain a number of natural materials that contribute to the overall flavor and consistency of the nectar including water, sugars, organic acids, and flavor compounds that are important to our diet. Besides water and sugar, it is an excellent source of vitamin C, potassium and folic acid, which are recommended for women who are pregnant or may become pregnant (Gunasena *et al*., 2003). Therefore, manufacturing of fruit nectar from Ambarella as a ready to serve value added product to the Sri Lankan food and beverage industry will be a good solution for the enormous wastage of local Ambarella fruits.

## Materials and methods

Immature, mature and completely ripen Ambarella fruits were selected for the sample preparation. The pulp of the each samples were extracted by removing the peel of the fruit and filtered through a sieve to separate coarse particles. Adequate quantity of pulp was mixed with the syrup, prepared using water and adequate amount of sugar. Sodium Metabisulphite (SMS) was added to the mixture as a chemical preservative. Then the pH and Total Soluble Solid (TSS) levels were measured and pulp percentage levels were determined as basic quality parameters.

In order to determine the sensory qualities, three samples were coded as;

123 - Nectar prepared from unripe fruits obtained from the market.

456 - Nectar prepared from Matured fruits selected before ripening on trees.

789 - Nectar prepared from completely matured fruits selected after initiating the ripening on trees.

Three samples were sensory evaluated using five point hedonic scale with the participation of 25 untrained panelists. Collected data were statistically analyzed using Friedman test with the confidence level of 95% in MINITAB version 14 statistical software. Chemical and microbiological analyses were performed for the selected best sample according to the results. Further, proximate analysis and shelf life analysis were also performed by comparing the variation of pH values and soluble solid value in the period of 84 days.

## Result and discussion

Pulp percentages of selected three samples varied between 38% - 60%. Juice content of completely ripen fruits was considerably high compared with pulp obtained from immature fruits. Sample prepared with fruits at the stage of completely matured and ripening initiated on the trees was selected as the best sample by the pair wise comparison using the critical difference value. TSS values and pH values didn't show much variation with the time and the values were in slandered levels. According to the Friedman statistical analysis, three samples were found significantly different for all sensory attributes. Therefore, sample 789 was considered as the best sample considering all the sensory attributes.

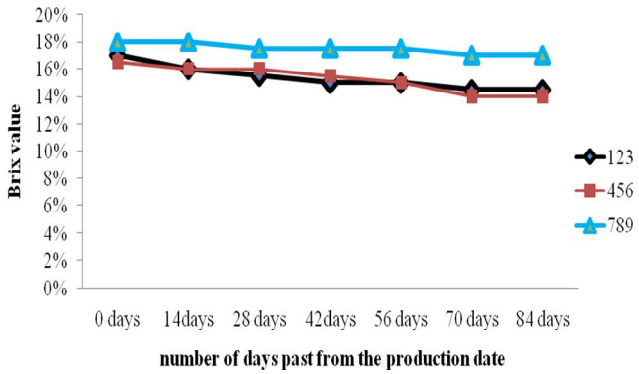


Figure 1: The graph of Brix value vs time

According to the obtained results, the Brix value variation was low in 789 sample compared with other two samples.

According to the proximate analysis, nutritional level of Ambarella nectar is as follows; Energy 69.12 K Cal/ 100 g, Carbohydrate 16.65%, Protein 0.45%, Fat 0.08%, Sodium 3018 mg/ Kg, Pottasium 344 mg/ Kg, Calcium 94.7 mg/ Kg. The total cost for the production of Ambarella nectar was calculated and it was 190.00 Rs per one liter.

## Conclusions

Ambarella can be recommended as a fruit source for fruit nectar production and the best maturity stage for the nectar production is the stage of fruits completely matured and ripening initiated on trees.

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# Development of Wine from Jack Fruit (*Artocarpus Heterophyllus*): A Value Added Product from a Tropical Fruit Crop in Sri Lanka

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## Introduction

Wine is an alcoholic beverage typically made from fermented grape juice or variety of fruits. Since fruits have natural sugar and natural acids, they provide all the required ingredients for making wine. The quality and the type fruit dictate the final quality of the fruit-based wine. Therefore, selection of correct fruit commodity is critical in wine making (Fithriadi *et al*., 1996).

Jackfruit is an extensively grown and very important tree for the people of Sri Lanka. It is essentially a carbohydrate food and is useful as a source of energy. The perianth is rich in sugars, contains carotene, protein, fat, calcium, phosphorous, and iron in quantities similar to those found in other fruits which are used to manufacture wine (Pushpakumara *et al*., 2007). Therefore, Jackfruit can be considered as a potential fruit crop in making wine. Wine is produced by fermenting crushed fruits using various types of yeasts. The majority of the yeast used in baking is *Saccharomyces cerevisiae*, which is the same species commonly used in alcoholic fermentation, and so is also called brewer's yeast (Pelcza *et al*., 1977).

Therefore, this study was carried out to prepare wine from Jackfruit juice with the baker's yeast as a low cost method for wine making and to compare the sensory attributes and quality parameters with commercial red wine from grape.

## Materials and methods

Completely ripened and undamaged jackfruits were collected from home gardens in Badulla area. The pulp of the jack fruit was crushed and juice was extracted. According to the pH and the Total Soluble Solid value (TSS), the most suitable pulp was selected for the fermentation. In order to prepare the suitable solution for the fermentation, the pulp was dissolved with distilled water according to 1:2 ratios until the brix value reaches to 20.

Three workable solutions were prepared with the pulp-water mixture and different amounts of sugar as follows.

Formulation (1) A = fruit juice 200 g and sugar 220 g, 400 mL of water.

Formulation (2) B = fruit juice 200 g and sugar 240 g, 400 mL of water.

Formulation (3) C = fruit juice 200 g and sugar 260 g, 400 mL of water.

Then all the mixtures were allowed for fermentation for four weeks, just after adding the starter culture, prepared with baker's yeast and sugar. Three replicates were maintained for each sample throughout the experiment. After four weeks, Sulfur dioxide bubbling was performed to stop the further fermentation and finally filtered using bacterial filters. Parameters such as pH level, Total Acidity (TA), TSS and alcohol content were measured in each sample. Sensory attributes such as taste, aroma, flavor, color/ appearance, and overall acceptability were evaluated using five point hedonic scale (where 1=dislike extremely and 5= like extremely) with thirty untrained panelist. Ranking test was applied to analyze the data obtained.

**Result and discussion**

According to the results obtained for the sensory evaluation, a significant difference ($p<0.05$) was obtained for all the attributes. (Figure.1)

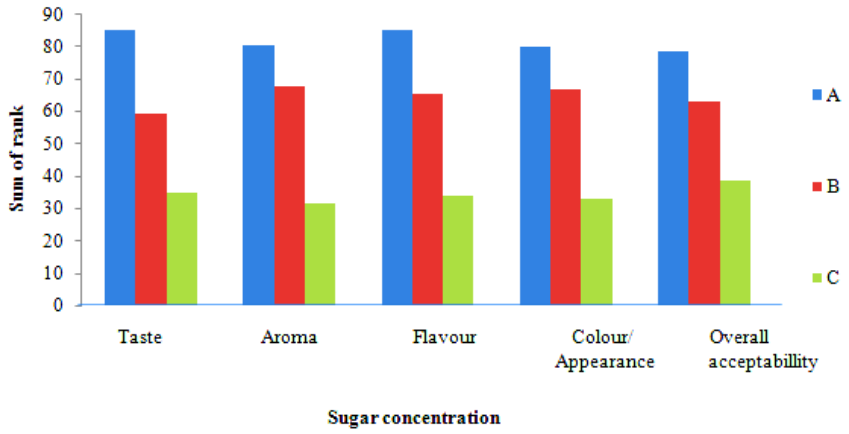


Figure 1: Preference for the wine sample series prepared using baker's yeast

Sample "A" had scored highest values for all the attributes compared with other samples (Table 1). According to the results, sensory attributes of sample B and C can be potentially improved. Considering all the sensory attributes and the quality parameters, sample A prepared with baker's yeast can be recommended as the most suitable product among the concentration series.

Table 1: comparison of wine sample A with red wine from grapes using Wilcoxon sign test

| Objective measurement | Standard wine | Wine sample A | | |
|---|---|---|---|---|
| | | Estimated mean | P value | Decision |
| Alcohol content | 14.5% | 14.5% | p=0.371 | Alcohol Content of the sample A = stranded red wine |
| pH | 2.9 - 3.3 | 2.805 | P=1.00 | PH of the sample A = standard red wine |
| TSS | 10 | 12 | P=0.181 | TSS of the sample A = standard red wine |
| TA | 0.6-0.7% | 0.6025% | P=1 | TA of the = standard red wine |

According to the above results there was no significant difference ($p<0.05$) between sample A and red wine sample from grapes.

**Conclusions**

Wine sample A has high sensory attributes, alcohol 14.5% and TA 0.60, pH 2.0, TSS $12^0$. According to the results, alcohol percentage, pH, TSS, TA and sensory attributes of wine sample A (jackfruit 200 g, sugar 220 g, water 400 mL) can be recommended for the preference and usage of underutilized fruit crops in Sri Lanka.

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# Development of Minimally Processed Banana Blossom (*Musa acuminata* colla)

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## Introduction

Minimally processing of fruits and vegetables is a rapidly developing segment of the food industry. The manufacturing steps of minimally processed products involve washing, sorting, peeling, slicing, blanching and packaging in films. The wound responses are the major problem of fresh cut development which cause the enzymatic browning (Wickramarachchi and Ranamukaarachchi, 2005) leading to the reduction of visual and organoleptic quality. Control of wound responses are the key to produce minimally processed product of good quality (Janisiewicz *et al*., 1999). Blanching is an important treatment, which primarily aims at inactivating the enzymes that cause undesirable changes (Kaur and Kapoor, 2000). The increase in cut damaged surfaces and availability of cell nutrients (Delaquis *et al*., 2003) and increased handling (Darmabandu *et al*., 2007) of the products provide greater opportunity for contamination by pathogenic organisms. Minimally proceed vegetables are rare in the Sri Lankan market. Therefore, the major objective of this research is to develop a minimally processed vegetable from a consumption constricted local banana blossom.

## Methodology

Fresh mature banana blossoms were purchased from the Badulla market and stored in a refrigerator until the processing initiated. The processing of banana blossoms involved removal of outer 2-3 outer bracts, washing by distilled water (8 ºC) followed by 100 ppm chlorinated water (8 ºC) for 5 minutes and sterile water (8 ºC) for 5 minutes. Then the banana blossom was cut into small pieces which are of 5 mm to 10 mm in thickness using a sharp stainless steel knife. Cut pieces were separately subjected to pre-treatments: T1 = distilled water (Control), T2 = 1g/l citric acid solution, T3=1g/l ascorbic acid solution, T4 = 1g/l sodium meta bisulfate (SMS) solution, T5 = 1g/l calcium chloride solution. All the treatments were given for five minutes under 8 ºC. The pre-treated samples were drained and packed in low density poly ethylene (LDPE) pouches (150 gauge). Packages were stored in a refrigerator at 8 ºC. The extension of browning was noted (none-1, slightly - 2, moderate - 3, high – 4 and very high - 5) until seven days of storage. The selected best treatments were carried out in increasing concentrations C1-1g/l, C2-2g/l, C3-3g/l, C4-4g/l, and C5-5g/l. For the best treatments the extensions of browning and aerobic & anaerobic microbial load were determined under the $1^{st,}$ $3^{rd}$, $5^{th}$ and $7^{th}$ days of storage. The experiment was repeated three times. The sensory evaluation was carried out for uncooked and cooked samples separately by involving 20 untrained panelists. The tested sensory attributes for cooked samples were taste, aroma, mouth feel, appearance and overall acceptability and it was a texture, color and appearance for uncooked samples. The five point hedonic scale was used to record the sensory attributes in both cases (like extremely – 5, like moderately – 4, neither like nor dislike –3, dislike moderately -2, dislike extremely – 1). Data were analyzed by Minitab statistical package version 14.

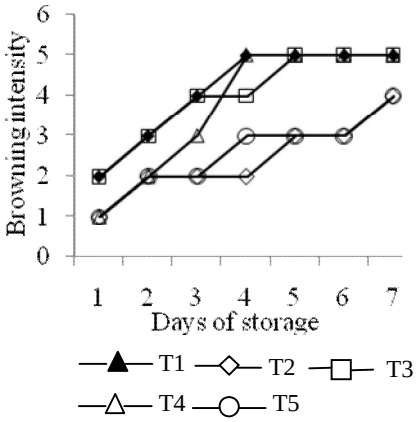



Figure 1: Browning intensity of minimally processed banana blossom against the four treatments and control under low temperature storage (8 $^{o}$C)

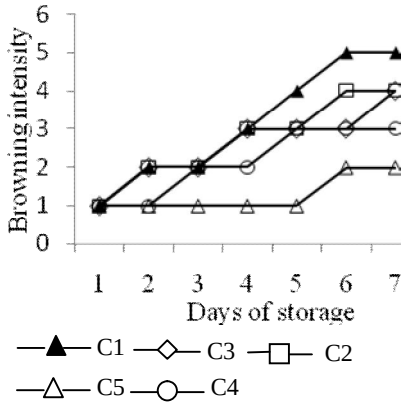



Figure 2a: Browning intensity of minimally processed banana blossom against the different concentrations of citric acid under the low temperature storage (8 $^{o}$C)

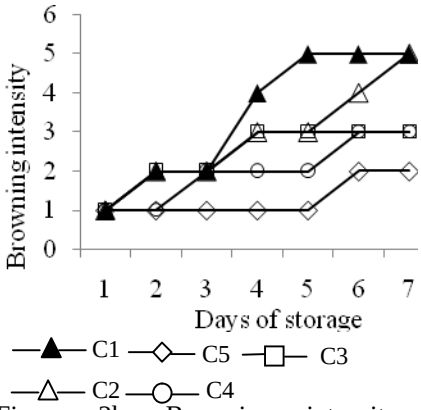



Figure 2b: Browning intensity of minimally processed banana blossom against the different concentrations of calcium chloride under low temperature storage (8 $^{o}$C)

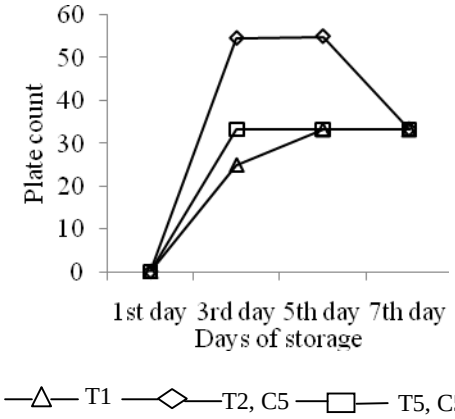
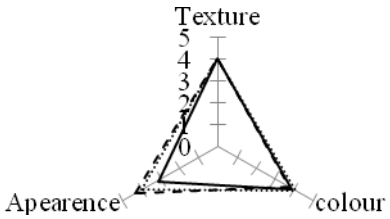



Figure 3: Anaerobic bacteria colony count of minimally processed banana blossom under the treatment of citric acid (5g/l) and calcium chloride (5g/l) under low temperature storage (8 $^{o}$C)

**Results and discussion**

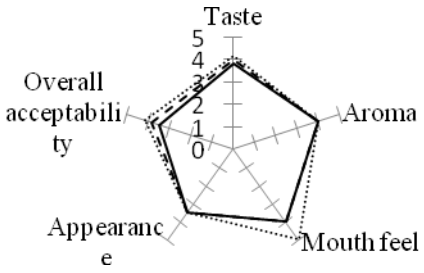
The cut surface discoloration is minimum with the citric acid and calcium chloride treatments (Figure 1). The effectiveness of browning prevention is higher with the increment of the concentrations of the both chemicals such as citric acid and calcium chloride. The similar results observed in all 3 trials (Figure 2a and 2b). According to the microbial determination, the aerobic colony count is an uncountable number. However, the number of anaerobic bacteria colonies is same under both treatments during the day

7 of storage (Figure 3). The Figure 4 and 5 show the sensory profile for the cooked and uncooked samples, respectively. In both sensory tests the sensory attributes were a significantly difference ($p<0.05$).




----- Citric acid …… $CaCl_2$
——— Control

Figure 4: Sensory profile diagram for uncooked minimally processed banana blossom




----- Citric acid …… $CaCl_2$
——— Control

Figure 5: Sensory profile diagram for cooked minimally processed banana blossom

**Conclusions**

Citric acid and $CaCl_2$ were the best treatments which control the browning in fresh cut banana blossoms. According to the sensory evaluation both citric acid and $CaCl_2$ treated uncooked samples were accepted by the sensory panel. Out from the cooked samples $CaCl_2$ treated sample was the best.

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# Development of Cocoa and Coffee Based Set Yoghurt According to Sri Lankan Consumer Preference

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## Introduction

Dairy products play a major role in human diet as milk is complete food. Yoghurt is one of the dairy products, popular as a desert which contains standardized full cream milk, sugar, gelatin, permitted stabilizer and colorings. Yoghurt market in Sri Lanka is still in growing stage compared with other countries. Consumer preference is due to increasing desire to take a more proactive role in optimizing personal health and wellbeing without relying on pharmaceuticals. The consumption of fermented yoghurt in which lactose has been converting to lactic acid has helped to reduce the risk of having pathogenic microorganism grow in the food (Baker and Miller, 1990).

Consumer preference is considered as one of the major critical parameters in food industry including dairy products. Vanilla, chocolate, banana, mango, strawberry are the different types of flavored yoghurts available in the market according to the consumer preference. Cocoa and coffee have lots of health benefits and can be used as flavors in yoghurt industry. Cocoa is rich source of antioxidant flavonoids, which may have beneficial cardiovascular effects on health and coffee contains caffeine, which acts as a stimulant. (Tamine and Robinsion, 1985). Therefore, the present study was carried out to produce cocoa and coffee based set yoghurt according to Sri Lankan consumer preference and as a new value added product for dairy industry.

## Materials and methods

Yoghurt mixtures were prepared under three different levels of cocoa and coffee concentrations. Each mixture were contained 3.25% fat, sterilized milk, skimmed milk, full cream milk powder, sucrose, gelatin, stabilizer, starter culture. Initially milk fat content and milk solid non fat were standardized by adding required amounts of sterilized milk and skimmed milk. For each experimental mixture, the same basic procedure was followed except the proper amounts of cocoa and coffee. Each treatment was replicated three times. Then the three mixtures were pasteurized at 95 °C for 15 s and homogenized. The yoghurt mix was cooled to 44 $^0$C and culture was added in proper amount. After incubation at 44 $^0$C for 2 hours and 30 minutes, the yoghurt mixtures were kept in refrigerator under 4 $^0$C.

Preliminary trial and error tests were carried out to find out suitable cocoa and coffee mixture for set yoghurt and suitable flavor percentage. Chemical and microbiological analyses were performed for the selected best sample according to the results. Three samples were sensory evaluated using six point hedonic scale with the participation of 25 untrained panelists. Collected data were statistically analyzed using Friedman test.

**Results and discussion**

Analysis of final product revealed that the incubation has not affected the fat content. Acidity of the final product has increased up to desired range due to incubation by rapid cooling after attaining the correct pH level.

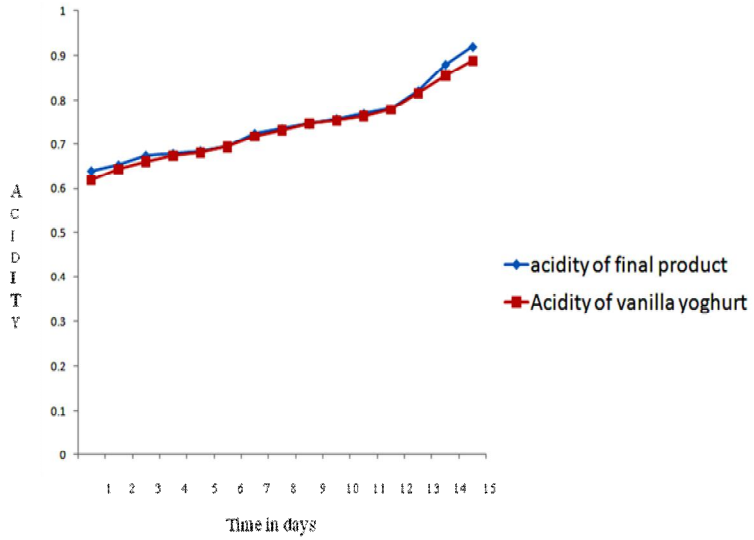


Figure 1: Changes of titratable acidity during the shelf life period

During the cold storage of 14 days at 4 $^{0}$C± 1, acidity has changed significantly ($P<0.05$); and were within the acceptable range of the yoghurt. According to the results, Titratable Acidity (TA) values for sample "B" ranged from 0.64-0.92%. The increase in TA values could be attributed to the activity of lactic acid bacteria which usually converts lactose to lactic acid. According to the SLS standard for yoghurt, recommended acidity level is 0.7% to 1.25 %.

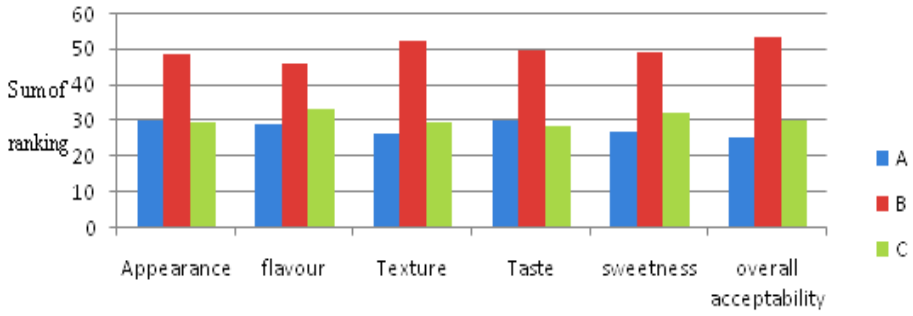


Figure 2: Sensory value according to sum of ranking

According to the results obtained, a significant difference was observed among three samples. Sample "B" was considered as the best formulation which contained (0.06%) cocoa flavor, (0.05%) permitted coloring and (0.1 %) cocoa powder and sample "A" was comparatively less in overall acceptability.

## Conclusions

Sample "B" was selected as the best ratio through sensory evaluation containing (0.06%) Cocoa flavor, (0.05%), permitted coloring and (0.1 %) cocoa powder. Product shelf life was 14 days under cold room storage where temperature was maintained at 4 °± 1 °C.

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# Bioactivity of Tithonia diversifolia (Hemsl), *Tagetes erecta* L. and *Lantana camara* L. against Grain Storage Pests *Tribolium castaneum* (Herbst)

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## Introduction

According to the viewpoint of economists, farmers have an opportunity to take advantages of seasonal price rises, but the benefits can only be achieved if grain is heal longer on the farm with no deterioration in quality. Then it appears the necessity of post harvest management. Post harvest deterioration of grains is principally caused by biological spoilage organisms including insects, fungi and small vertebrates (Golob *et al.*, 2002). Control of these insects relies heavily on the use of synthetic insecticides and fumigants. But their widespread use has led to some serious problems including development of insect strains resistant to insecticides, toxic residues on stored grain, toxicity to consumer and increasing costs of application (Jbilou *et al*., 2006; Golob *et al*., 1999). Thus, several of natural plant extracts had been tried in the control grain storage pest insects with the view of advantages such as local availability, little or no toxicity to humans and simple preparation procedures (Okigbo *et al*., 2009).

The present study was undertaken to analyze the insecticidal activity of *Lantana camara* L., *Tagetes erecta* L. and *Tithonia diversifolia* (Hemsl) with the aim of development of plant base insecticidal fumigants to apply on grain storage pests. These are underutilized plants in Sri Lanka which are heavily used in conventional farming to eliminate insects. Also they have been shown to have repellent and insecticidal activities against certain insects (Adedire *et al.,* 2004; Nikkon *et al.,* 2009; Ogendo *et al,*. 2004). Insects, red flower beetle, *Tribolium castaneum* (Herbst) is considered as a major pest of stored grains (Jbilou *et al*., 2006).

## Materials and methodology

Fresh leaves of *T. diversifolia*, *T. erecta* and *L. camara* and flowers of *T. diversifolia* were collected from Badulla area and plants were confirmed for the identification by the Herbarium, National Botanic Garden, Peradeniya. Adult insects *Tribolium castaneum* were collected and introduced to a ventilated container containing wheat flour. After 24 hours, adult insects were removed and allowed to incubate. Insect culture was maintained in the ventilated container containing wheat flour.

Part of the collected plant materials were shade dried for seven days. Both fresh and dried levees and flowers of *T. diversifolia* were extracted in to absolute methanol and fresh and dried leaves of *T. erecta* and *L. camara* extracted in to absolute ethanol by shaking the plant materials with solvent at room temperature for 72 h. Plant material: solvent ratio was maintained at 1 g : 5 mL. Each extract was evaporated, by rotary evaporator at or below 40 $^0$C to obtained crude extracts.

Bioassay for mortality and repellency was conducted with two factor factorial design. 7.0, 10.5 and 14.0 mg of crude extracts were taken and dissolved in a 1 mL of extracting solvent. Solutions were introduced to 5 cm diameter Petri dish and air dried at room temperature to remove solvents leaving extracts on the plates in doses (weight of the extract/surface area) of 350, 525 and 700 μg/cm$^2$. Ten randomly selected adult insects of

age 90 days were added to the Petri dishes and air tied using Para films. Dishes with solvent but without extracts were maintained as the negative control. Observations for mortality and repellency were taken at 24, 48 and 72 hours (Nikkon *et al.,* 2009).Motility percentages were reexamining at a randomly selected concentration series and using logit probit analysis median lethal doses ($LD_{50}$) values were calculated.

Statistical analyses were performed using Minitab Statistical Package and SAS Statistical Package.

**Results and discussion**

Mean mortality (M%) and repellency (R%) percentages with different type of plant crude extracts at 24 h exposure time (Table 1) indicates that there is a 100% repellency in most of the test samples. In control dishes there was no any mortality found even without food and air supplement. There is a gradual increment in the mortality percentage along with the concentration. ANOVA analysis on the results indicates there is a significant interaction effect of concentration and crude type to the mortality rate ($p < 0.05$). Further, the motility percentage is increased with time according to the observations at 48 and 72 h.

The highest mortality values were shown by crude extract of *T. diversifolia* dry leaves with the resulted mean mortality percentages of 90 %, 95 %, 95 % at 350, 525 and 700 µg / $cm^2$ doses, respectively. Also there is a considerable insecticidal activity of crude extract of *T. diversifolia* fresh flowers. Tukey mean comparison ($p < 0.05$) confirmed, these two crude extracts as the highest active among the studied crude extracts and Dunnet Test ($p < 0.05$) proved that there were no other effective extracts compared to best selected types. Thus, these two were selected for further analysis of $LD_{50}$ values.

Table 1: Mean mortality (M%) and repellency (R%) percentages with different type of plant crude extracts at different dose levels after 24 h exposure time.

| Crude extract | | | Mortality (M%) and repellency (R%) at different doses | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 350 µg/$cm^2$ | | 525 µg/$cm^2$ | | 700 µg/$cm^2$ | |
| Plant | Plant part | Fresh/ Dry | M (%) | R (%) | M (%) | R (%) | M (%) | R (%) |
| *T. diversifolia* | Leaves | Fresh | 15 | 100 | 65 | 100 | 80 | 100 |
| | | Dry | 90 | 100 | 95 | 100 | 95 | 100 |
| | Flowers | Fresh | 70 | 100 | 90 | 100 | 100 | 100 |
| | | Dry | 30 | 65 | 75 | 100 | 85 | 100 |
| *T. erecta* | Leaves | Fresh | 0 | 100 | 25 | 100 | 40 | 100 |
| | | Dry | 25 | 100 | 50 | 100 | 60 | 100 |
| *L. camara* | Leaves | Fresh | 0 | 100 | 20 | 100 | 95 | 100 |
| | | Dry | 10 | 75 | 75 | 100 | 45 | 100 |
| Negative control - Methanol | | | 0 | 0 | 0 | 0 | 0 | 0 |
| Negative control - Ethanol | | | 0 | 0 | 0 | 0 | 0 | 0 |

Based on the mortality values at twenty randomly selected concentrations of crude extracts (between 100 and 350 µg / $cm^2$) Logit Probit analysis results the $LD_{50}$ of crude extracts of T. diversifolia dry leaves as 209.17µg / $cm^2$ and fresh flower as 253.15 µg / $cm^2$.

These results indicate the high bioactivity of underutilized plant species, wild sunflower or tree marigold, T. diversifolia and the potential agricultural and industrial applications via the development value added products.

## Conclusions

*Lantana camara* L., *Tagetes erecta* L. and *Tithonia diversifolia* (Hemsl) have good insecticidal activity. Both flowers and leaves of *T. diversifolia* have high insecticidal activity and can be used in the control of *T. castaneum* in pest management system. However, more studies should be carried out to isolation of bioactive compounds as well as for field trials, followed by the development of insecticidal fumigant.

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## Acknowledgement

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# Inhibitory Effect of Essential Oils Extracted from *citrus* Peel on Microbial Growth of Bread

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## Introduction

Shelf life i.e. the period it will preserve an acceptable level of eating quality from a safety is very important for both manufacturer and the consumer. Bread occupies an important place amongst all bakery products, because the ingredients that are used and the water activity (0.96-0.98) of breads are supportive of the growth of microorganism reducing the shelf life of bread (Salim-ur-Rehman *et al*., 2007).

To enhance the shelf life of bread, chemical antimicrobial agents have been employed but they are considered responsible for many carcinogenic and teratogenic attributes and residual toxicity. Due to these reasons, consumers tend to be doubtful of chemical additives and thus the demand for natural preservatives has been intensified (Skandamis *et al*., 2001). The *Citrus* peel essential oil comprises one of the most versatile essential oils. It is well known that essential oils from *Citrus spp*. have pronounced antimicrobial effect against both bacteria and fungi (Sumonrat *et al*., 2008). Also the *Citrus* peels are non edible thus discarded after extracting the juice. *Citrus spp.* can be found in most of the regions in the world (i.e. commonly found in Sri Lanka).

## Methodology

Citrus peel essential oil extraction

*Citrus* peel EOs of lime (*Citrus aurantifolia),* sweet orange *(Citrus sinesis)* and pommelo (*Citrus maxima)* were extracted by using steam distillation method (Sumonrat *et al*., 2008).

Investigation of antibacterial and antifungal property of essential oil:

A preliminary test was conducted to bacteria and fungi which were isolated from spoiled bread that kept at room temperature (i.e. open environment for 5-days). Pour plate method was followed for the isolation of test micro-organisms with streak plate method. The isolated and identified fungi and bacteria were grown on PDA and NA media, respectively for the treatments of three groups of EOs. Triplicates were conducted to increase the accuracy of tests.

Application of *Citrus* peel EOs on bread:

The selected high effective EO was sprayed on whole bread, sprayed on slices and sprayed on bread wrapping along with the control bread. The EOs treatments were done under 0.1% (v/v) as described by Viuda *et al*. (2007).

Later, the plate count method was followed to take direct measurement of micro-organism count in each EO treated bread as well as for controls during 0 - 96 hr time period.

Sensory evaluation and data analysis:

The sensory evaluation was conducted for EOs treated breads during 0-96 hrs. The data obtained from bacterial and fungal count were statistically analyzed by ANOVA and Friedman's test, respectively.

**Results and discussion**

Identification of molds and bacterial morphology

On the basis of direct examination and gram staining, the arrangement and the colony morphology were taken into account and it was recorded that majority of the molds isolated during this study belonged to *Mucor spp.*, *Aspergillus spp.*, *Penicillum spp.*,and the bacterial *spp.* were gram-positive coccus as well as gram-positive coccus.

Effective essential oil for bread test micro-organisms

The 0.1% (v/v) *C. sinensis, C. maxima, C. aurentifolia* treated for identified bread fungi sp. such as *Mucor sp*. *Aspergillus sp*. *Penicillum sp*. and for bread bacteria *spp*. A with different volume (5 ml, 10 ml, 20 ml) and negative treatment, control treatment were showed significance difference ($p<0.05$) under the 95% confidence interval. The effective treatment for mold *sp*. and bacterial *sp*. were 10 ml and 20 mL *C. sinensis* among another treatments.

Bacterial and Fungal colony count at different storage intervals

Bacterial and fungal susceptibility to *C. sinesis* essential oil, as determined by the plate count technique, showed that treatments and storage periods had significantly ($p<0.05$) affected the bacterial count of bread. Maximum numbers of bacterial colonies were observed in bread containing no essential oil treatment ($T_0$). Spraying of *C. sinensis* peel essential oil on all slices ($T_2$) showed to be most effective treatment against bacterial spoilage than other two essential oil treatments showed in Figure 1 shown.

Sensory evaluation of bread 0 hr- 96 hr storage interval

According to the Friedman's test sensory evaluation results for each sensory attributes showed significantly different ($p<0.05$) with sum of ranks at 0 hr- 72 hr storage intervals. After the 72 hr storage interval control bread rejected due to spoilage and at 96 hr storage other treatment types (i.e $T_1$, $T_2$, $T_3$) taken for sensory evaluation.

As 0 hr - 96 hr storage interval's sum of ranks, 96 hr sum of rank for each sensory attributes of bread showed high values for $T_2$ - Spraying of *C. sinensis* essential oil on all the slices treatment type than other treatment types such as EO sprayed on whole bread and EO sprayed on wrapping of bread.

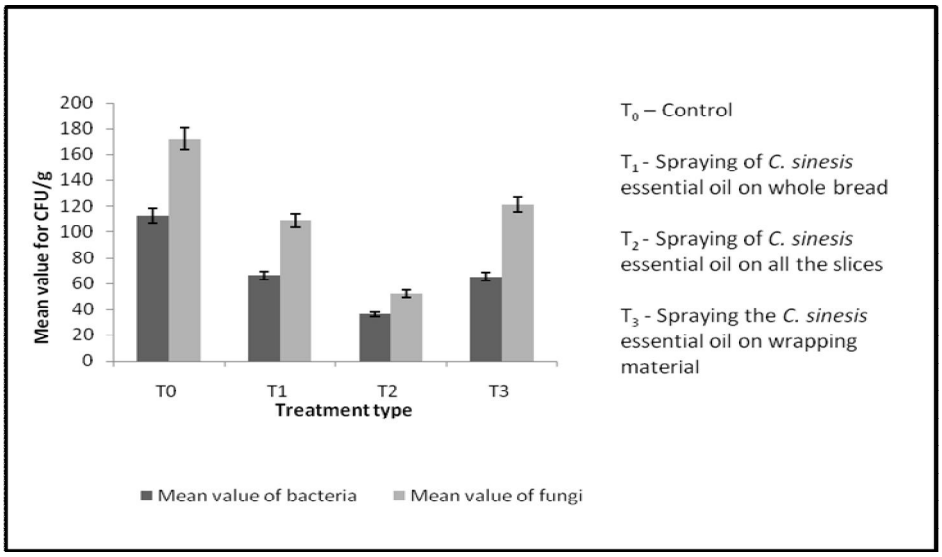


Figure 1: Mean value for colony forming unit per gram of bacteria and fungi with treatment type

## Conclusions

When considering all the analyzed data and results from three type of essential oil i.e. *Citrus sinensis* (sweet orange), *Citrus maxima* (pommelo), *Citrus aurantifolia* (lime) exhibit the antimicrobial ability against bread micro-organisms while *Citrus sinensis* illustrate the high efficacy than other two types against both bacteria and molds of bread showing significant difference ($p<0.05$). The highest bacterial and mold colony count were recorded in the bread treated without the spray of *Citrus* peel essential oils. Treatment $T_2$ in which *C. sinensis* peel essential oil was sprayed on all slices of bread proved the most effective inhibitory treatment against bacterial and fungal spoilage of bread significantly ($p<0.05$).

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# Effect of *Aerva lanata* in Controlling Root-Knot Nematode *Meloidogyne incognita* of Tomato in Sri Lanka

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## Introduction

The root knot nematodes (*Meloidogyne spp.*) are a group of endo-parasites which are among the most damaging agricultural pests, attacking a wide range of crops worldwide. In Sri Lanka, tomato (*Lycopersicon esculentum* Mill.) is a commercial and a widely grown vegetable which is often severely prone to attack by root-knot nematode, *Meloidogyne incognita*. Other than in tomato, the *Meloidogyne spp.* cause serious reduction in yield in several economically important plants such as potato, chillies, okra, mung-bean, rice, tea and tobacco (Akhtar, 2000).

Nematode control is mainly based on chemical nematicides, which present potential risk on non-target organisms and the environment. Their high costs, non-availability at the time of need and the hazards they pose, discourage most potential users. In the search for more environmental friendly and acceptable alternatives to chemicals, possibilities are being investigated to exploit nematode-anatagonistic plants for nematode control. Leaf extracts of certain plants are known to have nematicidal or nematostatic properties against several plant parasitic nematodes (Gapasin *et al*., 2002). The nematicidal activity of the plant extracts can leads for development of plant-based agrochemicals.

Weeds are usually aggressive growers with the presence in large quantities. These plants species may therefore contain active biological compounds to resist various nematode infections. Biological nematicides prepared with weed plant extracts have the advantage of readily availability, low cost and environmental safety over other conventional nematicides. Therefore, the main objective of this study was to evaluate the effect of *Aerva lanata* (Ameranthaceae) weed plant species for nematicidal activity against *Meloidogyne incognita*, root-knot nematode collected from tomato.

## Materials and methodology

According to Potenza *et al.* (2006) several families of plants are known to have compounds with insecticidal activity. Therefore, the weed plant species, *A. lanata* from family Ameranthaceae was selected for the study. The plants were collected from home gardens and university premises and were authenticated by the National Herbarium, Peradeniya. Second stage juvenile (J2) larvae of *M. incognita* which were collected from root knots of infected tomato plants and cultured under laboratory conditions were used for the investigation.

The leaves of *A. lanata* were separated and air dried for two weeks until complete removal of water. Then 15 g of dried plant leaves from each species were ground and extracted separately with absolute ethanol, ethyl acetate and dichloromethane solvents using a sonicator for 20 minutes. The extracts were filtered with filter paper (Whatman No. 1) and solvents were completely evaporated using a rotary evaporator at 40 $^0$C and 150 rpm. The solid residual was weighted and stored at 4 $^0$C until use. Measured quantities of plant leaves extract were dissolved with distilled water to make five different dilutions (100, 300, 700, 500, 1000 µg/mL). 3 mL of each dilution was poured into sterile petri dishes and ten freshly hatched J2 larvae of *M. incognita* were transferred into each petri dish. Distilled

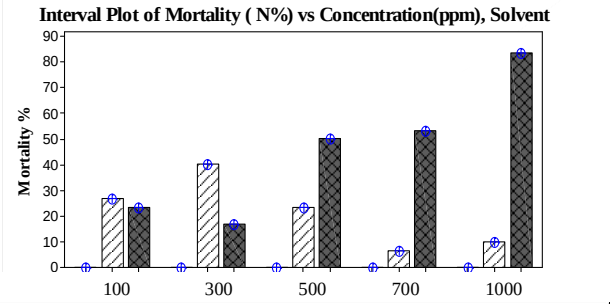
water was used as the control and each treatment was replicated thrice. The petri dishes were kept in the dark at room temperature (25-27 $^{o}$C) and the number of dead J2 larvae was counted by using a stereo microscope (Labomed, USA) after 24 and 48 hours.

The nematicidal activity of the plant extract was assessed based on the mean mortality percentage of the nematodes. General linear model was used for the data analysis using Minitab 14 software. Means were compared using Tukey comparison test while Probit Analysis was used to estimate the $LC_{50}$ (50 % killed lethal concentration) values. Paired T-test was used to compare nematode mortality after 24 hours and 48 hours of exposure.

## Results and discussion

Ethyl acetate extract of *A. lanata* leaves with a concentration of 1000 µg/mL showed the best nematicidal activity which is 83.33 $\pm$ 5.77 % after 24 hours (Figure 1a). This extract showed a highly significant difference ($P<0.05$) compared to control, and most of the other concentrations of other solvent extracts. 700 µg/mL and 500 µg /mL concentrations of ethyl acetate extracts also showed high *M. incognita* mortality of 53.33 $\pm$ 11.55 % and 50.00 $\pm$ 10.00 %, respectively. Hence, there was no significant difference between these two extracts and the best extract. Dichloromethane extracts of *A. lanata* showed no effect on *M. incognita* mortality after 24 hours. According to the probit analysis, the $LC_{50}$ values for ethyl acetate and ethanol extracts of *A. lanata* were 722.16 $\pm$ 0.25 µg/mL and 19211.00 $\pm$ 0.84 µg/mL, respectively. Thus, ethyl acetate extract showed the highest lethal effect to *M. incognita*. These results demonstrate that ethyl acetate is the most suitable solvent to extract the chemical compounds in the leaves of *A. lanata*.

(a)




(b)

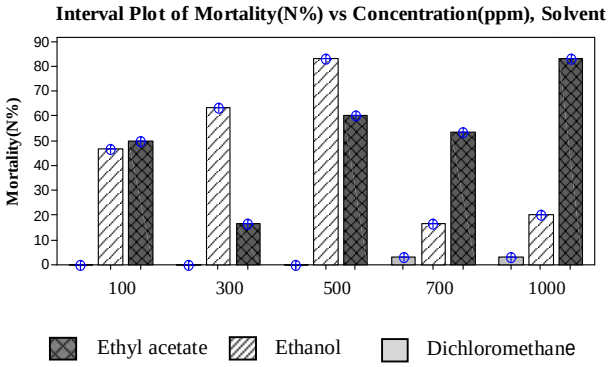



Figure1: Mean **%** mortality of *M. incognita* at 100, 300, 700, 500, 1000 µg /mL concentrations of dichloromethane, ethanol and ethyl acetate leaf extracts of *A. lanata* after 24 hours (a) and 48 hours (b) of exposure.

Figure 1b demonstrates that the nematode mortality percentage has increased after 48 hours of exposure to *A. lanata* leaf extracts. Nematode mortality showed a significant increase after 48 hours of exposure to 500 µg/mL and 300 µg/mL ethanol extracts ($T_{500}$=5.2, $T_{300}$=7.0, $P<0.05$). Though dichloromethane extracts had no effect on *M. incognita* mortality after 24 hours, 700 µg/mL and 1000 µg/mL concentrations of the dichloromethane leaf extract have shown nematode mortality with longer time of exposure.

The nematicidal activity of the *A. lanata* plant extract used in this study may serve as leads for development of plant-based agrochemicals. Thus, it is unknown whether the nematicidal activity was due to a single compound or to a complex of compounds, or other mechanisms and/or interactions. Therefore, future research should be carried out using bioassay guided fractionation methods to isolate the bioactive compounds responsible for the nematicidal activity.

**Conclusion**

This study showed that the leaf extracts of the weed plant, *A. lanata* posseses good nematicidal activity against *M. incognita* in tomato and the mortality of *M. incognita* increases with longer duration of exposure.

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# Production of Mosquito Repellent Body Lotion from the Species *Ocimum sanctum*

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## Introduction

Mosquito-transmitted diseases such as Dengue Fever, Malaria, Yellow Fever and Arboviral Encephilitides are major concerns, all over the world. Every year, it is developing new strains of mosquitoes and infecting viruses are reported that lead to the failures in treatments. The number of dengue deaths reported for the first seven months of 2010 in Sri Lanka is about 165 and the number of dengue patients reported during the same period is about 34,000 (Ministry of Health, Sri Lanka).The main precaution for these infections is prevention of mosquito bites using repellent methods such as mosquito coils which is also reported to have side effects.

Mosquito repellent ability of natural plant substances was well known for hundreds of years. In Sri Lanka plants such as *Ocimum sanctum,* has been used from the ancient times. *O. sanctum* (holy basil) is one of the common plant species which is having the mosquito repellent ability (Shankar *et al*., 2009). The active compound of this plant contains 7% eugenol, 4% caryophllene - 3.8 mg, 1% triterpenoic acids including ursalic acid, oleanolic acid, and rosmarinic acid (Prakash & Gupta, 2005). *O. sanctum* belongs to the family Labiatae characterized by square stem and specific aroma. Several medicinal properties have been attributed to the plant in ayurveda and unani systems of medicine. Juice of the leaves is used as demulcent, stimulant and expectorant (Shankar *et al*., 2009). *O. sanctum* is a common weed in Sri Lanka which is highly available and a low cost value added product can be produced in commercial scale.

*Aedes* species and *Culex* species are two common mosquito species act as vectors respectively for dengue and filarium. Current study is on production of a herbal based mosquito repellent body lotion which can be applied to human without any side effects to assure non toxicity and non irritancy.

## Materials and methodology

Leaves of *Ocimum sanctum* were collected from Sirimalgoda area, Badulla. The eggs of the two mosquito species: *Aedes* and *Culex* were obtained from the Medical Research Institute (MRI) Colombo. Medically recommended cream base ingredients were obtained from Teaching Hospital Kurunegala.

Leaves of *O. sanctum* (1 kg) were cut into small pieces and the essential oil was extracted using steam distillation by heating the plant material at 120 $^0$C in a liquid paraffin bath for 24 h. The extracted essential oil was stored at 4 $^0$C until use in repellency testing.

A two cage apparatus (24 cm x 24 cm x 24 cm) along with a connecting corridor (60 cm) was constructed to determine the repellency of two mosquito species in the presence of deferent volumes: 25, 50, 100, 150 and 200 µl of extracted oil. Different amounts of the essential oil were introduced to a petri dish kept in the cage with mosquitoes and the percentage repellency was determined to assess the effective oil amount. 3-5 day old mosquitoes were used for the assays and commercially available citronella oil was used as the positive control. All the readings were taken in triplicates. Data were analyzed

parametrically using the one way ANOVA table under 90% confidence level, and pooled standard deviation values using Minitab Statistical Package version 14.0.

The base cream was developed in 2 steps by producing the emulsifying ointment (emulsifying wax 30%, white soft paraffin 50%, liquid paraffin 20%), and aqueous cream (emulsifying ointment 69%, water 30%, phenol 1%). The essential oil was mixed well with the base cream using a homogenizer to produce the body lotion. Product was tested for the suitability of applying on skin under medical supervision of the University Medical Officer, Uva Wellassa University with 15 volunteers. Questionnaires were given to the respondents to assess the acceptability of odor, texture, mosquito repellency, occurrence of allergic reactions and overall acceptability.

**Results and discussion**

Yellow colored essential oil (3 mL) was obtained from steam distillation of leaves of *Ocimum sanctum* (1 kg).

Tables 1 and 2 show the repellency percentages of the two mosquito species *Aedes* and *Culex*, respectively at different volumes of extracted oils. Parametrical data which were analyzed using the one way ANOVA table under 90% confidence level indicates that there is no significant difference on repellency parentage between the oil amounts for *Aedes* species ($P < 0.05$) but there is a significant difference for *Culex* species ($P > 0.05$). Further, when analyzing the pooled Standard deviation values for *Culex* species, there is a significant difference between the repellency at 100 µl-150 µl and 100 µl- 200 µl. But there is no significant difference between 150 µl-200 µl. Thus, according to the statistical analysis and considering the cost of production, efficiency of essential oil extraction and raw material availability, 150 µl can be selected as the most effective essential oil amount, which has the maximum repellency for both species.

Table 1: Repellency percentage of *Aedes* species on different volumes of oil extract

| Volume / µL | Repellency percentage | | |
|---|---|---|---|
| | Trial 01 | Trial 02 | Trial 03 |
| 25 | 60.00% | 80.00% | 60.00% |
| 50 | 71.43% | 85.71% | 71.43% |
| 100 | 83.33% | 83.33% | 83.33% |

Table 2: Repellency percentage of *Culex* species on different volumes of oil extract

| Volume/ µL | Repellency percentage | | |
|---|---|---|---|
| | Trial 01 | Trial 02 | Trial 03 |
| 100 | 20.00% | 0.00% | 20.00% |
| 150 | 33.34% | 33.34% | 66.67% |
| 200 | 66.67% | 66.67% | 33.34% |

When considering the composition of prepared cream product, instructions should be given to apply about 1.5 mL to introduce a volume of 150 µL of extracted oil to earn maximum repellency from *Aedes* and *Culex* mosquitoes.

Responses from the volunteer participants given through the questionnaire indicate 67% of the respondents like the odour of the product, 20% like it moderately and 13% dislike the odour of the lotion. 70% of the respondents stated that the mosquito repellency is excellent and 30% said it is moderate. No one stated that the repellency is poor. When considering the texture, 40% like the texture of the lotion, 47% like the texture moderately and 13% of the respondents do not like the texture of the lotion. No allergy reactions were reported. When considering the overall acceptability 53% of the respondents accepted the product as excellent and 47% said it is moderate. No one stated that the overall acceptability of the lotion is poor. Final assessment of the product acceptability gave an 86.67% result, saying that the product is acceptable.

## Conclusion

This product is a low cost product which adds value to natural resources. This is also a good solution to the critical health issue of mosquito related diseases like "Dengue and Filaria.

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# Screening of Wood Rotting Basidiomycetes Fungi for Bioremediation Ability of Textile Dye Effluents

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## Introduction

Sri Lanka is considered to be one of the world's leading apparel producers. The textile industry utilizes large volumes of water in its processing operations and generates substantial quantities of dye containing waste water which is usually discarded into water bodies mostly without further treatments. About 10-15% of all dyes are directly lost to wastewater in the dying process and removal of color from effluent is one of the major problems that the textile industry faces. The presence of color in water hinders the absorption of solar radiation, thus reducing the natural photosynthetic activity, causing changes in aquatic biota. Furthermore, textile dyes pose serious health threats to humans due to their carcinogenicity and lead to mutagenic and toxic effects on organisms. Amongst many classes of synthetic dyes, triphenyl methane group of dyes such as crystal violet and malachite green are the most used in the textile and dyeing industries (Bumpus and Brock, 1988). The decolonization and degradation of textile dye effluent does not occur when treated with conventional effluent treatment systems (Murugesan *et al*., 2007). Use of microorganisms to remove dyes from industrial effluents or bioremediation is inexpensive and the end products of complete mineralization are nontoxic. Basidiomycete fungi produce an array of extracellular enzymes helpful in removing synthetic dyes from industrial effluents (Asamudo *et al*., 2005). This study investigates the ability of some selected Basidiomycete fungi to decolonize malachite green.

## Methodology

Basediocarps of wood rotting Basidiomycete fungi were collected from different localities and their shape, color and size were recorded separately. A square of 5 x 5 mm from each of the Basediocarp was cut, surface sterilized by dipping in 70% alcohol solution for 1 min and cultured separately in petri plates (100 x 15 mm) containing Potato Dextrose Agar (PDA) medium. Inoculated petri plates were sealed with parafilm and incubated at $30^0$ C. After two weeks, streak plates from isolated fungal colonies were prepared in fresh PDA medium to establish pure cultures. Colony morphology, pigmentation and ultra structure of the fungal mycelium and macro spores under the light microscope was recorded for each pure stain isolated and used in identification of the fungus.

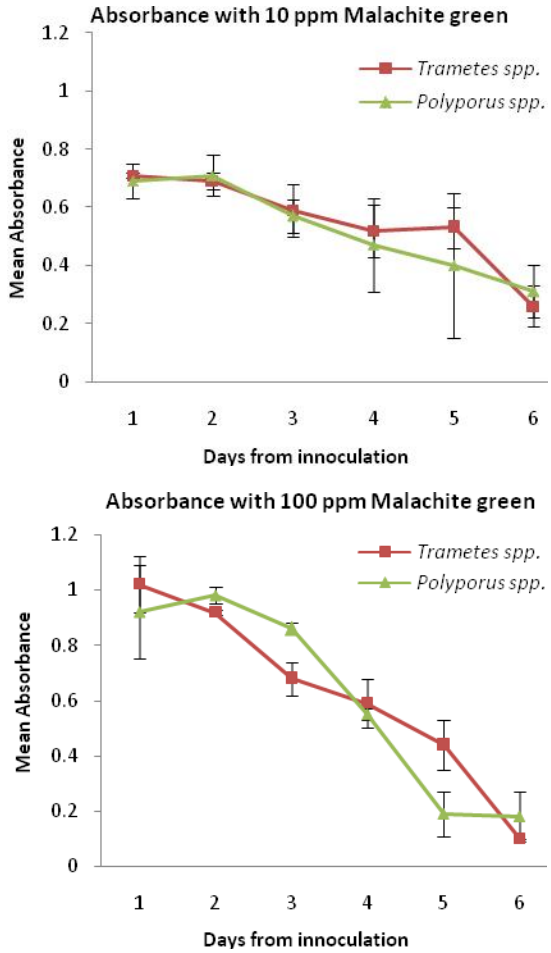
Ability to decolorize malachite green by sixteen isolated fungal strains was evaluated by culturing in PDA plates supplemented with 100 ppm of Malachite Green. A disc of fungal mycelia 5 mm in diameter was cut separately from fungal strains and placed in the center of petri plate. PDA plate containing either 10 ppm or 100 ppm of Malachite Green but no mycelium served as the control. All the plates were then incubated at $30^0$C and after for 10 days, ability of the fungi to decolorize the dye was established visually using a three scale scoring system (poor, average and good).

Two fungal strains *Trametes* spp. and *Polyporus* spp. with proven decolonization abilities were used for liquid phase assessments. Selected fungal strains were grown separately in 200 mL of Potato Dextrose Broth (PDB) medium in 500 mL Erlenmeyer flask containing 10 ppm or 100 ppm Malachite green and incubated at 30 $^0$C for 10 days. An aliquot of 15 mL was removed from cultures daily (up to six days) and centrifuged separately at 4000

rpm for 2 min. The maximum absorbance at 617 nm was measured using UV spectrophotometer and de-colorization of the dyes was calculated using Beer-Lambert law.

Experiments were arranged in a Complete Randomized Block Design. Data were analyzed using one way ANOVA and pair wise comparison was done by Tukey comparison test using MINITAB statistical package version 14.0.

**Results and discussion**




(Each data point represent mean of three readings. Vertical bars represent SD.)

Figure 01: Mean Absorbance of *Trametes* spp. and *Polyporus* spp. grown in PDB medium with 10 ppm and 100 ppm Malachite green.

The two fungus types *Trametes* spp. and *Polyporus* spp. showed significant ($p<0.05$) de-coloration effects when cultured in 10 ppm and 100 ppm Malachite green concentrations. *Trametes* spp. showed the lowest absorbance hence highest decoloration compared to *Polyporus* spp. Rate of de-coloration was low in the first two days of culture and thereafter

de-coloration was steadily accelerated (Fig.01). This could be attributed to the elevated level of exogenous enzyme production when fungal strains colonize the growth medium.

Lignin degrading enzymes produced by white rot fungi is capable of de-coloration of organic compound such as Malachite green. De-coloration ability of *Trametes* spp. has been well demonstrated by previous works (Wesenberg *et al*., 2003) whiles to our knowledge no such records available for *Polyporus* spp. This study was conducted under stimulated conditions in the laboratory using only one dye type. It is important to extend the study to check the de-coloration ability of other dyes associated with textile dye effluents. Identification of fungal stains remains a difficult task and this study identified strains only up to genus level. Species level identification could even necessitate molecular approaches and could be an important future perspective.

## Conclusion

This study identified two fungal stains belonging to wood decaying white rots with dye de-coloration abilities. The two fungi have the potential to be used in treating textile dye effluents. However, further studies are needed before industrial level applications to determine complete mineralization of dyes leading to the production of nontoxic compounds.

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# Antimicrobial Activity of *Plumbago Rosea* Root Extract against Human Pathogens

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## Introduction

*Plumbago rosea,* also known as *Plumbago indica* is an ornamental garden plant. Root of this plant with acrid, vesicant, alterative, digestive, stimulant abortifacient and oral contraceptive properties is used in Ayurvedic medicine (Okeyo, 2006). According to the previous studies root of *P. rosea* contains plumbagin or 5-hydroxy-2-methyl-1,4-naphthoquinone (Mallavadhani 2002). Plumbagin is present in all the varieties of genus plumbago to a maximum of about 0.91%. *Plumbago zeylanicas*, another species belong to genus plumbago has been reported for its antimicrobial properties. (Dhale, 2011).

The emergence of antibiotic resistant strains of human pathogens and side effects of currently available drugs are becoming a serious problem, for which alternative therapies are urgently required. Infections caused by *Staphylococcus aureus* especially due to methicillin–resistant *S. aureus* (MRSA) in immune compromised patients is continue to be a serious problem in worldwide. *Staphylococcus saprophiticus* is a cause for community-acquired urinary tract infections in young women. Opportunistic pathogens such as *Pseudomonas aeruginosa* which causes range of human infections and the *Escherichia coli* are also, being reported for the antibiotic resistance. Drug resistant strains of *Candida albicans*, which causes candidiasis is also a problem with global concern.

## Materials and methods

The roots of *Plumbago rosea* were collected around Haldumulla, Badulla, Sri Lanka. The collected plant materials were identified at Herbarium, National Botanic Garden, Peradeniya, Sri Lanka. Pure cultures of human pathogenic bacteria: *Staphylococcus aureus*, (NCTC 6571) *Staphylococcus saprophiticus* (clinical isolate), *Psudomonas aeruginosa* (ACTC 27853), *Escherichia coli*, (NCTC 10418) and fungi *Candida albicans* (clinical isolate) were obtained from Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka. Cultures were maintained on Muller-Hinton agar at 4 $^0$C.

The collected roots were air dried for two weeks and ground. 20 g of root samples were extracted separately with distilled water (100 mL) and ethanol (100 mL) using Soxhlet extractor for a period of 8 h and 48 h, respectively. The aqueous extract was concentrated up to 50 mL by continues maintaining of extract at ambient pressure in the same heating mantel for another 2 h. Ethanol extract was concentrated in vacuum under pressure using rotary evaporator to obtain the crude ethanol extract. Then the extracts were maintained at 4 $^0$C in capped vials.

Agar well diffusion assay was used to determine the antimicrobial activity of the aqueous extract and the crude ethanol extracts. Plates were inoculated with 0.5 McFarland microbial isolates using pour plate method and well diameter was 8 mm. The experiment was carried out in triplicate. Agar plate dilution method based on the BSAC standard (Andrews, 2001) was performed to determine the Minimum Inhibitory Concentration (MIC), lowest concentration which did not show any visible growth, with the dilution series: 8.192, 4.096, 2.048, 1.024, 0.512, 0.256, 0.128, 0.064, 0.032 mg/ml. The experiment was carried out in

duplicate. All the plates were incubated aerobically at 36-37 °C and read at 24 h. Samples were dissolved in sterilized distilled water mixed with Tween 20 (5:0.5) and same solution was used as the negative control.

Statistical analyses were performed using Minitab Statistical Package 14.0 version.

**Results and discussion**

The aqueous root extract of *P. rosea* (Table 01) showed high activity against *S. aureus* and for *S. saprophiticus* and moderate activity against *E. coli, P. aeruginosa* and *C.albicans* in agar well diffusion assay.

Agar well diffusion assays results of crude ethanol extracts with six months old and two weeks old samples (Table 1) indicates there is a considerable reduction of the activity with the age of the sample. With six month old samples at the concentration of 1 mg/ml, it did not show any antimicrobial activity. At the concentration of 5 mg/ml, it showed activity only against *S. aureus* (24.93 ± 0.08 mm) and at the concentration of 10 mg/ml only against *S. aureus* (25.13 ± 0.09 mm) and *S. saprophyticus* (13.03 ± 0.02 mm). With two weeks old extracts at 1 mg/ml concentration *S. aureus* and *S. saprophyticus* shows activity and at 10 mg/ml concentration E. *coli* shows activity where as *P. aeruginosa* was resistant even with the new extracts. Activity on two *Staphylococcus* species has significantly increased with new samples *(P = 0.002,* and *0.000).* Observations after 72 h with two weeks old crude ethanol extracts indicates that *C. albicans* also showed considerable sensitivity to root extracts of *P. rosea.* These results suggest that the antimicrobial active compounds in *P. rosea* may decompose with time at 4 $^{0}$C.

Table 1: Diameter of the inhibition zone in agar plate dilution assays with aqueous extract and six month old and two weeks old crude ethanol extracts

| Organism | Inhibition zone diameter /mm | | | | | | |
|---|---|---|---|---|---|---|---|
| | Aqueous extract | Crude ethanol extract | | | | | |
| | | Six month old samples | | | Two weeks old samples | | |
| | | 1 mg/ml | 5 mg/ml | 10 mg/ml | 1 mg/ml | 5 mg/ml | 10 mg/ml |
| *S. aureus* | 27.83 ±0.35 | - | 24.93 ± 0.08 | 25.13 ± 0.09 | 24.93 ± 0.11 | 30.40 ± 0.69 | 34.93 ± 0.11 |
| *S. saprophyticus* | 25.60 ± 0.52 | - | - | 13.03 ± 0.02 | 15.43 ± 0.40 | 29.66 ± 0.15 | 33.93 ± 0.05 |
| *E.coli* | 14.13 ±0.41 | - | - | - | - | - | 12.93 ± 0.11 |
| *P. aeruginosa* | 15.16 ±0.76 | - | - | - | - | - | - |
| *C. albicans* | 11.16 ±0.15 | - | - | - | 23.33 ± 0.57* | 31.33 ± 0.57* | 35.00 ± 0.10* |

(-) Absence of an inhibition zone
*Observations after 72 h

Minimum inhibitory concentration values resulted from agar plate dilution assay (Table 2) with two weeks old crude ethanol extracts of *P. rosea* indicates these is high antimicrobial

activity in root extracts of *P. rosea against S. aureus*, *S. saprophyticus* and *C. albicans (MIC:* 0.128 – 0.064 mg/ml). However, the sensitivity of *E.coli* and *P. aeruginosa* is less *(MIC:* 4.096 mg/ml). On the basis of the result obtained in present investigation, it implied that the gram-positive bacteria were more susceptible to the root extract of *P. rosea* than the gram-negative bacteria. Possibly because of the presence of outer membrane that serves as an effective barrier in gram-negative species (Girish, 2008).

Table 2: Minimum inhibitory concentration of crude ethanol extract on different organisms

| Organism | Minimum Inhibitory concentration /mg/ml | |
|---|---|---|
| | Trial 1 | Trial 2 |
| *S. aureus* | 0.128 | 0.064 |
| *S. saprophyticus* | 0.128 | 0.064 |
| *C. albicans* | 0.128 | 0.128 |
| *P. aeruginosa* | 4.096 | 4.096 |
| *E.coli* | 4.096 | 4.096 |

Further, root extract of plant P. rosea has potential to use as a chemotherapeutical agent against bacterial and fungal infectious diseases, indicating possible industrial applications with value added products.

## Conclusions

Root extracts of *P. rosea* has high antimicrobial activity in against gram positive bacteria: *S. aureus* and *S. saprophyticus* and fungi: *C. albicans*. However, the activity against the gram negative bacteria: *E.coli* and *P. aeruginosa* is less. *P. rosea* has potential to use for value added products with antimicrobial properties.

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# Phytoremediation Potential of Indian Mustard (*Brassica Juncea*) genotypes for Cr (Vi) Mitigation

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## Introduction

Phytoremediation is a low cost, environmental friendly technology of using plants to mitigate hazardous contaminants from the environment (Dushenkov *et al.*, 1997). Phytoremediation efficiency of selected nine accessions of Indian mustard (Brassica juncea) was examined at different experimental conditions, in vitro and in vivo. Phytoremediation ability of Indian mustard (Brassica juncea) is well established, thus a good candidate plant for phytoremediation. (Weerakoon and Somaratne, 2009). This investigation demonstrates phytoremediation potential of Brassica juncea genotypes for Cr (VI) mitigation.

## Methodology

Experiments were conducted to screen nine accessions (Ac. 501, 790, 1774, 1814, 5088, 7707, 7788, 7789, and 8831) and three accessions with high accumulation (Ac. 0501, 1774, 5088) were used for further experiments on hydrophonic system with parallel to *in vitro* experiments in liquid media. All experiments were designed with, three replicates with three plants per replicate. After three weeks of culturing, plants were exposed to five weeks with a growth media containing four different concentrations of Cr (VI), (0 ppm, 50 ppm, 150 ppm, and 200 ppm). Growth media for hydrophonically grown plants was Albert's solution while liquid MS media was used for *in vitro* grown plants. Performance of plants for Cr (VI) accumulation in hydrophonic system and *in vitro* was studied to select the accession with maximum accumulation.

The software program Minitab 14 (Minitab Inc., 2003) was used for the statistical analysis of metal accumulation data. Statistical analysis was done in three factor factorial design. Analysis of variance (ANOVA) was performed followed by multiple comparisons to determine which means differed significantly ($\alpha = 0.05$) by using Tukey and Dunnett tests.

## Results and discussion

The chromium accumulation is depending on genotype and growth media. Highest accumulation (3511 mg $kg^{-1}$) was shown in accession 1774 grown in MS media contains 200 ppm of Cr (VI) at *in vitro* conditions (Figure 1). Plants grown on *in vitro* showed high accumulation levels than plants grown hydrophonically (Figure 1). Seed of *Brassica juncea* were unable to germinate on the growth media contains Cr (VI) $\geq$ 250 ppm. *B.juncea* itself contains some amount of Cr (VI) which is negligible when compared with mean accumulation of Cr (VI) (Table 1). However, *Brassica juncea* did not show any toxic symptoms when media contains Cr (VI) $\leq$ 200 ppm.

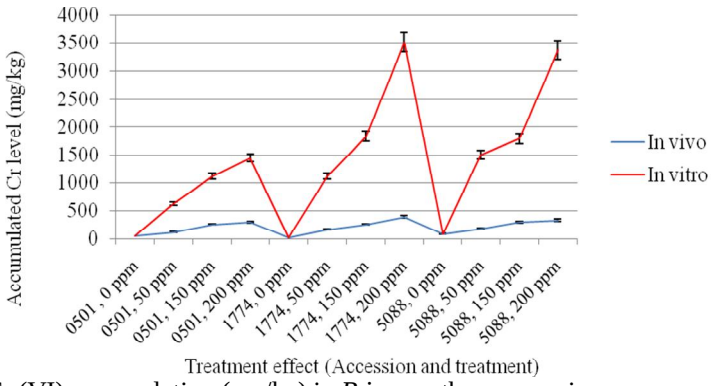


Figure 1: Cr (VI) accumulation (mg/kg) in *B.juncea* three accessions exposure to *in vitro* and *in vivo* conditions in different Cr (VI) treatments

Table 1: Mean Cr (VI) accumulations (in mg/kg) in selected best three accessions of *B. juncea* grown in *inviro* and hydrophonic systems with standard error of mean (n = 9).

| Treat. / Acc. | 0 ppm | | 50 ppm | | 150 ppm | | 200 ppm | |
|---|---|---|---|---|---|---|---|---|
| | Hydro: | *Invitro* | Hydro: | *Invitro* | Hydro: | *Invitro* | Hydro: | *Invitro* |
| AC | 22.4 | 49.9 | 125 | 621.8 | 257.1 | 1114 | 294 | 1446 |
| 0501 | ±0.033 | ±0.0433 | ±0.065 | ±0.072 | ±0.072 | ±0.08 | ±0.038 | ±0.01 |
| AC | 36.9 | 24 | 159.3 | 1112.3 | 257.3 | 1825.6 | 387.5 | 3511 |
| 1774 | ±0.0427 | ±0.0814 | ±0.028 | ±0.03 | ±0.069 | ±0.04 | ±0.071 | ±0.07 |
| AC | 90.9 | 89.4 | 175.1 | 1498.1 | 296.3 | 1788.4 | 330.6 | 3374 |
| 5088 | ±0.0468 | ±0.029 | ±0.023 | ±0.04 | ±0.032 | ±0.02 | ±0.064 | ±0.06 |

**Conclusions**

*Brassica juncea* is a chromium hyper-accumulator and the accumulation level is depend on the genotype, growth medium, and initial Cr (VI) loading. Maximum tolerance limit for Cr (VI) for selected accessions is 200 ppm and *B.juncea* accumulated high amounts of Cr (VI) at *in vitro* conditions compared to *in vivo.* Indian mustard accession 1774 is the best accession among selected accession for Cr (VI) mitigation. So, there is a potential for using accession 1774 of Indian mustard in the remediation of chromium contaminated sites.

Moreover, *B.juncea* itself contains some amount of chromium itself and the attention has to be made when *Brassica juncea* is used as a vegetable due to its high heavy metal accumulating ability.

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# Removal of Lead Metal Ion from Aqueous Solutions Using Zinc Oxide Nanoparticles and Zinc Oxide Bulk Material

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## Introduction

Many toxic heavy metals have been discharged into the environment as industrial wastes, causing serious soil and water pollution. Toxic metal compounds coming to the earth's surface not only reach the earth's waters but can also contaminate underground water in trace amounts by leaking from the soil after rain. Therefore, drinking water obtained from springs which may also be contaminated by various toxic metals. $Pb^{2+}$, $Cu^{2+}$, $Fe^{3+}$, and $Cr^{3+}$ are especially common metals that tend to accumulate in organisms, causing numerous diseases and disorders (Inglezakis *et al*., 2002). Among these lead ($Pb^{2+}$) is a highly toxic heavy metal which adversely affects the red blood cells of the human nervous system and kidneys (Potgieter *et al*., 2006). According to World Heath Organization, the maximum permissible limit of lead in drinking water is 0.05 mg/L (Kanawade and Gaikwad, 2011).

The adsorption of heavy metal ions onto various solid supports such as activated charcoals, ion exchange resins, zeolites and ion chelating agents immobilized on inorganic supports is the most common route among the different techniques applied to remove dissolved heavy metals from waste water and industrial effluents (Chen *et al*., 2003). Many methods using today for decontamination of waste water are not suitable in developing countries due to the high costs associated with production. Therefore, the use of alternative low-cost materials as potential sorbents for the removal of heavy metals should be investigated.

Among inorganic nanoparticles, the zinc oxide (ZnO) nanoparticle has received great attention because of its unique catalytic, antibacterial, electrical, electronic and optical properties as well as its low cost and extensive applications (Kathirvelu *et al*., 2009). Zinc oxide bulk material and zinc oxide nanoparticles are widely used in industry and daily life for various things including as absorbents for gases such as CO, $CO_2$, $O_2$, $H_2$, $SO_2$, $CH_4$ (Scarano *et al*., 2004). Therefore, the main aim of this study was to investigate the possibility of using ZnO nanoparticles and ZnO bulk material for the removal of $Pb^{2+}$ from aqueous solutions by adsorption.

## Methodology

Synthesis of ZnO nanoparticles were carried out according to the published procedure of Becheri *et al*., 2008 and ZnO bulk material were prepared using the same method but without the peptization process. To evaluate the effect of pH on removal of lead ions in water, pH 6-9 solutions of 10 mg/L $Pb(NO_3)_2$ were prepared adjusting the pH values using phosphate buffers. 50 mg of ZnO nanoparticles were dispersed in 10 mL of each pH solution. After shaking the suspension for 4 hours at room temperature (25 $^oC$) using an electric shaker the particles were separated via centrifugation (6000 rpm, 15 minutes), and the lead metal ion concentration was determined by atomic absorption spectroscopy (Varian Atomic Absorption Spectrophotometer with air-acetylene flame).

To evaluate the ability of ZnO nanoparticles and ZnO bulk material to remove $Pb^{2+}$ from water at pH 7.0, 50 mg of ZnO nanoparticles and bulk material were dispersed in 10 mL of 10 mg/L $Pb^{2+}$ solution separately and the same procedure as mentioned above was carried out to determine the remaining metal ion concentration in the aqueous solution. Six replicates and controls (without adding ZnO) were carried out using the same procedure

and the results were statistically analyzed with two sample T-test using Minitab 14 software.

The adsorption isotherm of divalent metal ion ($Pb^{2+}$) was further determined by equilibrating ZnO bulk material (50 mg) in 10 mL of appropriate metal ion solutions (concentration range 5-100 mg/L) for 4 hrs. The adsorption capacities Q (mg/g) were obtained as follows: $Q = [(C_0-C_f)V]/m$; where $C_0$ and $C_f$ are the initial and final concentrations (mg/L) of metal ion in the aqueous solution, respectively, V is the volume of metal ion solution reported in L and m is the weight of the adsorbent in g (Bystrzejewski *et al*., 2009). The experiment was carried out in triplicates and average results were used for data analysis.

The effect of contact time of ZnO bulk material on lead ion removal was determined by following a similar procedure with different shaking times (30 min, 1 hour, 2 hours, 4 hours, 6 hours and 8 hours). Reusability of the ZnO sorbents were studied using previous once utilized ZnO bulk material.

**Results and discussion**

Highest adsorption (99.3%) of lead ions using ZnO nanoparticles was showed at pH 7 and adsorption at pH 6 was also more than 60 %. The initial lead concentration of 10 mg/L was reduced to 0.025 and 0.048 mg/L after applying ZnO nano particles and ZnO bulk material respectively. This reduction of lead ions in the solution was significant compared to control and initial concentration ($T_{nano} = 17.77$, $T_{bulk} = 17.73$; $P < 0.05$). However, the amount of lead ion adsorption by ZnO nanoparticles compared with ZnO bulk material showed no significant difference. Accordingly at pH 7.0 in room temperature (25 $^oC$) the ZnO sorbents were very efficient and removed more than 99 % of lead ions in the solution. This amount is more than many conventional sorbents that have been used to remove lead ions in aqueous solutions (Payne and Abdel-Fattah, 2004).

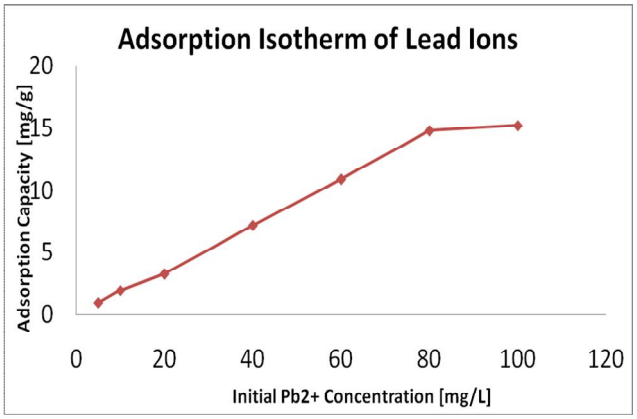


Figure 1: Lead ions adsorption isotherm of ZnO bulk material

The adsorption capacity is an important factor because it determines the amount of adsorbent, which is required for quantitative enrichment of the target analyte from a given solution. Figure 1 shows the adsorption isotherm of $Pb^{2+}$ at their initial concentration range 5-100 mg/L. According to Figure 1 adsorption capacity (15 mg/g) has become constant

after 80 mg/L concentration at pH 7 in room temperature. Adsorption capacity has become constant because, after 80 mg/L concentration, the sorbents have got saturated with lead ions, so that it is unable to absorb any more lead ions from the solution. The current adsorption capacity is very high compared to other heavy metal removing sorbents at similar concentrations (Bystrzejewski *et al*., 2009).

According to this study there was no effect from contact time on percentage removal of lead ions in water and also there was no significant difference in adsorption between previously utilized and newly prepared ZnO particles.

In conclusion, ZnO nanoparticles and bulk material both are prospective materials for efficient removal of lead ion ($Pb^{2+}$) from aqueous solutions and the findings in this research are very important from application point of view for water and waste water treatments in order to remove toxic lead metal ions.

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# Development of a Detector to Determine Presence of Formalin in Fish

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## Introduction

Formalin is an aqueous solution of the chemical compound formaldehyde. Formalin is used as a disinfectant solution. Formalin is commonly used in aquariums to prevent the growth of parasites. In some cases formaldehyde is produced naturally by certain types of fish.

Overuse of formalin causes many health risks for human. Many of these health issues may occur due to illegal use of the formalin. Recently some cases of illegal use of formalin were reported in many coastal areas in Sri Lanka. However, there is no rapid and economical method for formalin detection. Most of the detection methods require expensive and high technology apparatus, a large amount of samples, long sample preparation and detection time. Therefore, it is necessary to develop a simple, rapid and a convenient method avoiding the above mentioned disadvantages. The instrument designed is a convenient, rapid and a simplified method of detecting formalin in fish and can be handled easily. It works at a high accuracy in a range between 100 ppm to 1000 ppm. The design is portable. Samples can be tested within a short period of time. The design is also cost effective and simple.

## Methodology

Used a chemical method. The chemical which reacts with formalin was Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) and it is used to specify the formalin. Purpald reacts with formalin and forms a Tetrazine which is in purple colour in the presence of oxygen. Purpald reacts with different formalin concentrations and the color development of the reaction was proportional to the formalin concentration.

The colour development of the reaction was detected by a Light Dependent Resistor (LDR). Variation of resistance in LDR converted into a voltage variation, which is proportional to the color development of the reaction. Voltage variances of different formalin concentrations were recorded and plotted with respect to the time.

The voltage difference at the seventh minute was distinguishable from each other (Figure 1). There was a linear positive strong correlation between the formalin concentration and the voltage. Pearson correlation of Formalin concentration and Voltage in the seventh minute was 0.986. So the regression analysis was carried out. This regression model was used to program the microcontroller.

Detected voltage difference, which is an analog voltage variation, was fed in to the microcontroller. Using a microcontroller program, analog signal was converted into digital form and formalin concentration was calculated as parts per million (ppm) value. Detected formalin contamination was displayed on Liquid Cristal Display (LCD).

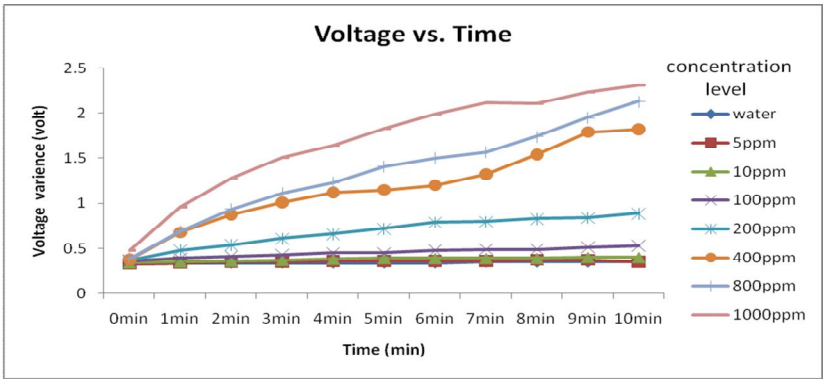


Figure 01: Voltage at different formalin concentrations with respect to the time

**Results**

Samples were taken from two locations, one from a fish market and another from International fish Export Company. Tuna (*Thunnus* sp.) was taken as the fish variety. These samples were taken by washing the skin (outer most layer) and gills of the fish. Three standard formalin concentration series were tested with the chemical to check the repeatability and accuracy.

The reproducibility of the results was high and the precision is very high in the instrument. The accuracy of the instrument was 70.9%.

**Discussion**

A rapid and simple method was developed to determine the formalin content in fish. Designed instrument is user friendly and less time consuming instrument with low energy consumption. Another advantage of this machine is that it is portable and can be operated with a battery DC current.

Only one chemical is used and no other chemicals are needed to prepare the sample. Another advantage is that there are no any specific conditions for sample preparations and by pressing just one switch the user can get the accurate reading within seven minutes. User interface displays the result as a numerical value and the led series also indicate the result. Another crucial advantage of this model is that the users do not need technical skills to operate the instrument.

**Conclusions**

The accuracy of the machine can be increased. High sensitive RGB sensors and image processing techniques can be used. The accuracy of the machine will be increased through image processing technology. Another advantage of image processing is that it can store relevant data which are taken from the machine using a microprocessor.

This model can be developed as automated design. A machine consists of stock tanks with an automatic system can be introduced to measure the quantities of solutions used in the machine.

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# Investigating Properties of Rice Husk for Contaminant Removal from Polluted Water

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## Introduction

Contamination of ground and surface water by different pollutants is a major environmental problem. These pollutants are discharged by sources such as industries into natural water streams. Water pollutants are toxic to most aquatic organisms, human body and may cause denaturing of protein, tissue erosion, and paralysis of the central nervous system and also damage the kidneys, liver and pancreas. Most of the pollutants are toxic even at very low concentrations. Therefore, designing effective strategies to remove pollutants from water is of practical interest (Zhang *et al*., 2011).

Utilization of one waste material to control pollution caused by another is of high significance in the remediation of environmental problems. Rice husk is an abundantly available agricultural waste. The compositions of rice husk are 32% cellulose, 21% hemicellulose, 22% lignin and 15% mineral ash (Nakbanpote *et al*., 2007). Rice husks can be used as a low cost adsorbent (Tarley and Arruda, 2004). This research aimed to chemically modifying surface properties of rice husk to be used in waste water treatment. It would help to increase the volume of purified consumable water.

## Methodology

Rice husk ash was prepared by heating rice husk at 500 $^0$C for 2 hours in box furnace. Subsequently, it was reacted with $CH_3COOH$ (1M, 24 hrs, 1g/10 mL, Room Temperature) to made surface modified rice husk ash. Their physical and chemical properties were determined by using Fourier transform infrared (FTIR) spectrum, zero point charge ($pH_{ZPC}$), zeta potential, methylene blue method and Boehm titration method.

## Results and discusion

### FTIR Spectra study

The FTIR spectra are virtually identical (Table 1). Since, -COOH groups are originally contain in rice husk, FTIR spectra of modified rice husk ash does not show any new peak but some shifts can be seen. Therefore, we can not confirm about surface modification on rice husk based only on this analysis. Since the quantity of samples used in test were not the same, it is difficult to quantify the amount of new –COOH groups using obtained FTIR spectra alone.

Experimental results of the determination of $pH_{ZPC}$ are illustrated in Figure 1 and 2. It was found that pHzpc values of rice husk ash before and after modification as 8.3 and 6.5, respectively. These data show that the pHzpc is more acidic after modification. Similar results were reported elsewhere in literature (Chen et al., 2003). Such decrease in pHzpc confirms the surface modification. Determination of pHzpc in aqueous solutions of different ionic strength gave nearly equal results (Figure 2). This finding shows that $pH_{ZPC}$ does not depend on the ionic strength of the solution. The significance of this kind of plot is

that a given material surface will have positive charge at solution pH values less than the pHzpc and thus be a surface on which anions may adsorb. On the other hand, that material surface will have negative charge at solution pH values greater than the zpc and thus be a surface on which cations may adsorb.

Based on Figure 3 and 4, specific surface area of the unmodified and modified rice husk is 0.52 $m^2/g$ and 2.81 $m^2/g$, respectively. Considering zeta potential values, 8.29 mV increment can be shown after the modification. The results of Boehm titration clearly show that the acetic acid treatment has increased the amount of –COOH groups present in rice husk ash.

Table 1: FTIR spectrum values & functional groups for unmodified & modified rice husk.

| Unmodified Rice Husk ($cm^{-1}$) | Modified Rice Husk ($cm^{-1}$) |
|---|---|
| 3443.7 | 3447.9 ➔ OH |
| 1637.9 | 1639.2 ➔ C=O |
| 1094.4 | 1093.1 ➔ Si-O-Si |
| 799.6 | 801.8 ➔ Si-H |
| 463.4 | 467.1 ➔ Si-H |

**Zeta potential study**

The zeta potential of unmodified rice husk and modified rice husk were -44.50 mV and -36.21 mV at pH 7 buffer solution, respectively. An increment of 8.29 mV was obtained after the modification.

**pHzpc study**

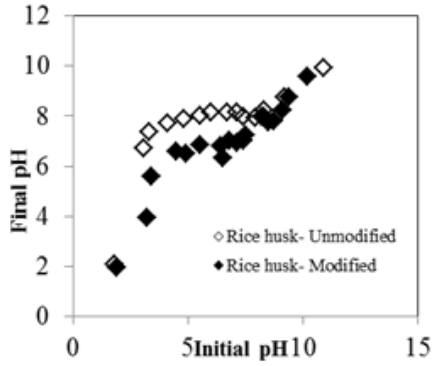


Figure 1: Determination of pH $_{ZPC}$ of unmodified rice husk and modified rice husk in pH solutions without ionic strength. (ZPC of unmodified rice husk= 8.3, ZPC of modified rice husk = 6.5)

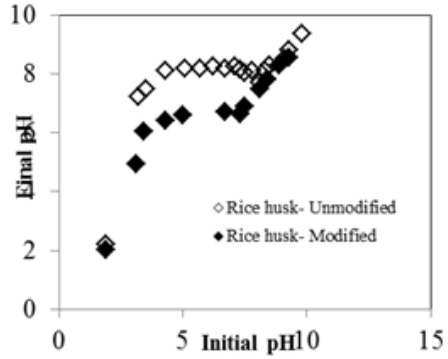


Figure 2: Determination of pH $_{ZPC}$ of unmodified rice husk and modified rice husk in pH solutions with 0.05 M $NaClO_4$. (ZPC of unmodified rice husk= 8.3, ZPC of modified rice husk= 6.7)

**Quantitative determination of –COOH**

Quantity of –COOH groups present in unmodified rice husk= 2.384 x 10-3mol/g, modified rice husk= 2.488 x 10-3mol/g.

**Surface area determination**

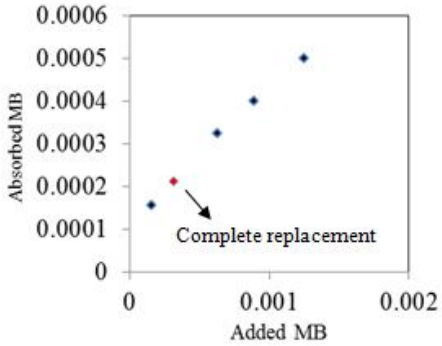


Figure 3: Determination of the point of complete replacement of cations from the titration curve of parent rice husk

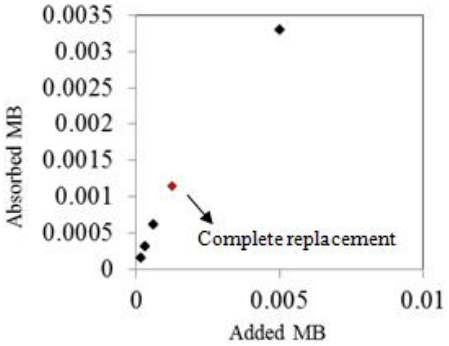


Figure 4: Determination of the point of complete replacement of cations from the titration curve of modified rice husk

Specific surface area of unmodified rice husk= 0.52 $m^2/g$, modified rice husk= 2.81 $m^2/g$.

**Conclusions**

The FTIR spectrum of modified rice husk was shifted slightly from the FTIR spectrum of unmodified rice husk. Considering those graphs it can be concluded that the rice husk ash and modified rice husk ash contain OH, C=O, Si-O-Si, and Si-H groups. The Zero Point Charge ($pH_{zpc}$) of modified rice husk using Batch equilibrium method was reported as 6.5 while the pHzpc of unmodified rice husk was 8.3. Introduction of ionic strength to the background electrolyte does not have significant impact on pHzpc.

At neutral pH, parent rice husk ash can adsorbs anions while modified rice husk ash can adsorbs cations. Zeta potential of modified rice husk was increased by 8.29 mV. So Zeta potential and ZPC values confirm the chemical modification. The modification improves the surface area of rice husk ash. Boehm titration shows the increment in –COOH functional group after chemical modification. The rice husk ash was modified and the surface properties have been changed.

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**Acknowledgement**

Institute of Fundamental Studies, Hantana Road, Kandy is acknowledged for their support and encouragement for the research work.

# Modification of Natural Rubber using Grafting Technique

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## Introduction

With growing demands for new products and technologies there has been continued interest in the field of modification of rubber. A considerable amount of published information is available on the modification of synthetic and Natural Rubber (NR). Further, with increase in price of products based on petroleum, some of the synthetic rubbers which were commercially available subsequently replaced by modified natural rubbers.

Carboxylated synthetic rubbers mainly Nitrile Butadiene Rubber (NBR) and Styrene Butadiene Rubber (SBR) exist in the market. Main advantage of introducing extra carboxylic link on the synthetic rubber could be explained in terms of mechanical and physical properties of the final product.

The aim of this project was to introduce such a system (extra carbonyl group) to natural rubber, which is not available at present. The properties of natural rubber have been modified by several methods. These include, either change the chemical nature of the rubber molecule or alter its structure. A useful method of modification involves the grafting of monomers on to natural rubber backbone.

This study is based on the graft copolymerization of Methyl Methacrylate (MMA) and acrylic acid on to NR by emulsion polymerization using Tertiary Butyl Hydroperoxide(TBHP)/ Tetra ethylene Pentamine (TEP) and Potassium persulfate/ Sodium thiosulfate initiator systems, respectively. Introduction of COOH groups using monomers on to the main back bone of the polymers would create new sites for cross links to be formed by metal oxides (ZnO, MgO etc.) or any other chemicals present in the formulation. Those new cross links formed in the system would enhance mechanical properties of the final product compared to C-S-C cross links formed by conventional sulphur vulcanization.

## Methodology

Methyl Methacrylate Grafted Rubber (MG rubber)

The monomer emulsion was prepared by first mixing the monomer with TBHP (0.25% on rubber), Oleic acid (1.0% on monomer) and then with water (1/2 volume) containing ammonia (0.8% on water). Vigorous agitation was needed to form a good emulsion. Then the monomer emulsion was added slowly to the latex while stirring and the stirring was continued for fifteen minutes.TEP solution was then added slowly to the mixture and stirring was continued for a further ten minutes. The mixture was left for at least eighteen hours to permit completion of polymerization; during this time, the latex was stirred as gently as possible. Finally MG rubber extracted using acetone and petroleum ether.

Acrylic acid grafted rubber

Cationically, stabilized latex (Positex) was used during grafting of acrylic acid on to natural rubber. Potassium persulfate/ Sodium thiosulfate initiator systems and nonionic surfactant

was used to initiate above grafting reaction. Grafted rubber was extracted using distilled water in order to remove un- grafted monomers and homopolymer from the system.

Analysis

The effect of grafting monomers on to NR back bone was investigated by FT-IR spectroscopy. The FT-IR spectrum of MG film after extraction was compared with the spectrum of NR film. The FT-IR spectrum of acrylic acid grafted rubber was compared with the FT-IR spectrum of positex film.

MG rubber was chosen to blend with natural rubber in the latex stage in order to improve strength and aging properties. The cast films prepared using the above blend were air dried and aged at 70 $^0$C. Then mechanical properties of the cast films were determined using Hounsfield Tensile Testing machine. The physical properties of MG /NR blended films were compared using tensile strength, tare strength and modules values.

**Results and discussion**

The polymerization reaction is an exothermic reaction and the temperature of the MMA grafted mixture increased during polymerization up to 42$^0$ C but temperature increment could not be observed for acrylic acid grafted system.

The presence of extra carbonyl group in the spectrum provides the evidence for grafting of MMA on to NR (Figure 1). However, presence of carboxylic link has not appeared in the FTIR spectrum of acrylic acid grafted rubber (Figure 2).

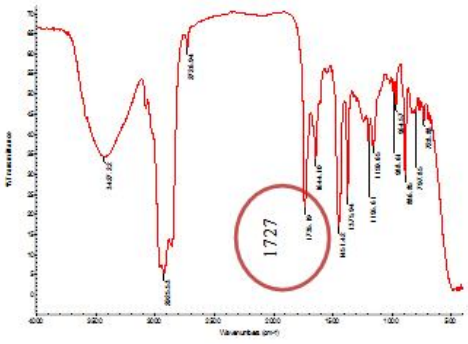


Figure 1: FI- IR spectrum of MG rubber after extraction

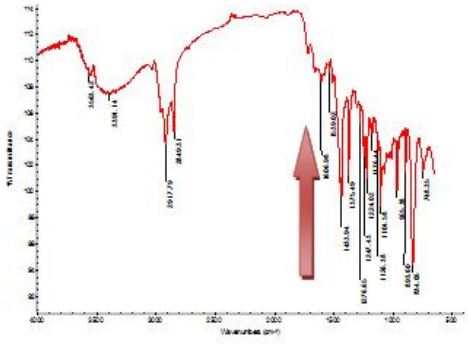

Figure 2: FT- IR spectrum of acrylic rubber after extraction

Only the homopolymer formation was succeeded using hydrophilic initiator system (Potassium persulfate/ Sodium thiosulfate) in acrylic acid grafting. During that reaction temperature has increased rapidly as expected from the polymerization reaction. Simply it means acrylic acid grafting on to NR could not be achieved using the grafting conditions applied during this research because acrylic acid monomers have failed in entering in to the rubber particles in the presence of said initiator system. However, copolymerization reaction has occurred outside of the rubber phase. Therefore, it is essential to explore another method to initiate this grafting reaction.

Mechanical property analysis of the MG/NR blend revealed that NR: MG, 60:40 blend has very good physical properties. This blends with optimized elastic and plastic properties from NR and MG rubbers, respectively. As MG rubber provides polar properties to the blend, mixing of compounding ingredients (especially accelerators) will be very effective. Additional reinforcement from MG rubber will decrease the filler loading of the compound.

Further, many advantages could be achieved through the MG/NR blend. Natural rubber latex prepared with NR: MG, 60:40 blends will give extra stiffness to gloves that are used for industrial purposes. Adhesive made from this blend will enhance bonding properties of the substrates made out with textiles. Hence this could be used in textile supported glove industry and shoe manufacturing industry.

**Conclusions**

MMA grafting on to natural rubber was achieved using hydrophobic initiator system. Mechanical property analysis revealed that NR: MG, 60:40 blend has very good tensile and tare properties.

The both initiator systems (hydrophilic and lipophilic) that were used in grafting of acrylic acid on to natural rubber were not effective. Main drawback of this grafting reaction was the difficulty in entering acrylic acid monomer into the natural rubber phase as acrylic acid is hydrophilic in nature.

However, using hydrophilic imitator system acrylic acid was homopolymerised. Hence further studies on this system have to be done to investigate suitable mechanism to graft natural rubber with hydrophilic acrylic acid monomer.

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# The Performance of Raw Rubber Dried Using Different Drying Systems

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## Introduction

Drying and smoking are two operations carried out simultaneously during the manufacture of smoked sheet rubber. Smoke acts as the carrier medium for heat and the chemical substances presence in the smoke. Heat removes the moisture presence in the sheets which is approximately amounts to 30% (wb). Some of the chemical substances with antioxidant and antimicrobial properties available in the smoke deposit on the sheet rubber and enhance their resistance to oxidation and mould growth. Ribbed smoked sheets which are the major contributor for local rubber production, are conventionally dried for 4-5 days at 48 -54 $^0$C in a smoke house with intermittent interruptions of drying for the purpose loading unloading of sheets. Recently introduced single day smoke drying system (SS drying system) dries wet sheets continuously at a rather higher temperature of 55 – 65 $^0$C. In this system, wet sheets are exposed to hot smoke at a higher drying temperature, however, for a shorter period. Sheets are also dried in open sun without control and in a stream of warm air (34 $^o$C) for about 6-8 days to produce smoke free sheet rubber. Therefore, according to drying system used, sheet rubbers may be categorized as; Conventionally Smoke Dried sheets (CSD), Sun Dried Sheets (SDS), Air Dried Sheets (ADS) and Single day Smoke dried sheets (SS). Different drying systems may vary the quantity of smoke adsorbed to the surface of the sheets and duration and temperature to which sheets are exposed to. Consequently, the sheets dried using different drying systems may have different degree of resistance to the oxidation and fungal attack affecting the raw rubber and rubber vulcanizate properties. This study therefore, attempts to study the raw rubber and rubber vulcanizate properties of the sheet rubber dried using selected four drying systems.

## Methodology

Wet sheet rubber samples were prepared using standard sheet rubber manufacturing procedures. They were dried using four drying systems namely; single day smoked drying (SD1), three day smoked drying (SD3), five day smoked drying (SD5) and hot air drying (ADS). Raw rubber properties and rubber vulcanizate properties (ageing and un-ageing) were tested. The FTIR spectrums of raw rubber samples were obtained in an attempt to identify organic materials presence on them. Acetone extractions test was also performed on each sample to measure the smoke content. Mould growths of the samples were examined under 100% humidity conditions and under ambient conditions (28 $^o$C, 80% humidity).

**Results and discussion**

Raw rubber properties of the sheet rubber produced at different conditions and their vulcanizates properties are presented in Tables 1 and 2, respectively.

Table1: Raw rubber properties of sheet rubber

| Type of sheets | Wallace plasticity number ($P_0$) [min. 30] | Plasticity retention Index (PRI) [min. 60] | % (W/W) Volatile Matter (VM) [max.0.80] | Dirt (% w/w) [max. 0.05] | Ash (% w/w) [max. 0.40] |
|---|---|---|---|---|---|
| **SD1** | 49 | 89 | 0.69 | 0.041 | **0.35** |
| **SD3** | 51 | 82 | 0.72 | 0.038 | **0.34** |
| **SD5** | 48 | 83 | 0.60 | 0.052 | **0.36** |
| **ADS** | **45** | **97** | **0.88** | **0.032** | **0.42** |

- [ ] accepted standards

It can be seen that overall raw rubber properties of the samples dried in different drying systems have not seen any significant difference. All the raw rubber properties tested lie within the accepted standard ranges agreed for natural rubber. However, VM of the air dried sheet which was dried at a lower temperature has shown a higher value than the accepted maximum value. All smoked and dried sheets show lower PRI values (resistance to oxidation) than the ADS rubber which was not exposed to smoke. Smoked sheets were dried at higher temperatures and they might have been subjected to a thermal oxidation to a certain extent than the ADS, making PRI values of former are low. However, smoke should provide a resistance to the sheet rubber as reported in the literature. Therefore, the highest PRI value registered for ADS should be further investigated.

Table 2: Vulcanised properties of rubber sheets dried at different drying conditions

| Sample | Tensile strength | | Elong. @ break | | Hardness | Compres-sion set (%) | Abrasion weight loss % (wt) |
|---|---|---|---|---|---|---|---|
| | unaged | aged | unaged | aged | | | |
| **SD1** | 19.35 | 21.06 | 675 | 575 | 43 | 7.39 | 7.07 |
| **SD3** | 18.06 | 22.02 | 760 | 720 | 45 | 7.49 | 6.5 |
| **SD5** | 23.98 | 23.60 | 740 | 700 | 49 | 7.28 | 5.5 |
| **SD4** | 23.28 | 17.08 | 640 | 600 | 47 | 6.53 | 6.5 |

Comparison of physical and mechanical properties of the vulcanizate shows that variation of the properties (except for abrasion loss) has not been affected at any appreciable level. The lowest abrasion weight loss level of SD5 sample suggests that smoking for five days has a notable effect on the resistance to oxidation at high temperature even though it was not reflected when oxidation is taken place at low temperature as indicated by the PRI values of smoked rubber. Tensile drop of the ADS sample after ageing again confirms the resistance offered by smoke to the smoked sheets for high temperature oxidation.

Studies carried out on the resistance to mould growth on sheet rubber clearly show that the duration of smoking has a clear improvement in offering resistance to mould growth at

100% humidity. However, there is no any appreciable effect of smoking on the same at ambient conditions (RH 80%). FTIR analysis and acetone attraction experiments did not yield any good results to identify the effect of smoking on their performance.

## Conclusions

It can be seen that smoking at deferent levels has no significant effect on the raw rubber properties. However, exposure to high heat level tends to lower the PRI values. Mechanical properties of the samples do not show any significant adverse effect other than for abrasion weight loss.

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# Kinetics Modelling of Partial Degradation of Carbofuran by Pyrite

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## Introduction

Fenton Process is initiated by the formation of hydroxyl radical in accordance with the classical Fenton's reaction:

$Fe^{2+}_{(aq)} + H_2O_2 \rightarrow OH^{\bullet} + Fe^{3+}_{(aq)}$

If the product $Fe^{3+}$ is reduced, the $Fe^{2+}$ is regenerated for the next Fenton cycle; hence, $Fe^{3+}$ acts as an auto-catalyst. This reaction is also known to occur when $Fe^{2+}$ is present in solid phase (Cohn *et al*., 2006; Kwan and Voelker; 2003; Watts *et al*., 2003). The slow conversion of $H_2O_2 \rightarrow OH^{\bullet}$ when magnetite, iron hydroxides, or pyrite are present is ascribed to a Fenton like mechanism (Cohn *et al*., 2006).

In nature pyrite is the most abundant of metal sulfides. In Sri Lanka pyrite is an unwanted substrate in graphite industry. It is intimately associated with graphite by degrading the quality of the nation's graphite resource and it is confirmed that pyrite can be used as a starting material for purification of the water polluted with organic pollutants in both the presence and absence of light (Weerasooriya *et al*., 2006). When properly fabricated, pyrite based $OH^{\bullet}$ generation technology will hold a great promise in water treatment industry due to its simplicity, cost-effectiveness, and environmentally friendliness.

Carbofuran (2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate) is a broad spectrum carbamate pesticide. Because of high water solubility of carbofuran (320 mg/liter at 25 °C) the risk of ingestion in to the human body is high Kidd and James (1991). Carbofuran, was selected for the present study with the aim of investigating its degradation in pyrite mediated aqueous environments under anaerobic conditions due to its wide use as an insecticide and nematicide in agricultural applications and high toxicity (Bachman *et al*., 1999).

## Methodology

Zeta potential values of pyrite were measured using a ZETA-METER SYSTEM 4.0 in various pH values in the presence of carbofuran. Infra red (IR) analyses were carried out using a Fourier Transform Infra Red Spectrometer at 4 $cm^{-1}$ spectral resolutions (Nicolet 67000). To elucidate degradation products of carbofuran, the pyrite mediated systems were monitored with RP-HPLC (Reversed phase High-Performance Liquid *Chromatogrphy*) and gas chromatography-mass spectroscopic (GC-MS) methods.

## Results and discussion

Surface charge, hence the zeta potential of pyrite is a function of potential determining ions, in the present case, i.e. $H^+$, $OH^-$, $S^{2-}$ and $Fe^{2+}$ (Weerasooriya and Tobschall, 2005). Any specifically adsorbed species on the surface will also affect the zeta potential (or surface charge). Figure 01 shows zeta potential as a function of pH for three $NaClO_4$ concentrations. The iso-electric point is observed around pH 1.40-1.70. The curves show a symmetric behavior over the common cross point. That observation is typical for an electrolyte that do not specifically adsorbed. According to the Figure 02, in the presence of carbofuran, the values of zeta potential moved towards a less negative direction. It indicates the direct surface interactions. Upon the carbofuran loading from ~20 – 200 µM, $pH_{IEP}$ was shifted from 2.4 to 4.5. That provides confirmatory evidence of its intimate surface interactions, and these interactions ultimately enhanced upon auto-oxidation of carbofuran by pyrite.

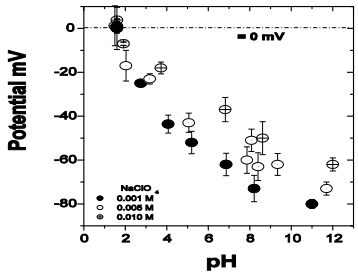



Figure 01: Variation of zeta potential of pyrite as a function of solution pH for $NaClO_4$ solutions of 0.001M, 0.005M and 0.01 M

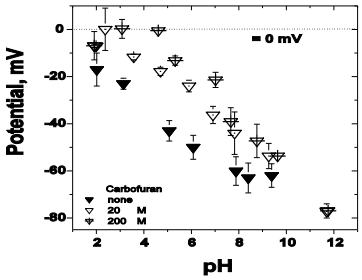



Figure 02: Variation of zeta potential of pyrite as a function of pH in 0.01M $NaClO_4$ in the presence of carbofuran

As shown in the Figure 03 the variations of IR spectrum of pyrite – carbofuran mixture were monitored with respect to time for a period of 0.5 hour. With the time a clear decrease was observed in the intensity of the IR band at 3363$cm^{-1}$ due to $–NH_2$ stretching. According to the IR spectral data of bare pyrite, it does not show any variations in the vicinity of 3363 $cm^{-1}$.The band at 3363 $cm^{-1}$ appeared as a result of carbofuran and pyrite interactions.

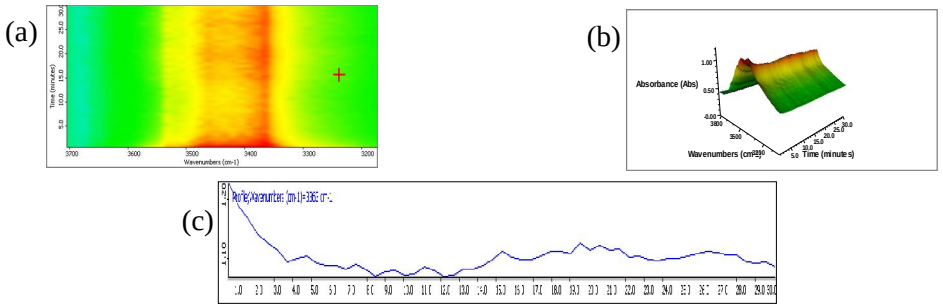
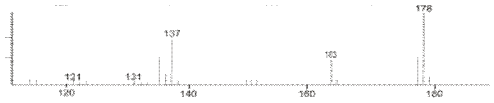
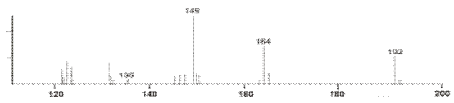



Figure 03: Time resolved IR spectra of pyrite-carbofuran system. The data collection under the transmission mode; time-step 0.5 s. Red circled spectral region monitored for rate determination. (a) Band intensity variation with time. (b) Variation of absorbance with time. (c) Intensity variation of the band at 3364/3 $cm^{-1}$ with time.

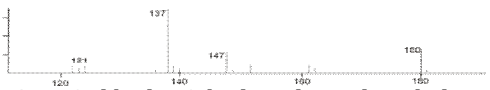
Initial conditions: $\frac{[carbofuran]}{[pyrite]} \sim 5$



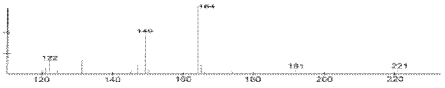
A - 2,3-dihydro-3-oxo-2,2-dimethyl benzofuran-7-ol



B - 2,3-dihydro-2,2-dimethylbenzofuran-7-yl-formate



C - 2,3-dihydro-3-hydroxyl-2,2-dimethyl benzofuran-7-ol



D - 2,3-dihydro-2,2 dimethylbenzofuran-7-yl methylcarbamate

Figure 04: Mass spectroscopic fragmentation patterns of carbofuran and it degradation products.

**Conclusions**

When the pH ~1.7-2.0 the partial degradation of carbofuran occurs with a highest efficiency in the presence of pyrite. Dominant degradation products of the carbofuran degradation process are A, B, C with some unidentified intermediates due to low resolution of the mass spectrometer. In the presence of natural pyrite, nearly 40% of carbofuran degraded with an hour.

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**Acknowledgement**

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# Development of Lithium Ion Rechargeable Batteries by Using Sri Lankan Graphite and Locally Synthesized Low-Cost Materials

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## Introduction

Lithium-ion battery (LIB) is a family of rechargeable battery types in which the lithium ions move from the negative electrode to the positive electrode through the electrolyte. LIBs are currently one of the most popular types of battery for portable electronics with one of the best energy-to-weight ratios, no memory effect and a slow loss of charge when not in use. However, the present generation of LIBs has many limitations, such as high internal resistance, expensiveness, temperature effect, aging effect, short circuiting, environment effect and over heating. The present inferior electrode materials are the main reason for these drawbacks and hence the main obstacle to achieve reliable and cheaper lithium-ion batteries (Pushpaka *et al*., 2008).

This abstract presents a study of developing $Li(Ni_{1/3}Co_{1/3}Mn_{1/3})O_2$ based cathode electrodes and $NiO\text{-}LiFeO_2\text{-}LiCoO_2$ based anode electrodes with the Sri Lankan graphite as electrical conductivity enhancer. These novel electrode materials were synthesized by using Glycine-nitrate and Pechini methods, which are low cost techniques but can result in powders with high purity, homogeneity and particle morphology that are highly desired for LIB electrodes (Wijayasinghe *et al*., 2006).

On the other hand, the expensive synthetic carbon materials are now being used as electrical conductivity enhancer of LIB electrodes, currently. In a recent research work on Sri Lankan graphite at the Uva Wellassa University with the collaboration of Institute of Fundamental Studies our group indicated of having sufficient electrical conductivity in Sri Lankan graphite to be used as conductivity enhancer (Geethika *et al*., 2010). Sri Lanka has been well known for processing high purity graphite, which is mainly being exported as cheap raw materials. Introducing this cheaper material for the high-tech energy conversion devices will definitely reduce the cost of these devices while also adding value to our mineral resources. By considering these factors, Sri Lankan Bogala graphite was investigated in this study as a conductivity enhancer in LIB electrodes.

## Methodology

Two wet-chemical powder synthesis techniques, Glycine-nitrate and Pechini methods, were employed to prepare the novel electrode materials. Metal Nitrates, $LiNO_3$, $Ni(NO_3)_2.6H_2O$, $Co(NO_3)_2.6H_2O$, $Mn(NO_3)_2.4H_2O$ and $Fe(NO_3)_3.9H_2O$ of analysis grade were used as starting materials with the organic precursor solutions of citric acid, Glycine and ethylene glycol.

In the Pechini method, powders were prepared with ethylene glycol to citric acid ratio of 1:4. In the Glycine-nitrate method powders were prepared by keeping glycine:nitrate ratio as 0.6:1. Subsequently powders were calcined at 900 °C in static air in a box furnace. The calcined powders were pressed in to green pellets of 12mm in diameter, followed by sintering at 1000 °C in a horizontal tube furnace in static air. The electrical properties of the selected materials were determined by using d.c. four-probe techniques. Specimens for measuring d.c four-probe conductivity were prepared by coating both end surfaces of the pellet with gold paste to provide better contact with the electrodes of the sample holder.

Fabrication of solid flexible electrodes from the selected materials was done via tape casting.

Bellcore-type electrodes of about 0.1 mm thickness and 10 mm diameter, were prepared for electrical measurements by mixing the electrode material with PvdF-HFP (KynarFlex 2801), a carbon source in proportions 1:0.25:0.10 by weight. Those proportions mixed and blended with propylene Carbonate (PC, Merck) Corresponding to 40% of active material weighted and dissolved with acetone. As the carbon sources for enhancing conductivity, Sri Lankan graphite samples obtained from Bogala mines were investigated. To compare their performance, synthetic graphite was used as a reference material. The details of the selected graphite samples are given in Table 1. In order to measure the electrical conductivity of these selected Sri Lankan graphite and synthetic graphite, the DC four Probe measurements were performed on the pellets prepared by these samples.

Table 1: Selected Sri Lankan Bogala graphite samples for conductivity enhancing in LIB electrodes

| Sample | Position | Grade | Carbon% |
|---|---|---|---|
| BA | BR Mill | BP 8085 | 80-85 |
| BB | Micron Mill | BFPH 99 | 99 |
| BC | | BFP 9799 | 97-99 |

Solid flexible tapes were prepared by using these selected cheaper local graphite by replacing expensive synthetic graphite and changing the proportion of other chemicals. The electrical conductivity of the prepared solid flexible electrodes was measured by employing the vander Paw method at room temperature. For these fabrication and characterization, a manual tape caster and a vander Paw measuring setup designed and constructed under this project work.

**Results and discussion**

Under the powder synthesis work, $LiCoO_2$, $LiFeO_2$, and some other novel oxides powders were prepared. The details of these prepared novel compositions are given in Table 2 and Table 3. The measured room temperature d.c electrical conductivity of the synthetic graphite used as a reference material for this study was $1.43\times10^2$ S/cm. In comparison, the measured room temperature conductivities of Sri Lankan BA, BB, and BC-Bogala graphite were $0.80\times10^2$ S/cm, $1.15\times10^2$ S/cm and $1.37\times10^2$ S/cm, respectively. This reveals that the conductivities of these selected Sri Lankan graphites are almost close to that of the synthetic graphite, which is the state of the art conductivity enhancer of LIB electrodes.

Of the solid flexible cathode electrodes prepared with the expensive synthetic graphite as a reference materials, the room temperature electrical conductivity of Li $(Ni_{1/3}Mn_{1/3}\ Co_{1/3})O_2$ and Li$(Ni_{0.33}Mn_{0.33}\ Co_{0.22}Fe_{0.11})O_2$ electrodes were $10.9\times10^2$ S/cm and $15.8\times10^2$ S/cm, respectively. Very interestingly, as seen in Table 2, the cathode electrodes prepared with the same electrode materials but by using cheaper local graphite added electrode also show of having comparable electrical conductivities

Furthermore, the room temperature electrical conductivity of 63.75% NiO+11.25%$LiFeO_2$+15% $LiCoO_2$ anode electrode which prepared by using expensive synthetic graphite was $14.63\times10^2$ S/cm. Interestingly as seen in Table 3., the local graphite added electrode of the same material composition shows of having sufficient electrical conductivity.

Table 2: The electrical conductivity of cathode electrodes developed by using Sri Lankan graphite

| Electrode materials for cathodes | Sri Lankan Bogala graphite/ grade | Conductivity (S/cm) at 25 $^0$C |
|---|---|---|
| $Li(Ni_{1/3}Mn_{1/3}\ Co_{1/3})O_2$ | BA/BP8085 | $6.00 \times 10^2$ |
| $Li(Ni_{1/3}Mn_{1/3}\ Co_{1/3})O_2$ | BB/BFPH99 | $3.61 \times 10^2$ |
| $Li(Ni_{0.33}Mn_{0.33}\ Co_{0.22}Fe_{0.11})O_2$ | BB/BFP9799 | $6.88 \times 10^2$ |

Table 3: The electrical conductivity of anode electrodes developed by using Sri Lankan graphite :

| Electrode materials for anode | Sri Lankan Bogala graphite/grade | Conductivity (S/cm) at 25 $^0$C |
|---|---|---|
| 63.75% NiO+11.25%$LiFeO_2$+15% $LiCoO_2$ | BA/BP8085 | $1.86 \times 10^2$ |

.

As it is stated elsewhere (Pushpaka *et al*., 2008) in order to get the optimum performance, the LIB electrodes should possess an electrical conductivity of the order $10^2$ S/cm at room temperature. In the light of this, all the electrodes developed in this study with locally synthesized low-cost materials and cheaper Sri Lankan graphite show all most similar electrical performance as of the expensive state of the art electrode system of present day Lithium-ion batteries.

**Conclusions**

The electrical conductivity of the Sri Lankan graphite selected for this study shows of having appropriate conductivity to be used as a conductivity enhancer in electrodes. Further, this study revealed the possibility of fabricating solid flexible electrodes with the locally synthesized novel oxides using low-cost synthesis techniques. The electrical performance of the electrodes prepared with the cheaper local graphite are almost comparable with those prepared with expensive synthetic graphite, which is the state of the art conductivity enhancer of LIB electrodes. As a whole this study indicates the possibility of preparing cheaper electrodes for LIB with locally synthesized low-cost materials and Sri Lankan graphite.

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**Acknowledgement**

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# Investigation of Electrical Properties in Different Structural Varieties of Sri Lankan Graphite

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## Introduction

Graphite is a crystalline polymorphic form of elementary carbon and it consists of parallel sheets of carbon in a hexagonal arrangement. Graphite is a soft mineral with black lead streak having a metallic lustre. Sri Lanka is the world's only significant source of crystalline vein graphite. It is very popular all over the world for its high purity and its high carbon content graphite (97-99%) (Herath, 1995). Sri Lankan natural graphite is exported as a cheap raw material and the only local graphite based industry is the pencil industry. Sri Lankan natural vein graphite is found in various morphologies with different structural and physical characteristics (Balasooriya and Bandaranayake, 2010). Four common morphologies of vein graphite have been identified from the Bogala and Kahatagaha-Kolongaha mines. They are coarse flakes of radial graphite, coarse striated-flaky graphite, needle platy graphite and shiny-slippery-fibrous graphite.

Natural graphite is a host material for lithium intercalation and there is a potential of using it as an active anode material for the rechargeable lithium cells. Among the requirements to be such an active anode material, electrical conductivity is a main factor and the candidate material should possess sufficient conductivity in the order of around $10^2$ $Scm^{-1}$ at operating temperature to support the anode function (Pushpaka *et al*, 2008). Further, there is only very limited work reported on morphology and structural characteristics of Sri Lankan natural graphite. Any information on the electrical behaviour of them, specially the electrical conductivity, has not yet been reported elsewhere. By considering these factors, this study was performed to identify suitable verities of Sri Lankan graphite based on their electrical conductivity.

## Methodology

Natural Sri Lankan vein graphite from the Bogala mine situated in Kegalle district was used for this study. Identification of different structural varieties was done by visual inspections and then the selected structure variety samples were separated as small chips. These chips were then crushed in to powders by using a disk mill, after that the powder samples were rained by a centrifugal ball mill (Model 06.102/2188).

Then the selected graphite powder was subjected to mild oxidation by heat treating at 550 $^0$C in air for 6 h in a tube furnace (model CTF/12/65/550). Thereafter both the raw powder and heat treated powder were pelletized by pressing cold uni-axially at a pressure of 100 MPa. The electrical conductivity measurements on them were performed by using the d.c. four probe techniques at room temperature. The schematic diagram of the d.c. four probe techniques developed for this study is given in Figure 1.

## Results and discussion

Four common morphologies of vein graphite were identified from the graphite investigated from Bogala mines. They were coarse striated-flaky (BNG03), coarse flakes of radial (BNG04), needle-platy (BNG05) and shiny- slippery-fibrous graphite (BNG06). These four common morphologies of vein graphite are shown in Figure 1.

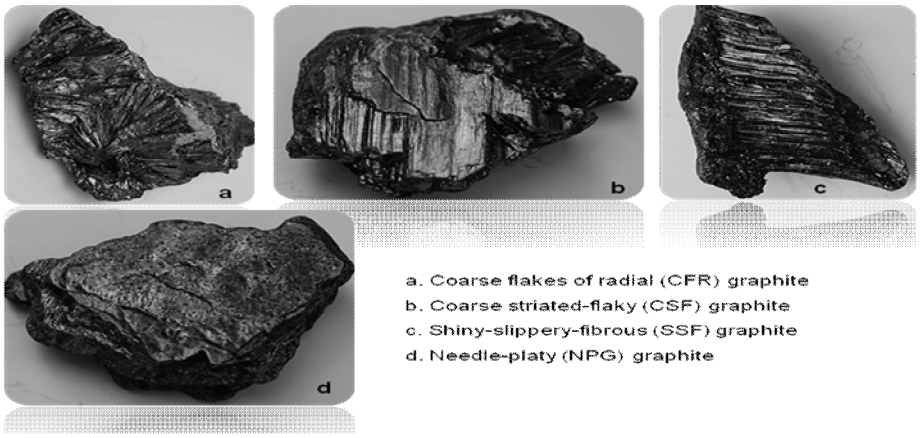


Figure 1: The four common morphologies of vein graphite identified in this study from the graphite investigated from Bogala mines.

Table 1: Electrical conductivity of different structural verities of Bogala graphite

| **Samples** | **Electrical conductivity σ[SCm$^{-1}$]** | |
|---|---|---|
| | **Raw samples (25$^0$C)** | **After heat treated (at 550$^0$C for 06 h)** |
| Coarse striated-flaky graphite | $1.16\times10^3$ | $3.99\times10^2$ |
| Needle-platy graphite | $9.24\times10^2$ | $1.20\times10^3$ |
| Shiny- slippery-fibrous graphite | $1.24\times10^3$ | $1.86\times10^3$ |

The electrical conductivity details of these structural varieties are given in Table 1. As seen in the table all these structural varieties of Bogala graphite possess the electrical conductivity in the order of around $10^2$ Scm$^{-1}$ at room temperature. Hence, it fulfils the electrical conductivity requirement to be an active anode material for the rechargeable lithium-ion batteries.

Further among these selected structural varieties, the Shiny-slippery-fibrous graphite structure shows the highest electrical conductivity. The mild oxidation by heat treatment of these structural varieties increases the electrical conductivity mostly in needle-platy graphite and shiny- slippery-fibrous graphite.

The present study reveals the promising characteristics of Sri Lankan natural graphite as intercalation anode material in rechargeable lithium battery applications due to its appropriate electrical conductivity, suitable morphology, low cost and high purity.

**Conclusions**

In the graphite samples collected at Bogala mines, four different structural varieties have been identified there are coarse flakes of radial graphite, coarse striated-flaky graphite, needle-platy graphite and shiny-slippery-fibrous graphite.

All these investigated Bogala graphites show of having sufficient electrical conductivity in the order of around $10^2$ $Scm^{-1}$ and show the potentiality for using as active anode materials for the rechargeable lithium-ion batteries.

**Acknowledgements**

The authors wish to thank the Institute of Fundamental Studies, Kandy and the Uva Wellassa University.

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# Surface Modification of Activated Carbon to Treat Polluted Water Streams

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## Introduction

Water pollution due to the industrial applications, agro chemicals, etc. is a serious environmental problem which creates health, economical, and ecological impacts worldwide. The presence of toxic compounds, both organic and inorganic, in water streams creates significant threats to man and nature. Therefore, polluted water streams should be purified before releasing to the environment (Akhtar *et al.*, 2006); (Massa *et al.*, 2004). Adsorption is one of the most versatile and effective method, among other different methods. Adsorption is a natural process by which molecules of a dissolved compound collect on and adhere to the surface of an adsorbent solid. Activated carbon has a great potential for effectively removing contaminants from water by adsorption process due to its electrochemical surface properties. Most forms of activated carbon are non-polar in nature, so they have the greatest affinity for other non-polar substances. As a result, they are most effective in the removal of a variety of organic contaminants. However, activated carbons do not effectively remove trace metals, contaminants of high solubility or inorganic salts like nitrates. Hence, modifying the surface chemistry of activated carbon becomes an attractive route towards novel applications in enhancing the efficiency in water treatment (Chen *et al*., 2003). In this research, chemical treatment was used to modify the commercially available activated carbon and the modified material was characterized.

## Methodology

Five grams of commercial activated carbon (Aldrich) and 50 ml of 1M acetic acid were stirred in magnetic stir plate at room temperature for 24 h. Then it was repeatedly washed with 50 ml of distilled water to obtain constant pH value. Finally, the modified activated carbon (MAC) sample was centrifuged (Beckman CP centrifuge) and dried at vacuum desiccator at 60 $^0$C temperature for 4 h until get powdered.

Both parent activated carbon (PAC) and MAC were characterized using Fourier Transform Infrared Spectrometry (FTIR) (Nicolet 6700 FTIR), Zeta-Meter 4.0. (Zeta Meter Inc), pH of zero point charge (ZPC) and Methylene blue (MB) absorption measurements.

## Results and discussion

Fourier Transform Infrared Spectrometry (FTIR) analysis:

Figure 1 shows four major peaks at 3465.3 cm$^{-1}$, 1635.3 cm$^{-1}$, 1384.5 cm$^{-1}$, 526.1 cm$^{-1}$ of FTIR spectrum of original PAC. The broad and strong absorption peak at around 3465.3 cm$^{-1}$ is O-H group and peak at 1384.5 cm$^{-1}$ is O-H group which is derived from deformation modes of alcoholic and phenolic type O-H with a sterically hindered. The strong peak at around 1635.3 cm$^{-1}$ appeared in spectra is the functional groups of C=O (Coates, 2000). The strong peak at around 526.1 cm$^{-1}$ is also due to the simple hydroxyl

compound. It is indicated ‘‘free’’ OH groups, either on the surface, or embedded within a crystal lattice, and free from interactions with other ions or groups (Coates, 2000). Figure 2 shows the FTIR spectrum of the modified activated carbon which is very similar to that of PAC. However, the strong peaks appeared in spectra of PAC is shifted in MAC indicating surface modification on activated carbon.

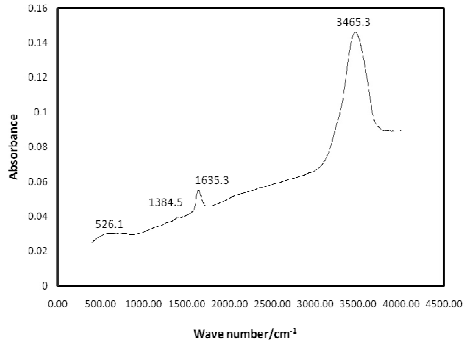


Figure 1: FTIR spectrum for parent

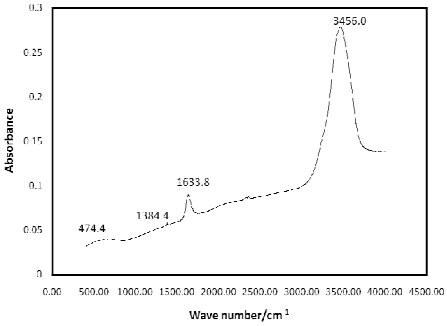


Figure 2: FTIR spectrum for modified activated carbon

Zeta-Meter 4.0. analysis:

At pH 7, Zeta potential of PAC and MAC were found to be -132.2 mV and -172.6 mV, respectively. Zeta potential was reduced by 35.4 mV after the surface modification. The results obtained have a good agreement with that of Thielbeer *et al*. (2011). Hence, zeta meter 4.0 analysis also confirms the surface modification of activated carbon.

pH of zero point charge:

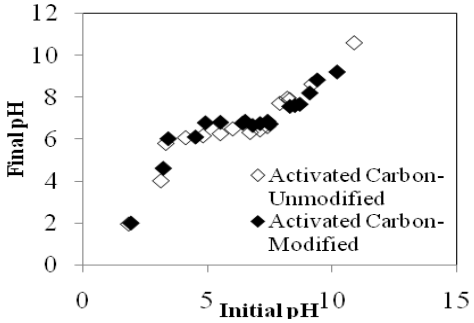


Figure 3: Determination of $pH_{ZPC}$ of PAC and MAC without introducing ionic strength

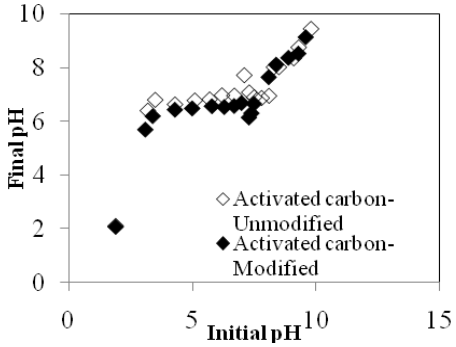


Figure 4: Determination of $pH_{ZPC}$ of PAC and MAC with introducing ionic strength 0.05 M of $NaClO_4$ solution.

The pHzpc value of PAC was found as 6.9 and that of MAC was 6.7 without background ionic strength. Therefore, in MAC, at pH values greater than 6.7, the surface charge is negative. At pH values lower than 6.7, the surface charge is positive. Similar experiments were repeated by introducing ionic strength to the background electrolyte solution. The results show that ionic strength does not have a significant impact on the ZPC. The result of ZPC is in good agreement with that of Chen and Lin (2001).

Methylene blue absorption measurements:

Methylene blue (MB) method has been used to determine the surface area of activated carbon. Using Figure 5 and 6, the specific surface area of PAC and MAC were calculated as 188.87 $m^2/g$ and 132.12 $m^2/g$, respectively. As such, after the modification the surface area was reduced. This could arise from pore blockage by adsorbed acetic acid molecules. This finding is consistent with the results reported elsewhere (Chen *et al.*, 2003).

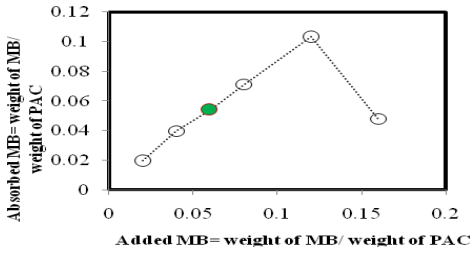


Figure 5: Determination of the point of complete replacement of cations from the titration curve of PAC.

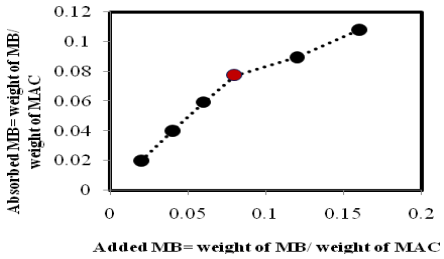


Figure 6: Determination of the point of complete replacement of cations from the titration curve of MAC.

## Conclusions

Commercial activated carbon was chemically modified. Several characterization processes were used to investigate the surface properties of modified and unmodified materials. Investigations confirmed the occurrence of surface modification.

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# Erosive Wear Resistance of Sri Lankan Rocks Used for Flooring

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## Introduction

Varieties of floor tiles are amongst many of the specialties in ancient Sri Lankan architectural wonders. Even today floor tiles in Sri Lanka claim a high regard in world export market being one of the biggest equity grosser in commodities. Stone floors are admired for their splendid appearance as well as for higher durability. Natural stones such as slate, granite, gneisses and marble are commonly used for interior designs and flooring.

Stone floor tiles being materials which are always in physical contact with moving objects, are prone to wear, although at a comparatively slower rate. Therefore, the durability of tiles should also be of concern in addition to their aesthetic appearance. However, wear resistance of stone floor tiles has not been attracted adequate attention in material testing. Hence, it is essential to ascertain tribological properties, including resistant to wear, when selecting the most suitable flooring material. This research aims at investigating and documenting the wear resistance of commonly used Sri Lankan rocks in flooring.

## Experimental Procedure

Sri Lankan rocks used in floor tile industry are mainly high-grade metamorphic gneissic rocks having varying mineralogical compositions and textural characteristics. Granites and granitic gneiss, charnockites and charnockitic gneiss, migmatitic gneiss, garnet granulite, and garnet-biotite gneiss were used for testing. In addition an imported slate sample was also tested. Small thin slabs of rocks with dimensions 5 mm x 5 mm x 1 mm were prepared as the testing materials. Quartz slabs of same dimensions were used as the control material. A slurry pot tester was used where silicon carbide particles suspended in distilled water was used as the erodent. Specimens were mounted on a nylon disk which was rotating at a constant speed while being immersed in the slurry. Erosion of the specimens was determined as a function of time by measuring the mass loss of all eight materials at regular time intervals.

Erosion of the specimens was determined by measuring the mass of loss as a function of time. For this, the test was interrupted after suitable time intervals and the specimens were unclamped from the rig and dried in the oven before measuring the weight.

## Results and discussion

As expected, quartz specimen showed the highest resistance to erosive wear. Therefore, its use as the control material can be justified. Figures 2a, 2b and 2c show cumulative erosion of the tested samples plotted as functions of time. Granite specimen shows the least erosion among the test samples in the first batch of tests (Figure 2a). Therefore it has a high resistance to wear compared to the other test samples. Slate is considered as comparatively a soft rock. It shows the least resistance to wear.

In the second batch of tests, the medium to fine-grained granitic gneiss specimen indicates higher resistance to wear. Coarse-grained granite is relatively prone to erosion and thus has a low erosive wear resistance (Figure 2b). As shown in Figure 2c, in the

third set of tests, garnet granulite shows the lowest erosion rate and it has a higher resistance to wear while garnet-biotite gneiss has a relatively lower resistance

Figure 3 summarizes cumulative erosion of all tested samples. Medium to fine-grained graintic gneiss indicates highest resistance to wear among all samples.

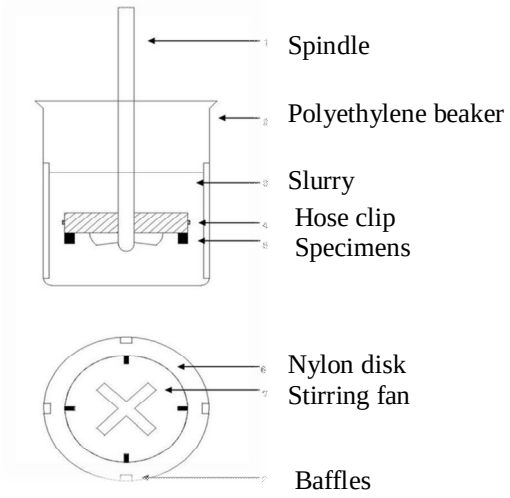


Figure 1 : Schematic diagram of the slurry pot tester (after Wijayasinghe, 1999)

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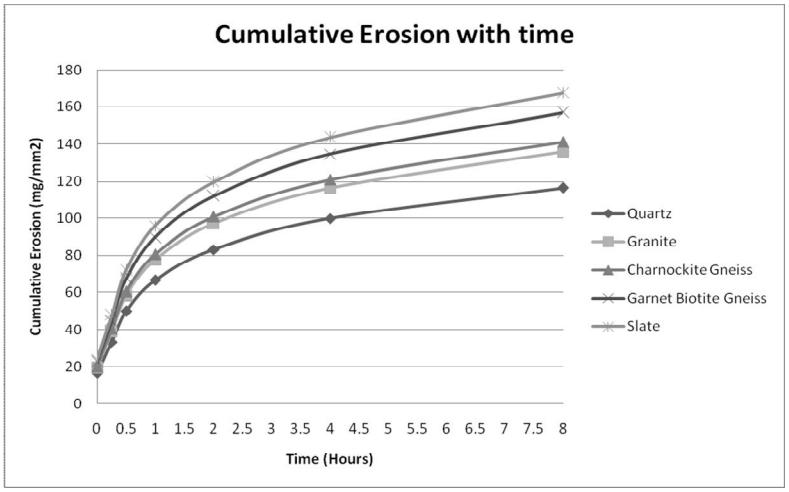


Figure 2 (a): Cumulative erosion of selected rock specimens.

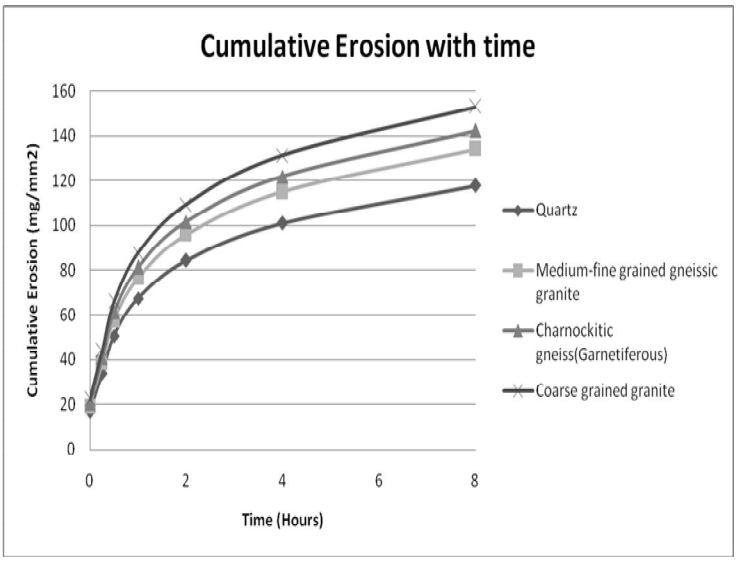


Figure 2 (b): Cumulative erosion of selected rock specimens.

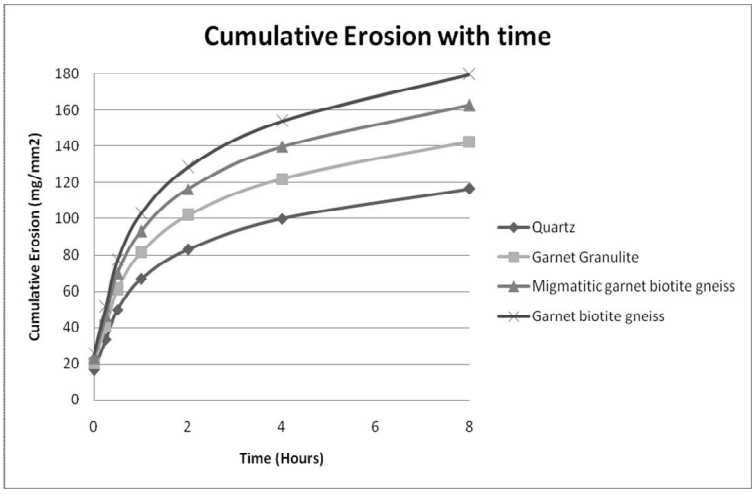


Figure 2 (c): Cumulative erosion of selected rock specimens.

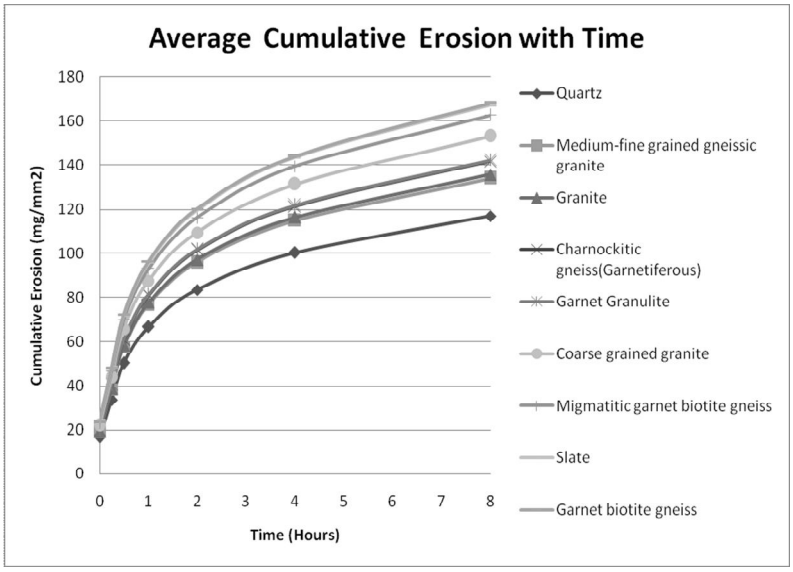


Figure 3: Average cumulative erosion of all tested samples.

## Conclusions

This study indicates the possibility of applying this cheaper slurry erosive pot tester method to distinguish the resistance to erosion between different natural flooring materials. The medium to fine-grained granitic gneiss shows the least erosion and the highest resistance to erosive wear compared to the other materials tested in this study. Hence this study suggests the medium to fine-grained granitic gneiss having a higher durability is the most suitable as flooring material considering its durability aspects.

## Acknowledgement

Mr. Upendra De Silva, Research Assistant of Geological Survey & Mines Bureau (GSMB) is thanked for preparing the rock specimens for testing. Star Granite (Pvt) Ltd., kindly provided the rock samples.

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# Designing Low Cost Smart Energy Meter

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## Introduction

Smart energy meters are the next generation of electricity or gas meters which are software-based, power efficient devices that accurately track the energy consumption. The Smart energy meter offers major benefits to both the customers and companies, in terms of efficiency, reliability, and cost saving. There are three kinds of electricity meters available in the world. They are electromechanical meters, Electronic meters and multiple tariff (variable rate) meters. Electronic meters or smart energy meters, as opposed to traditional mechanical and/or electromechanical solutions in use, provide benefits as more accurate bills, lower bills, sell energy back to the grid, and flexible tariffs. Introducing smart energy metering to domestic electricity consumption has many advantages like reducing electricity consumption in peak hours, drawing consumer attention on their energy consumption and etc. These energy meters are commercially available in the market today. But to replace all the existing manual electrical meters with smart energy meters, large amount of money has to be invested. Therefore, designing a low cost smart energy meter will be the best solution for issues of increasing electricity consumption in the country. Acordingly, this project aimed to design a low cost smart energy meter to minimize energy consumption and energy costs for consumers by introducing a system to save energy and control the utilization of electricity at peak hours. And this project also focused to introduce a degree of automation, including Automated Meter Reading (AMR).

## Methodology

The energy meter was designed using MCP3906 energy metering IC, Current Transformer to measure the load current, Resistor voltage divider network to measure the voltage, PIC18F2550 USB microcontroller for control unit, a LCD display, a Real Time Clock and EEPROM.

The MCP3906 is a Digital Signal Processor based instantaneous power integrator that supplies a pulse output proportional to the amount of energy consumed. The MCP3906 uses an internal 14-bit ADC to sample the voltage and current.

The software is to have an overall control of the hardware at all time and to determine the energy (counting the pulse) and save with different location in EEPROM (peak and off-peak energy). Hardware controlling consists of input devices, power circuit, EEPROM, RTC and LCD with external peripherals. In addition, base on the selected algorithm, the software must be able to continue tracking the input Energy meter IC pulse signals, convert to energy and send those data to LCD display. (Time, Date, currently usage Energy, once the button pressed shows -Total Peak energy usage and Total off-peak energy usage).

After overall planning, the principle of energy calculations is in first priority to work on, and follow to instruct the microcontroller to execute for Initialize, pluses check, communicate with RTC and EEPROM, button check, USB check and data sending to LCD and MS Access database.

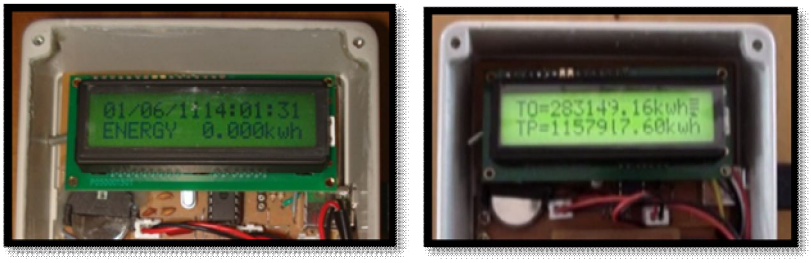


Figure 1: LCD user interface

**Results**

Figure 2: Designed smart energy meter

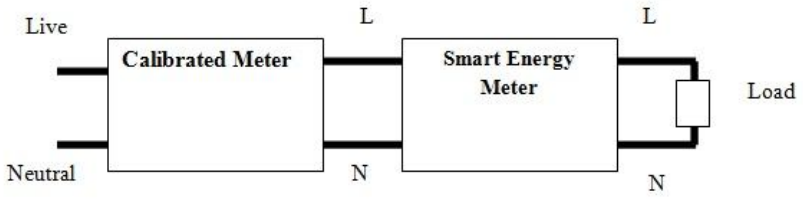


Figure 3: Test setup for Experiment

An experiment was carried out to determine whether the energy measurement circuitry was able to measure the overall energy usage up to the required accuracy. Specifically, the accuracy maintained between loads of different magnitude was determined, in effect measuring the linearity of the energy meter's accuracy. As both the device under test and the control device output a pulse proportional to the amount of energy consumed, it is merely necessary to check that the amount of pulses that both meters output over time and with different loads stay proportional

Although the testing performed was limited, from the results, during an each time interval the pulses counted from the control circuitry were constant (constant load). And it can thus be concluded that the pulses counted by the control circuitry are a very good (if not perfect) representation of the energy consumed (according to the energy metering circuit).

## Discussion

The energy measurement accuracy test of the designed meter performed good results in order to be accepted in working environment. The designed system uses a Current Transformer to measure load current. This Current Transformer is specially designed for this system to overcome the problems of unavailability of required shunt in local market for the purpose and available Current Transformer in local market are bigger in size (this will ultimately increase the size of the product and cost). By using required standard Current Transformer (specially designed for energy meters) can increase the accuracy of the system as well as it will increase the capacity of the meter.

The system is capable of storing the pulse readings in EEPROM within short time period. There is higher potential of increasing this capacity and efficiency of the meter by using a backup power supply. This may help to avoid any inconvenience of missing data/readings of energy consumption during billing.

The meters currently in use are only capable of recording kWh units. The kWh units used still have to be recorded monthly by meter readers on foot. The recorded data need to be processed by a meter reading company (Ceylon Electricity Board) for processing bills. There is a good potential that meter readings can be transmitted to distributors/utilities over wireless media thus, eliminating the need of a manual meter reading collection process.

Available smart energy meters in nowadays are equipped with a range of communication technologies including Low Power radio, GSM, GPRS, Bluetooth and etc. The designed system has USB communication facilities. Therefore using USB GSM modem can easily achieve this target.

The results of the overall system of the designed smart energy meter were acceptable and can be thus be accepted to be in a working condition with further modifications, based on the duties that the system should perform.

## Conclusions

Introducing smart energy metering to domestic electricity consumption has many advantages like reducing electricity consumtion in peak hours, drawing consumers attention on their energy consumption, avoiding meter reading cost and etc. Designing a low cost smart energy meter will be a best solution for issues of increasing electricity consumption in Sri Lanka. This project has been successfully developed and implemented. To reduce electricity consumption in peak hours and to draw consumers' attention on their energy consumption this kind of smart energy meter is a better solution. The system designed was low cost which can be built around Rs.3000.00. This is cheaper than those smart energy meters available in market.

# Car Park Navigation and Management System: A Software Solution

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## Introduction

The Car Park Management and Navigation System is a system designed to reduce the number of problems associated with parking cars. Aims of this project are to create a systematic method of parking cars, to reduce congestion in parking lots and to prevent the occurrence of indiscriminate parking.

Parking in the urban areas has become increasingly part and parcel of successful business; more and more shoppers get attracted to organized shopping complexes where ample, secure and effectively managed parking is provided. This project has focused on developing software that will effectively manage the parking facilities and manage parking in order to optimize the use of limited parking spaces and increase customer satisfaction. To achieve this objective, a qualitative and quantitative research design was used whereby survey used as the research instruments in data collection.

## Methodology

Basically, the entire project was divided in to five main sub modules for the convenience of proper development. They are Number Plate Recognition System, Payment System, Car Detection System with a model car park, Image Capturing System, and the Database.

The system displays free spaces in the car park using green color indicator lamps. Customer entering to the system can select a slot by pressing a push button associated to the particular free slot and the indicator lamp will turned to red color. When the customer is in front of the entrance gate it captures an image of the car number plate and save it in the disk. Saved image processed by the number plate recognition application and gives out the output as ASCII characters and will be saved in the database. After that the customer gets permission to get into the car park.

When customer moved away from the car park particular space is updated automatically switching on the green indicator at the relevant space on the display board. When car is at the exit gate, it again captures the image, processes the number plate and update the database with the exit time. At that time it calls the payment systems where the customer can pay his/her bill. When customer clicks the 'Show My Bill', it shows the customer bill and asks to select a payment method. Customer can press a button and select a payment method he/she likes. Customer has options for three payment methods namely, credit card, SMS and cash. After the withdrawal process GUI displays a message which shows that the customer can exit.

## Results and discussion

The parking management system is suited for the current Sri Lankan society with the technology and usage. The prototype would help the parking authorities as well as the customers of the car park in the following ways-

- Reduction in time as well as fuel spent by customers to search for parking spaces.

- Reduction in congestion caused due to cars driving around and looking for parking spaces.
- Facility to pay in automatic machines via SMS, Credit Card, which would save customers from looking for change to buy tickets at the ticketing machines.

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# Development of a Virtual Dressing System

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## Introduction

Recent years there have been an overwhelming growth in the usage of internet based systems to sell appareled based products to online customers. One of the major problems that experienced by both electronic apparel merchants and customers is the difficulty in determining how a cloth will fit to the customer. Customers are reluctant to purchase garments online because they are unsure about the exact size to order, and how that cloth will look on them. Virtual dressing room is a relatively new concept which is slowly becoming more popular. This new technology allows the customer to fit on the virtual garments on a live video model of the customer. Therefore, the customer can be certain that both the style and the fit are matched before garments are purchased online.

The need for the virtual dressing systems is becoming more essential with the rapid growth of online marketing systems. This will benefit the customers by saving the don and doff time. Also customers can order clothes online without going to a shop and try clothes. In addition customers can save lot of time that is wasted in fit on queues. Also shop owners can reduce the extra cost that is involved in fitting rooms and quality of the clothes will not be deteriorated by frequent touches by customers. Instead of operating in a limited geographical area shop owners can use virtual dressing rooms to sell their garments in the internet to a large customer base around the world.

## Methodology

This work developed an argument reality virtual dressing room system that can dress up a virtual model of the customer using image processing techniques. There are two stages in the modeling process namely, image selection and image processing. First live video stream of the customer is loaded to the image processing application which is run on a computer. In the computer interface where the video stream is running there are buttons with various functionalities like garment selection and zooming. Next the customer needs to hold and move a color tag that can be detected by the image processing software. When the customer selects the suitable garment color tag is over layered by the chosen garment. Therefore, when the customer move the color tag in the real world garment is moved left-right, front and backward in the virtual dressing room environment. Therefore, by using color tag as a guide customer can virtually dress the clothes in the virtual environment.

The proposed system used Visual Studio 2008 with openCV image processing libraries in HSV color spectrum. It is observed even a low resolution web camera can be used to input live video stream to the system. Also by using this interactive software user can zoom in out the dress in order to fit it to his body.

## Results & discussion

Subsequent testing of the system showed virtual dressing room system works at an acceptable level. Major drawback is computation and image processing time are too high and it limits the real time usage. Image processing time can be further reduced by optimizing the codes and memory usage. Computation burden is further reduced by the

fixing a single colour background in the dressing room. This will reduce the burden of detecting the colour tag and the user from the live video stream.

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# The Interactive Surface System: Concept and Development

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## Introduction

In the present digital era, the integration of natural interfaces between humans and machines is becoming more important than before. This is particularly relevant to education and business domains as the utilization of interactive tools can provide clear advantages by improving teaching practices, facilitating the comprehension of complex concepts and permitting collaborative work etc. More particularly, interactive whiteboards are gaining importance in our society, both at a business level and, especially, at educational level. However, the products offered by the major manufacturers have a major barrier to their acquisition: very high prices. There is an urgent need to use new technologies in order to provide solutions that present the same performance levels than typical interactive whiteboards, but with a significantly reduced cost.

Major drawback of projection screen is that the presenter should come to the computer to change the projected screen. This usually distracts the presenter's as well as audience's focus. While the presenter is at the computer he/she cannot simultaneously use the white board. The interactive surface system gives the solution for that drawback.

## Methodology

The interactive surface system is a system for controlling the computer remotely. This system can be used mainly in class room, lecture room, and any other place where projector is used to discuss or teach something. Design of the system is based on assigning mouse functions to finger movements. Without using mouse, user can control projected screen in front of the web cam. The main challenge of this method is environment brightness and quality of the web cam. If user can find high quality web cam and good lighting environment this system works properly.

The proposed system is based on the Open Source Computer Vision (OpenCV) library and some of the image processing techniques such as Thresholding, RGB and HSV color space etc. The system control is based on a webcam, based on color, which supports most of the mouse events such as mouse pointer movements, single click, double click.

Similar systems such as "Smart Board" have been deployed. The Smart Board is an interactive whiteboard that uses touch detection for user input – e.g. scrolling, right mouse-click – in the same way normal PC input devices, such as a mouse or keyboard, detect input. A projector is used to display a computer's video output on the interactive whiteboard, which then acts as a large touch screen.

## Discussion and conclusions

There are many advantages of using this proposed system. Firstly, the cost of this system is much lower than the existing interactive systems, because there is no much expensive equipment needed in the system as in the similar existing systems. Secondly, the key components of the system are webcam, computer and a projector. Computer and the projector are already installed in most universities\company discussion rooms and educational classes so we can easily upgrade the existing systems to the proposed

interactive system. Thirdly, for the same reason, this system is more portable than the other systems. Fourthly, there is no theoretical limit in the size of the projected image so we can use the proposed system for size and dimensions that most of the existing interactive systems cannot act. In many interactive systems, the size of image is related to size of display that is used in that system.

Having most of the advantages, this system also may have some disadvantages too; if brightness of environment is too high, the system will not operate very well and if there is an obstacle between finger and the camera, system will not be able to receive user's instructions, which is occlusion problem.

In future we can extend this system as a fully remote controlled system for the computer. From this kind of an improvement the user of the system needs to wear multiple color clips in hand and all function in computer can be handled remotely.

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