出國報告（出國類別：國際會議）

參加 2016 第 18 屆環境和水資源管理國際會議（ICEWRM 2016: 18th International Conference on Environment and Water Resource Management）

服務機關：行政院環境保護署
姓名職稱：范楓旻技士、羅偉僑技士
派赴國家：日本
出國期間：105 年 5 月 24 日至 5 月 28 日
報告日期：105 年 8 月 19 日

摘　　要

第 18 屆環境和水資源管理國際會議(ICEWRM 2016: 18th International Conference on Environment and Water Resource Management)，主要針對水資源永續利用、廢水再利用、環境影響評估、永續發展、生物科技、能源…等不同領域之議題，發表相關之研究論文，並提供廣泛的交流管道與經驗分享，其中參與對象包括學者、研究機構、公部門、商界人士…等。本次會議在日本舉行，會議主軸為環境和水資源議題，會議論文分為 17 項主題，包括水資源永續利用、廢水處理、生物科技、食品營養科學、能源電力工程、化學分子工程、人類社會科學...等。除瞭解國際水資源再利用之趨勢外，亦以展示電子海報方式，分享我國致力於落實環評監督，使水資源永續再利用之成果。

目　　次

壹、出國目的

第 18 屆環境和水資源管理國際會議(ICEWRM 2016: 18th International Conference on Environment and Water Resource Management)由科學工程與技術世界學院(World Academy of Science, Engineering and Technology, WASET)主辦，該單位為一群關注亞洲、歐洲和北美的學者與商業人士所組成之非營利組織。主要針對水資源永續利用、廢水再利用、環境影響評估、永續發展、生物科技、能源…等不同領域之議題，發表相關之研究論文，並提供廣泛的交流管道與經驗分享，其中參與對象包括學者、研究機構、公部門、商界人士…等。

本次除參與研討會外，並與其他國家經驗交流，以電子海報說明方式，分享我國致力於落實環評監督，使水資源永續再利用之成果，並期能藉由參與本次會議，瞭解國際水資源再利用趨勢。

貳、行程

日期	地點	工作內容
5 月 24 日	臺北至日本東京	啟程
5 月 25 日	日本（東京）	報到及開幕式
5 月 26 日	日本（東京）	研討會（分組論文平行會議）
5 月 27 日	日本（東京）	研討會（分組論文平行會議）
5 月 28 日	日本東京至臺北	返程

參、會議地點及內容

「第 18 屆環境和水資源管理國際會議」於西元 2016 年 5 月 25 日至 27 日於日本東京 NARITA TOBU HOTEL AIRPORT 舉行。

圖一、第 18 屆環境和水資源管理國際會議舉辦地點-NARITA TOBU HOTEL AIRPORT

圖二、第 18 屆環境和水資源管理國際會議舉辦地點-
NARITA TOBU HOTEL AIRPORT

圖三、第 18 屆環境和水資源管理國際會議舉辦地點-
NARITA TOBU HOTEL AIRPORT

本次會議包括 17 場分組論文發表平行會議，每組約 12~18 篇，共 278 篇（口頭發表 163 篇及電子海報發表 115 篇）。會議主軸為環境和水資源議題，會議論文分為 17 項主題，包括水資源永續利用、廢水處理、生物科技、食品營養科學、能源電力工程、化學分子工程、人類社會科學...等（會議論文資料詳如附錄一）。共有來自世界 20 餘國約 300 人與會，我國除本署派 2 人代表參加外，亦有國內大專院校教授、研究生及行政院原子能委員會核能研究所研究員參與會議。

圖四、本署代表參加第 18 屆環境和水資源管理國際會議

（圖中包含國內大專院校教授及原子能委員會核能研究所研究員）

肆、 會議參與情形摘述

一、 本次會議主題與專討論文

本次會議專題研討分為水資源永續利用、廢水處理、生物科技、食品營養科學、能源電力工程、化學分子工程、人類社會科學等。分組論文皆以平行會議方式進行，故須取捨參與聆聽的演講，本署代表針對水資源利用及環境影響評估之議題等論文探討，以下就各國針對相關議題探討之摘要如下：

（一）發展中國家環境影響評估之挑戰：以泰國為例，泰國之環境影響評估在西元 1970 年已開始施行，目前僅有 7 大類別開發行為須實施環境影響評估，而統計 2011 年至 2015 年共有 2418 件開發計畫通過環評（平均每年 466~596 件），惟能源、交通、石化工業及水資源開發計畫通過環評的比率卻逐漸下滑，對於發展中國家經濟發展影響甚大，另報告中分析造成通過環評比率下降因素，包括專家委員會個人因素、居民反對、利益關係人反對…等，另外私人開發計畫與政府部門開發計畫環評程序不同，例如地方政府部門環評開發案件通過後，需再送至泰國政府內閣會議決議，各種原因造成開發案件之環評成效不彰，間接影響到該國家經濟發展，故報告中說明該國家環評制度上仍有一些制度設計需再檢討改善。(詳附錄一第 32 頁)。

（二）斯里蘭卡乾旱地區之水平衡，斯里蘭卡為一典型熱帶型氣候，以氣候條件可將該國區分為 3 大區域（乾旱區、潮溼區、介於兩者中間），在乾旱區缺水情形明顯相較過去更為嚴重，甚至導致嚴重缺水危機，故作者研究該國乾旱地區(Maravanpulo Jaffna)之水平

衡，以用水與供水之關係、供水與需水之關係、居民實際用水與居民可以獲取水源之關係來分析，因該乾旱區用水來源主要為地下水（挖井），作者選該乾旱區之 3 個不同地區做為研究該區域水平衡狀況，透過水平衡（自然進流、排放、人為用水…）計算，以更精準掌握該區域用水平衡，計算該區供水與用水是否充足，可提供相關單位做為用水開發政策之參考。（詳附錄一第 69 頁）。

（三）泰國 Royal LERD 都市污水處理廠，將處理後廢水，經自然處理設計（濕地、氧化塘、草地過濾）系統後，將處理後的廢水排入海灣的濕地（泥地），本身透過自然處理設計可以節省能源及設備維護成本，此外，廢水經過自然處理後，排入海灣的濕地，當作浮游植物之營養鹽，作者在濕地監測分析土壤底泥、海水水質之重金屬濃度，低於法規規範值，另外分析該濕地文蛤，發現文蛤腸胃道存在 70.46%浮游植物（某特定物種），後續探究處理後廢水排入濕地，可以增加浮游植物所需之營養鹽，進而增加文蛤豐富度，作者藉由本研究說明利用自然工法設計處理廢水，除達到法規標準外，亦可較節省能源，另將處理後廢水排入濕地，也增加海域沿岸生態豐富度，因此，如果妥善處理廢水與再利用，對於海域生態維護也有正面價值。（詳附錄一第 36 頁）。

圖五、第 18 屆環境和水資源管理國際會議研討情形

圖六、第 18 屆環境和水資源管理國際會議研討情形

圖七、第 18 屆環境和水資源管理國際會議研討情形

圖八、第 18 屆環境和水資源管理國際會議研討情形

二、 本署發表內容

本次會議除瞭解國際水資源再利用之趨勢外，另外亦分享我國致力於落實環評監督，使水資源永續再利用之成果，本署代表以展示電子海報，分享主題為「臺灣環境影響評估制度對於珍惜水資源之貢獻」，主要展示本署 104 年度執行全國科學工業園區「環評開發案廢水及用水回收率查核專案計畫」之查核目的及成果，內容如下：

(一) Introduction －臺灣水資源不足是亟待解決的問題，然對於要求水回收及再生利用方面，卻缺乏有效並強制之法規，且固然部分法規強調用水回收率的要求，卻無具體查核執行成效的方式，實無法有效達到管制目的，為突破此一困境，本計畫尋找適合之執法工具，並精進查核作為及創建查核制度，為珍貴水資源之永續發展盡一份心力。

(二) Methodologies －臺灣的環評制度(EIA)於 1994 年建立。EIA 的目的是為了避免及減輕開發行為對環境造成之不良影響，以落實環境保護的目的。透過環評審查，可考量個案特性不同，要求企業一定比率之用水回收標準，以推動污水源頭減量兼收節水效益。為了查核企業針對廢水及用水回收率環評承諾的執行情形，以有效掌握其水資源回收比率及再利用狀況，檢視這項對環境友善措施之執行成效，本計畫邀請相關領域之專家學者講解水回收再利用相關課程，加強執法人員查核知能，並撰寫查核參考手冊，及邀請專家學者及相關機關召開研商會議完備手冊內容，做為執行查核之依據。

(三) Major findings －本計畫查核科技部 3 個科學工業園區共計 65 家

每日用水量達 1,000 噸之高科技廠，回收水量成效顯著，每年回收總量近 4 億噸，足可供全臺灣民眾用水 2.5 個月，另將回收總量換算成可供 4 種用水標的使用之量化數據，可製作 7 千 1 百億瓶 600 毫升可樂、供 17 萬座 2,500 噸之國際標準游泳池使用、灌溉 4 萬公頃稻田及相當於 1.7 座翡翠水庫蓄水量。

(四)Conclusions -量化結果在在顯示用水回收環評承諾對於珍惜水資源之重要性，亦顯示出環評制度對於環境保護之價值。於經濟開發的同時，我們亦需追求環保及經濟並進的永續發展，此為現今人類社會的主流趨勢。環評制度為追求環境永續性的手段之一，環評法令的規範要求，將對環境的傷害性減至最少，以追求水資源的永續發展。

本署代表於會議中說明查核結果顯示 3 個科學工業園區執行本項環境友善措施之執行成效高於環評承諾最低標準，其為達環評承諾之規定，持續推行之用水回收制度，已有相當顯著之成效，不僅可符合法規規定，亦可減輕開發行為對環境造成的影響，顯見落實環評承諾對於珍惜水資源是相當具有成效的制度，對水資源永續發展確有正面效益。現場係以口頭說明方式簡報成果約 5 分鐘，當日參與情形熱烈，參與者對於我國致力於取得經濟發展與環境保護平衡之努力表示贊同與肯定，其中一位參與者 R.kandiah（來自斯里蘭卡）亦表示對於環評制度亦感興趣，另有兩位參與者提問關於回收率計算公式如何訂定、如何查核、計畫執行期間、人力及成本等問題，本署則將本計畫如何自行研究訂定合宜之查核點、引用參數、計算公式及查核方式，做為執行查核依據之歷程，一一回覆說明。

圖九、電子海報發表情形

圖十、電子海報發表參與者提問情形

圖十一、電子海報發表參與者提問情形

伍、心得及建議

(一) 本次會議主軸主要聚焦在環境與水資源議題，藉由各國研究者關心的領域、環境問題解決方案及環境管理分析，可瞭解世界各國趨勢與發展。就目前趨勢來觀察，在全球水資源有限現況下，如何有效整合資源管理、發展綠色經濟、發展永續生態環境等之政策方針，並建立民眾的環境友好習慣，為目前應積極發展之方向。

(二) 聯合國於西元 2015 年 3 月 20 日發布年度報告警告，如果世界各國再不採取任何措施，世界將陷入水資源危機，而首當其衝的自然是本身就已經遭遇乾旱問題困擾的國家，由於世界各國都有嚴重浪費水資源的現象，如果照此趨勢下去，到西元 2030 年全球可用的淡水資源將減少 40%。臺灣年平均降雨量達 2,510 毫米，高於世界平均值，惟降雨分布不均，多集中於五月至十月，因此可資利用之降雨量不多，加上地形之起伏變化大，集水區地勢陡峭不易涵蓄水份，而河川源短流急，雨量豐沛時河川流量暴增，在短時間內即奔流入海，水資源不易蓄存利用，且近年來臺灣地區經濟蓬勃發展，工商業發展迅速，工業用水逐年成長，其所排廢水甚而造成水質污染問題，已降低水資源之供應量，再者，國內水價水準偏低，亦導致企業在投資時因為低水價而不一定選擇用水最省的生產技術及投資用水回收設備，因而造成重複耗水的循環，因此，在先天自然環境條件上已然不足，加以在人為影響下，水資源之調配運用更是雪上加霜，臺灣已被聯合國教科文組織列為全球第 18 缺水國家的事實，已是全民必須正視之問題，亦需大家共同努力，為臺灣的水資源永續發展共盡一份心力。

(三) 綜觀各國因全球氣候變遷的影響，除節省寶貴的水資源外，已開始拓展並推廣其他水源，臺灣水資源面臨先天自然環境水資源匱乏、用水回收再利用法源不足及用水回收率查核方式不明等三大問題，而陷入缺水危機困境，為突破此一困境，本署尋覓適合之執法工具，期望藉由環評審查，考量個案特性不同，要求企業一定比率之用水回收標準，以推動污水源頭減量兼收節水效益。本署於 104 年度執行全國科學工業園區「環評開發案廢水及用水回收率查核專案計畫」之查核成果，顯示用水回收環評承諾對於珍惜水資源之重要性，亦顯示出環評制度對於環境保護之價值。於經濟開發的同時，我們亦需追求環保及經濟並進的永續發展，此為現今人類社會的主流趨勢。環評制度為追求環境永續性的手段之一，環評法令的規範要求，將對環境的傷害性減至最少，以追求水資源的永續發展。

(四)本署 104 年 7 月 3 日已修正發布「環境影響評估法施行細則」，除建立更廣泛與開放的公民參與程序，亦加強公眾與目的事業主管機關之對話，強化目的事業主管機關扮演之角色 ，對於環評審查與環評監督業務具有正面的助益。本署精進查核作為，期藉由革新執法作為，從水資源不足的這片臺灣旱地裡守護藍精，以改善臺灣水資源環境，未來將進一步分析並歸納查核成果，以提出建議供未來環評審查階段針對用水回收環評承諾決議之參考，將執行環評監督所獲得之成果及經驗回饋至環評審查端，冀對於我國環評制度之發展亦能有所助益。

附錄一、會議論文資料

Organic Substance Removal from Pla-Som Family Industrial Wastewater through APCW System

W. Wararam, K. Angchanpen, T. Pattamapitoon, K. Chunkao, O. Phewnil, M. Srichomphu, T. Jinjaruk

***Abstract*—**The research focused on the efficiency for treating high organic wastewater from pla-som production process by anaerobic tanks, oxidation ponds and constructed wetland treatment systems (APCW). The combined system consisted of 50-mm plastic screen, five 5.8 m^3 oil-grease trap tanks (2-day hydraulic retention time; HRT), four 4.3 m^3 anaerobic tanks (1-day HRT), 16.7 m^3 oxidation pond no.1 (7-day HRT), 12.0 m^3 oxidation pond no.2 (3-day HRT), and 8.2 m^3 constructed wetland plot (1-day HRT). After washing fresh raw fishes, they were sliced in small pieces and were converted into ground fish meat by blender machine. The fish meat was rinsed for 8 rounds: 1, 2, 3, 5, 6 and 7 by tap water and 4 and 8 by rice-wash-water, before mixing with salt, garlic, steamed rice and monosodium glutamate, followed by plastic wrapping for 72-hour of edibility. During pla-som production processing, the rinsed wastewater about 5 m^3/day was fed to the treatment systems and fully stagnating storage in its components. The result found that, 1) percentage of treatment efficiency for BOD, COD, TDS and SS were 93, 95, 32 and 98 respectively, 2) the treatment was conducted with 500-kg raw fishes along with full equipment of high organic wastewater treatment systems, 3) the trend of the treatment efficiency and quantity in all indicators was similarly processed and 4) the small pieces of fish meat and fish blood were needed more than 3-day HRT in anaerobic digestion process.

***Keywords*—**Organic substance, Pla-Som family industry, wastewater, APCW System.

I. Introduction

KWAN Phayao is one of three natural lakes in the north of Thailand which is important reservoir to provide resources for local consumption and serve livelihood. Moreover, another function is irrigation for support paddy rice growing in downstream areas and be water resources for waterworks. Nowadays, the water problem was shined on quantity, quality, and timing due to siltation, wastewater from community, unbalancing of aquatic plants to herbivore fish growth rate and invasion of water hyacinth, which make a long cut in using Kwan Phayao benefits, polluted reservoir, low fish productive resources and low water quality for waterworks.

Pla-som or traditional fermented fish product is the local preserved and protein food which have been cooked for very long period of time owning to natural abundance of scale fishes living in Kwan Phayao lake. According to delicious food, the production was begun from family consumption and gradually spread to another northern province, finally to all parts of the country [1]. Unfortunately, the producers did not know the consequent problems of high protein contaminant in wastewater for pla-som producing process [2] which drained directly to Kwan Phayao lake. Although, some producers tried to treat wastewater from producing process, but they do not know how to treat it, so water resources increasingly turning to worsening quality. Noticeably, water quality of Kwan Phayao lake has been identified as water surface quality standards on third to fourth levels (BOD equivalent between 2.5-4.0 and 4.0-6.0 mg/L) after research team made primary survey. Those results were a little far from raw water quality requirement for waterworks but it was on border line for survival of aquatic lives. Accordance with planning construction of sewer systems, Phayao municipal sewerage is expectedly drained out at downstream a few kilometers from Kwan Phayao lake outlet (fish ladder of Fisheries Department Station), the point sources of wastewater mainly come from pla-som family-industrial factories which exist more than 30 factories and tendency increase without treating wastewater care. Consequently, wastewater from pla-som production process has to be treated before releasing into Kwan Phayao lake in order to keep water resources clean for serving any purposive consumption, especially aquatic lives surviving.

Pla-som, one kind of traditional fermented fish and sometime called sour fish, has been made since in the ancient time for preserving fishes as the protein food. In the past, pla-som used to be necessary traditional preserved food like pla-ra, pla-jom, pla-kem (salted fish), pla-haeng (dried fish) and even bamboo. As take away protein food for the farmers and travelers whenever they were away from homes for their missions [1]-[7]. Among those preserved fish, pla-som is the most favorite protein food due to sour taste which is accepted as good appetizer as well as easiness in cookery. Normally, pla-som could be cooked by mixing raw slice-off fishes (scale fishes preferable) with garlic, salt, steamed rice, and a pinch of seasoning powder along with fresh banana leaf for 4 days [8]-[12]. In doing so, there was a small number of fish scarps, solid waste and wastewater (for washing raw fishes) in which they did not evidently pollute to the environment due to only home-made production, low-populated density, and biotic potential surroundings at that period. However, the pla-som production process could be encouraged to increase all kinds of pollutants in spreading in the environment, especially wastewater. In the same manners, lakefront people around have modified pla-

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som cookery instead of slice-fish to small piece together with rinsing by water of washing rice for adding up sour taste before grinding fine-textured fishes by manual. Certainly, wastewater plus the contaminants of fish scarps, blood, and fats are produced during the production processes which are surely composed of high nitrogen from protein in fish meat, phosphorus and iron as same as another elements from blood, and oil and grease from fats [13]-[15].

In principle, the high concentrated community wastewater is usually treated by anaerobic processes in order to decrease the organic matters, especially high content of nitrogen and phosphorus, down to standard values before releasing into public water sources and the length of hydraulic retention time (HRT equivalent to volume anaerobic tank divided by influent flowrate) plays vital role in treatment [16]-[21]. In other words, longer HRT is high treatment efficiency [16], [22]-[24]. If the concentration of contaminants is not decrease in effluent standards, the oxidation pond technology should be used for effluent retreating in order to allow hydrogen sulfide, ammonia, methane, carbon dioxide, and others moving up to sky, while sinking oxygen in above air can penetrated into bottom by photosynthesis and thermo-siphon processes [16], [25]-[29], together with growing water hyacinth in bamboo-frame raft for 20% of oxidation pond surface [30]-[31]. If the retreated effluent quality is still over standards but BOD has to be less 300 mg/L, the constructed wetland (either one or both vertical and horizontal flow) is recommended to absorb nitrogen and phosphorus for decreasing down to standards before drain into public streams [32]-[37]. Accordance with research in action is requirement to eliminate contaminants in high concentration of fresh organic matters both the solid and liquid states through anaerobic digestion processes which are expected consuming more HRT period. So, it has to bear in mind in all-time HRT that anaerobic processes are characterized by completing three-consecutive biochemical reactions: firstly, hydrolysis as the process of bacterial enzymes breaking and dissolving complex organic compounds (particularly glucose and amino acid) into simple compounds; secondly, acidogenesis as the process of bacterial converting simple compounds into substrates for methanogenesis, and also acetate, formate, hydrogen, carbon dioxide, hydrogen sulfide, ammonia and organic acids; and finally, methanogenesis as the process of bacterial converting methanogenic substrates into methane, carbon dioxide, and water under sensitive action to pH less than 6.5 and high pH more than 8 [4], [13], [16]-[17], [20], [24], [38].

The aforesaid information is expectedly applicable for high concentrated wastewater treatment from pla-som production process by using grease trap, anaerobic tanks, oxidation ponds and constructed wetland before using aqua-culturing herbivore fish pond and growing edible-leaf and fruit vegetables. This experimental research expected not only to find out effectively anaerobic technology system in treating high organic concentrated wastewater in cases of 500-kg pla-som production processes but also use this research knowledge to construct the handbook for high-organic-content wastewater treatment and use to train the owners of pla-som production factory to know how to treat wastewater in order to improve water quality of Kwan Phayao lake and surviving aquatic lives.

II. Materials and Methods

The experimental system was comprised of one 50-cm plastic screen, five 1.2-m ferro-concrete-circle tanks in using for five oil-grease trap tanks and four anaerobic tanks, 16.7-m^3 first oxidation pond, 12m^3 second oxidation pond, and 8.2-m^3 plant filtration plot, as shown in Fig. 1. An application program was illustrated in Fig. 2 which started up with wastewater obtaining from rinsing water of carp fishes (*Hypophthalmichthys nobilis*) before and after slicing to separate fish meat plus skin and by-products (heads, intestines, fishbone, skeletons, and scales). Then after, the sliced fish meat and skin were ground into fine texture by blender in which the fish meat was required to rinse for 8 rounds: rounds1, 2, 3 and 5, 6, 7 washing by tap water, and specifically rounds 4 and 8 washing by rice-wash water. In experimental point of views, the 100-kg fish meat need 5.2-kg garlic, 4.4-kg salt, 1.7-kg steamed rice and 1.6-kg monosodium glutamate. The ingredients were mixed together before wrapping by plastic bag or fresh banana leaf, and sending to wholesaler for selling on cash. For by-product benefits, fish head was sold out for delicious boil rice cooking, intestines directly using for raising ducks, while fishbones, skeletons, and scales were ground to make tablet food for raising catfishes. For focusing on this research paper, it must be paid more attention on finding out HRT period for treating on high concentrated-content wastewater from pla-som production process which presented in Figure 1 and 2 in terms of production processes from starting up until to water body of Kwan Phayao lake.

Treatment type	treatment	
	volume (m^3)	HRT (day)
oil & grease trap	5.8	2
anaerobic tank	4.3	1
oxidation pond1	16.7	7
oxidation pond2	12	3
constructed wetland	8.2	1
total	47.0	14.0

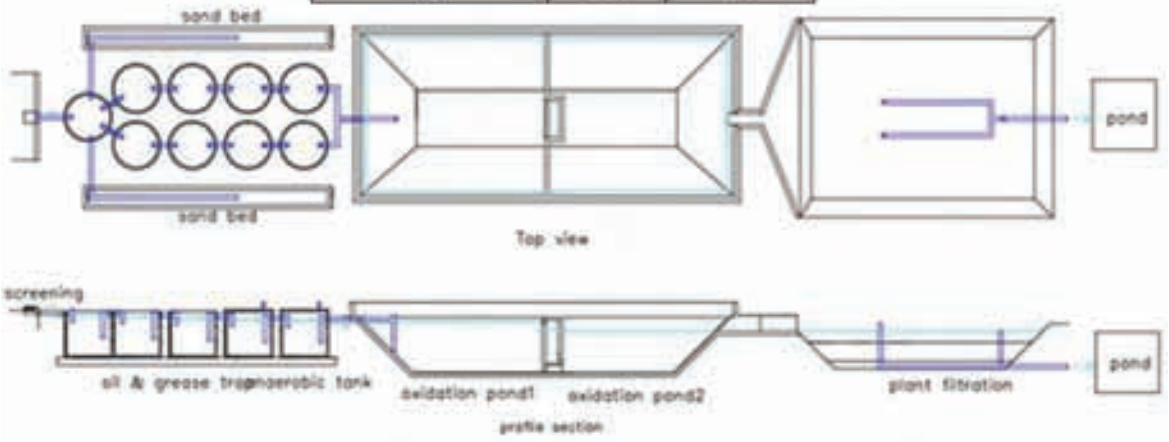

Fig. 1 Schematic illustration of onsite treatment system for high concentrated wastewater from pla-som production factory at Kwan Phayao lake, Phayao province, Thailand

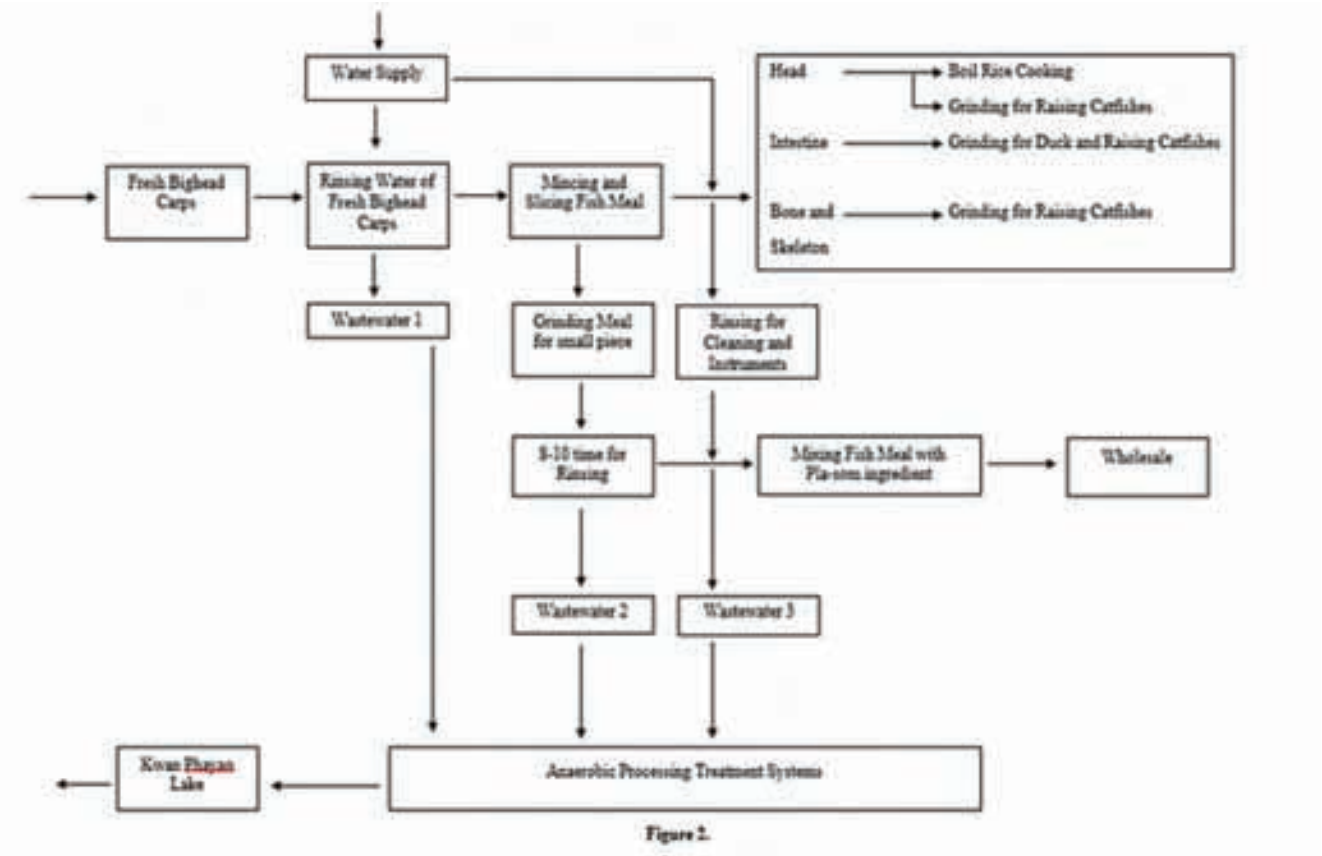

Fig. 2 Flow chart of onsite treatment system for high concentrated wastewater from pla-som production factory at Kwan Phayao lake in northerly of Thailand

Following APHA, AWWA, WEF [39], water samples were taken at outlets of production process, screen, before and after oil and grease traps, outflow from anaerobic ferro-concrete circular tanks, 30-cm and 0.8-h depths of oxidation ponds no.1 and 2, constructed wetland at top, middle, tip, and outlet. Water samples were analyzed on the water quality indicators of temperature, pH, DO, EC, salinity, TDS, SS, BOD, COD, TOC, FOG, TKN, NH_3, TP, and PO_4^{3-}.

Owning to find out the hydraulic retention time (HRT), the experiment was divided into phase by phase until the final effluent as drained out from the treatment system met the standard values under the various adding conditions of anaerobic organic digestion process. In doing so, there may be taken in more time because of high concentrated wastewater from pla-som production process which found from primary analysis, especially BOD at higher than 10,000 mg/L.

III. Results and Discussion

Since the determining HRT of anaerobic digestion processing system for high concentrated wastewater was specified before conducting on experiments in order to obtain the end products of effluent water quality indicators under standards. The details as presented in the following sections.

An application of 14-day HRT efficiency was utilized only nine-ferro-concrete circle tanks, five for oil and grease trap tanks and another four anaerobic tanks (Fig.1). In principles, the water supply is utilized for three main rinsing activities i.e. cleaning fresh fishes (averaging 60 L/100-kg fishes), washing off fish meat (averaging 800 L/100-kg fishes), cleaning floor and instruments (averaging 140 L/100-kg fishes). According to research requirement to determine the HRT for anaerobic organic digestion processes inside anaerobic tanks under stagnating condition, the amount of 5 m^3/day was used for washing fresh fishes (totally 500 kg) and fish meat which drained out by gravitational force to fill in full storage 47 m^3 for five oil and grease trap tanks 5.8 m^3 (2-day HRT) before storing in four anaerobic tanks 4.3 m^3 (1-day HRT) then moving to oxidation pond one 16.7 m^3 (7-day HRT) oxidation pond two 12.0 m^3 (3-day HRT), and constructed wetland 8.2 m^3 (1-day HRT), and in accumulative 14-day HRT as shown in Fig. 1, and the full scheme of processing production system as presented in Fig. 2.It was visualized by eyes during researching in action that the production process produced high contents of oil-grease, fish blood and small pieces of fish meat mixing in concentrated wastewater after taking pla-som cookery processes from production area. However, the results of experiment were presented in Table I which were provided to the conditions for treating efficiency of high organic-concentrated wastewater from pla-som production process as shown in Table II.

TABLE I
Treatment-One Water Quality as Significant Conditions for Providing Treatment Potentiality of High Organic-Concentrate Wastewater from Pla-Som Production Processes of Family-Industrial Factory at Kwan Phayao, Phayao province, Thailand

Sampling Point	Ambient T (°C)	Water Quality Indicator				
		T (°C)	pH	DO (mg/L)	EC (µS/cm)	Sal (g/L)
Effluent of Production Process	26.0	24.3	6.3	0.00	2,760	1.3
Effluent of Screening	26.0	25.9	6.5	0.00	3,730	1.0
Influent of Oil and Grease Trap Tanks	26.0	26.4	6.6	0.00	3,650	1.1
Effluent of Oil and Grease Trap Tanks	25.3	27.8	6.2	0.06	2,433	1.2
Effluent of Anaerobic Tanks	24.7	28.3	6.4	0.06	2,650	1.4
-30cm Depth of Oxidation Pond 1	23.9	28.9	6.5	0.10	3,093	1.6
0.8 h Depth of Oxidation Pond 1	23.9	26.4	6.6	0.11	3,053	1.6
-30cm Depth of Oxidation Pond 2	23.8	26.6	7.1	0.07	2,733	1.4
0.8 h Depth of Oxidation Pond 1	23.8	26.1	7.2	0.05	2,790	1.4
Constructed Wetland) Top,1-m(	23.8	26.1	7.5	0.00	2,423	1.2
Constructed Wetland)middle,3-m(	23.8	26.4	7.5	0.00	2,406	1.2
Constructed Wetland) Tip,5-m(	23.8	26.6	7.5	0.00	2,108	1.1
Effluent of Constructed Wetland	23.8	27.3	7.3	0.06	2,193	1.1
Water Quality Standard	-	40.0	5.5-9.0	-	-	-

Remarks: Measurement of ambient air temperature at 07.00 am. T = Temperature, DO = Dissolved Oxygen, EC = Electrical Conductivity, Sal = Salinity

It is quite clear that the ambient temperature were placed on the narrow ranges of 2.2 °C (between 23.8-26.0 °C) which is supposed to biochemical reaction influence of both the anaerobic and aerobic processes inside the fermented tanks and oxidation ponds as same as the growth of Typha aquatic plants (*typha angustifolia* Linn.) as grown in the constructed wetland. Salinity which ranged 1.0-1.6 µS/cm seemed to take an action on biochemical processes by inhibiting microorganisms for digesting small pieced of fish meat in terms of slowing down the process and taking more HRT periods. Expectedly, there were some gases emitting to the open area due to mostly digestion was not reach at the end of biochemical processes (methanogenesis) but it could be on hydrolysis and acidogenesis processes [10], [13], [16], [22], [29], [38]. Furthermore, the values water quality indicators in Table I were influenced in minor negative biochemical interactions, they were temperature, pH, EC, and DO in all instruments of treatment system [2]-[3], [5], [9]-[10], [14]-[18], [20], [38], [40]-[41]. In other words, the water quality indicators in Table I could be pointed out that they were in minor impacts on treatment efficiency due to

dilution of water supply as used for washing fresh fishes, fish meat, and cleaning floor and instrument. Besides salt as one of the ingredient of pla-som cookery, it also was added garlic for serving the purpose of smell, taste, and physical healthcare but it plays vital role in inhibiting bacterial decomposers as found in wastewater from pla-som production process [6], [10]-[12]. Luckily, washing water was supported the dilution of garlic content in wastewater that should not strong enough to kill all species of microorganisms.

TABLE II
WATER QUALITY OF TREATMENT ONE IN QUALITATIVE VALUES AND TREATMENT EFFICIENCY IN PERCENTAGE UNDER THE CONDITION OF CONSECUTIVELY FLOWING FROM PLA-SOM PRODUCTION AREA TO THE 50-MM PLASTIC SCREEN, 5 OIL AND GREASE TRAP THANKS, 4 ANAEROBIC TANKS, OXIDATION PONDS1 AND 2, AND CONSTRUCTED WETLAND

Sampling Point	Water Quality Indicator (mg/L)									
	TDS	SS	BOD	COD	TOC	FOG	TKN	NH_3	TP	PO_4^{3-}
Effluent of Production Process	2,208	11,963	10,633	20,200	2,874	1,878	274	286	22	19
	-	-	-	-	-	-	-	-	-	-
Effluent of Screening	2,984	10,706	9,633	19,850	1,654	1,566	528	270	24	27
	(-34)	(11)	(9)	(2)	(42)	(17)	(-93)	(6)	(-10)	(-44)
Influent of Oil and Grease Trap Tanks	2,920	2,396	3,710	6,720	930	336	235	256	15	66
	(-32)	(80)	(65)	(67)	(68)	(82)	(14)	(10)	(31)	(-245)
Effluent of Oil and Grease Trap Tanks	1,629	253	1,518	2,158	513	32	226	265	31	61
	(26)	(98)	(86)	(89)	(82)	(98)	(18)	(7)	(-42)	(-223)
Effluent of Anaerobic Tanks	1,783	247	1,289	1,403	612	29	215	243	30	121
	(19)	(98)	(88)	(93)	(79)	(98)	(22)	(15)	(-38)	(-538)
-30cm Depth of Oxidation Pond 1	2,070	122	1,216	1,828	855	16	261	209	24	23
	(6)	(99)	(89)	(91)	(70)	(99)	(5)	(27)	(-8)	(-23)
0.8 h Depth of Oxidation Pond 1	2,046	101	980	1,905	856	14	259	197	33	23
	(7)	(99)	(91)	(91)	(70)	(99)	(5)	(31)	(-49)	(-23)
-30cm Depth of Oxidation Pond 2	1,845	120	897	1,435	733	9	231	183	23	21
	(16)	(99)	(92)	(93)	(74)	(100)	(16)	(36)	(-4)	(-12)
0.8 h Depth of Oxidation Pond 1	1,835	231	965	1,602	741	11	243	162	48	86
	(17)	(98)	(91)	(92)	(74)	(99)	(11)	(43)	(-120)	(-351)
Constructed Wetland) Top,1-m(	1,614	264	764	1,150	607	3	188	145	27	101
	(27)	(98)	(93)	(94)	(79)	(100)	(31)	(49)	(-22)	(-432)
Constructed Wetland)middle,3-m(	1,629	144	970	1,167	600	4	183	168	18	97
	(26)	(99)	(91)	(94)	(79)	(100)	(33)	(41)	(17)	(-410)
Constructed Wetland) Tip,5-m(	1,432	152	765	951	401	3	131	158	23	64
	(35)	(99)	(93)	(95)	(86)	(100)	(52)	(45)	(-5)	(-236)
Effluent of Constructed Wetland	1,499	256	780	1,097	547	2	157	98	11	87
	(32)	(98)	(93)	(95)	(81)	(100)	(53)	(66)	(50)	(-357)
Water Quality Standard	3,000	50	20	120	-	5	100	-	-	-

Remarks: Numbers in parenthesis identified as percentage of treatment efficiency. TDS = Total Dissolved Solids, SS = Suspended Solids, BOD = Biological Oxygen Demand, COD = Chemical Oxygen Demand, TOC = Total Organic Carbon, FOG = Fat, Oil and Grease, TKN = Total Kjeldahl Nitrogen, NH_3 = Ammonia, TP = Total Phosphorus, PO_4^{3-} = Ortho Phosphate

The original wastewater was directly obtained from the pla-som production processes inside the factory as shown in Table II. They were found as follows, TDS 2,208 mg/L, SS 11,963 mg/L, BOD 10,633 mg/L, COD 20,200 mg/L, TOC 2,874 mg/L, FOG 1,878 mg/L, TKN 274 mg/L, NH_3 286 mg/L, TP 22 mg/L, and PO_4^{3-} 19 mg/L. Those values could be accepted in extremely high concentration and so difficulty to treat them by nature process but they were really needed not only anaerobic digestion processing tanks but also oxidation ponds aerobic process through thermo-siphon process and phytoremediation techniques through thermo-osmosis process [3], [26]-[28], [32]-[37], [42].

The study on consecutive decreasing and increasing as indicated by water quality in Table II found that the end products were extremely decrease but they were still out of standard values. Also, an unpleasant smells of gases emitted from wastewater treatment system that felt annoyed to surrounded people in working and sleeping. In fact, the most important role in the whole anaerobic treatment system was placed on the efficiency of anaerobic ferro-concrete circle tanks due to the existence of its function. By those reasons, reconsideration of treatment trends at the anaerobic outlet was pointed out that the decreasing values were high efficiency such as 19% for TDS, 98% for SS, 88% for BOD, 93% for COD, 79% for TOC, 98% for FOG, 22% for TKN, 15% for NH_3, -38% for TP, and -538% for PO_4^{3-} as shown in parenthesis of Table II. It would be stressed that the percentages were satisfied efficiency but the amount of water quality values were found still very high above the standards. It should be noted that nitrogen content varied in various forms as same as happening on total-P and phosphate due to quantitative blood in wastewater and also the area of onsite wastewater treatment being used for raising ducks and hens for long time. Such soil contaminants and existing blood as well as other organic matters could provide the variation of nitrogen, total-P and phosphate forms one way or another. However, the results were satisfied with treatment efficiency but the quantitative values were not accepted because they did not meet the standards of effluent quality.

The research observed more water existence in pla-som wastewater contaminating with small pieces of fish meat. It did not enhance an increasing digestion rate, because very small pieces of fish meat and oil-grease were still needed longer HRT for completion of bacterial organic digestion

process [4], [10], [13], [18]-[20], [22], [23], [30], [38]. Eventually, treatment cannot treat lower concentrated-organic contaminants both small pieces of fish meat and fish blood in pla-som production process wastewater by using raw fishes 500 kg along with 2-day HRT in oil-grease trap tanks and 1-day HRT in anaerobic tanks as well as 7-day HRT in oxidation pond first, 3-day HRT in oxidation pond second, and 1-day HRT in Typha constructed wetland. In principles, small pieces of fish meat and blood are required to convert them from organic matters to inorganic materials through anaerobic process rather than aerobic processes. So, only 3-day HRT (2-day HRT in oil-grease trap tanks and 1-day HRT in anaerobic tanks) were implemented for converting the small pieces of fish meat and fish blood into inorganic materials but it could not decrease wastewater quality to meet the effluent water quality standards after draining out from Typha aquatic plant constructed wetland. In order to applicability for high concentrated-organic contaminants, treatment-next experiment is recommended to conduct by increasing HRT period for anaerobic process, for implementing, the oxidation pond one should be covered for anaerobic process which provides another 7-day HRT for digesting small pieces of fish meat and oil-grease contaminants without any doubts.

IV. Conclusion

High concentrated organic content in wastewater from pla-som family industrial factory was treated under the combination of anaerobic process, oxidation ponds, and constructed wetland by dividing the experiment, the results as followed:

1. The 500-kg raw fish usage for pla-som cookery produced wastewater with the contaminants of TDS 2,208 mg/L, SS 11,963 mg/L, BOD 10,633 mg/L, COD 20,200 mg/L, TOC 2,874 mg/L, FOG 1,878 mg/L, TKN 274 mg/L, NH_3 286 mg/L, TP 22 mg/L, and PO_4^{3-} 19 mg/L. After treating with 2-day HRT for 5 oil-grease trap tanks, 1-day HRT for 4 anaerobic tanks, 7-day HRT for oxidation pond one, and 3-day HRT for oxidation pond two and 1-day HRT for constructed wetland, the treating efficiencies were 32% for TDS, 98% for SS, 93% for BOD, 95% for COD, 81% for TOC, 100% for FOG, 53% for TKN, 66% for NH_3, 50% for TP, and -357% for PO_4^{3-}.
2. The findings were satisfied in terms of efficiencies but the experimental values of effluent quality did not meet the standards.
3. Accordance with small pieces of fish meat and fish blood were really needed an organic digestion processes in anaerobic tanks. Dilution of wastewater did not enhance rate of organic digestion process either in anaerobic tanks or in oxidation ponds. Only prolonging 7-day HRT to 10-day HRT is very necessary for converting small pieces of fishes and blood into inorganic materials for high concentrated wastewater from pla-som production process.

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Open Lids and Watering into Concrete Box Technology for Enhancing Aerobic Process to Digesting Organic Waste in Producing High Content of Solid Compost

C. Chanthasoon, K. Chunkao, S. Thaipakdee, N. Semvimol

***Abstract*—**Composting of municipal solid waste in concrete box is the applied landfill technology. This study tried to determine the effect of application of artificial rainwater on the change in heat of compost in the concrete box. Factors and appropriate condition for composting and quality of compost were also studied. Treatments were applied the quantity of water at 0, 60, 120 and 180 liters as artificial rainwater. The result indicated the heat generated in each concrete box which attributed to the decomposition activity of microorganisms and the temperature showed peak at the first week of composting process. Thereafter, the temperature changed responds to microbial activity level in concrete box, and temperature declined at the end of composting. Besides, the result shows that the best time to measure temperature was suitable in the morning due to the effect of atmospheric heat because the solar radiation is the lowest at this time. In terms of microbial activity in concrete box, the study revealed that artificial rainwater application amount 60 L was the best appropriate condition for microbial activity. In case of rain, temperature in concrete box declined during the first 4-5 hours after the rain and it increased up to optimal condition thereafter. This is due to heat generated from decomposition activity of microorganisms and soil that acted as a temperature change buffer. Compost qualities were analyzed and reported as average values from all treatments. Percentage of nutrients were 0.23-0.37 for nitrogen, 0.04-0.06 for phosphorus, and 1.29-1.46 for potassium. C/N ratio ranged from 10.86 to 14.86 and moisture percentage content ranged from 54.75 to 59.64. Most values were below the standard value of compost which set by the Land Development Department. However, this compost can be used to increase soil porosity and as supporting agent. In terms of heavy metal, the compost contains 0.002 ppm of cadmium and 0.001-0.003 ppm of lead, which were lower than standard value set for heavy metal in compost by Germany.

***Keywords*—**Rainwater falling, concrete box technology, organic waste, compost.

I. Introduction

COMMUNITY garbage disposal has been popularly used sanitary landfill before the country confronted with its problem, approximately more than 30 years, due to day-to-day dumping them into the big holes without separate the organic wastes, recycled and hazardous wastes into their parts. There have been a lot of problems from none-separated community garbage that could be various disease carriers, toxic leachate, visual pollution and losses of good land for agriculture. In principles, the recycled garbage can used as raw materials for production the industrial products through recycled technology, while the hazardous wastes have to eliminate by specific technology and requirement before, during and after disposing them. In part of organic wastes are normally disposed by landfill rather than burning in common furnace or in incinerator because of too high moisture content to be burnt. Better way to eliminate community organic wastes is to make compost which convert organic solid, liquid, and gases into maturing compost as useful fertilizer for plant growth. Actually, it has been estimated that Thai people generate the community garbage about 15.1 million tons/year (record in 2009) for the whole country, and for Bangkok 10,000 tons/day or 3.5 million tons/year (record in 2014). By these garbage generating, it is generally composed of organic wastes between 40-55% of total garbage per day which had better used for organic compost rather than making dirty urban areas. [1]-[6]

In general, community garbage disposal is utilized the common landfill method by digging a hole with removing soil and sand before throwing the garbage in it. The said method is not faced on problem if it conducts in the rural areas owning to plenty land areas in forming a hole for bacterial organic digestion process under soil surface, but it is hard to find soil space in the city. According to the previous reason, The King's Project designed the concrete box technology (RCB model) which was created for community garbage disposal which can be successfully used for any areas either limit soil space or not in the households, community, villages, municipals and others. It has been accepted the RCB model that can applicable in all parts of Thailand.

In reality, organic wastes are composed of food scarps that might be included rice, fish meat and fishbone, shrimps, beef, shells, muscles, vegetables and its root, flowers and fruits, chili and chili sauce and candy, in which their properties are favored with specific micro-organic decomposers, their quantity, appropriate environment and periods of conversion processes through organic digestion processes [7]-[10].

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Anyhow, the temperature change in concrete box seems to influence on psycrophilic, mesophilic, or thermophilic bacteria for mixing organic wastes. Without any doubt, the proportion of organic wastes play a significant role in the conversion rate of fermenting process for pertaining to the composting quality. Theoretically, the concrete box is required an aerobic process for organic digestion which provides more solid products (compost), while an anaerobic process organic digestion provides less solid product due to non-metal elements (mostly N, C, S) in organic wastes transforming to gases such as ammonia, nitrogen oxides, methane, hydrogen sulfide, etc. Certainly, all gases are not only to decrease the solid-product compost but also to create the bad smells which push out from inside the concrete box. In theoretical point of view, the digestion process is really needed some amount of water to moisten the organic garbage for stimulate fermenting process. There is no data available of moisture level in garbage digesting process to reach the compost maturity. This is the basic need for conducting the research on amount of water and proper ranging periods for watering and water quantity (both water and amount of rainfall) as well. So, the research paper focused on appropriate condition for composting by study application effect of artificial rainwater on the heat change in compost in the concrete box and also studied the quality of compost.

II. Method and Procedure

A. Project Site

The King's Royally Initiated Laem Phak Bia Environmental Research and Development Project (LERD) is located at Laem Phak Bia Sub-District, Ban Laem District, Phetchaburi Province, about 16 km from Phetchaburi municipal. The project site is placed behind the second growth of mangrove forest which settles along mud beach adjacent to the Gulf of Thailand far from Bangkok 160 km, about 2-hr drive as shown in Fig. 1. The LERD project is aimed to study, research and development on community garbage disposal and wastewater treatment under the King's initiation on nature-by-nature processes, simplicity technology and possible using local materials. This paper will focus only on community garbage disposal by RCB technology as described in the following section.

B. Construction of Community Garbage Disposal Technology

The construction of community garbage disposal technology was concrete box with the size of 3-m length, 2-m width, and 1.5-m depth in which the accommodation of 2-ton fresh organic waste as well as one hole at the box bed on slope of 1:1,000 scale as shown in Fig. 2.

Fig. 1 The project site locations in Phetchaburi province

C. Experimental Design

The experiment was planned to take randomized complete design as the main plan for launching the research on making compost as by-product of community organic garbage under nature-by-nature processes along with concrete box technology. There are 12 concrete boxes for conducting research on how to manage community garbage. The randomized complete design is mainly expected to take 4 treatments and 3 replicates in determining amount of watering related to compost maturity, community garbage compost quality and heavy metal contamination. The layout of 12 concrete boxes for conducting the research on community garbage disposal can be seen in Fig. 2.

Due to wet climate in Thailand, the concrete box opening would be convenient for garbage composting. At the same time the bacterial digesting in concrete boxes are needed to keep in consistent processes without interruption of rainfall. Therefore, the amount of watering has to determine in order to take it for easy practicing to users. However, the experiment was applied randomized complete box design for 4 sets of watering 0, 60, 120, and 180 L with 3 replications by applying 2 tons of 3 level sorted garbage from the surface to the bottom about 660, 670, and 670 kg respectively, and topping with paddy soil 15 cm at the surface, 5 cm at the middle and 5 cm at the bottom which is paved sand to protect leachate leakage as shown in Fig. 2.

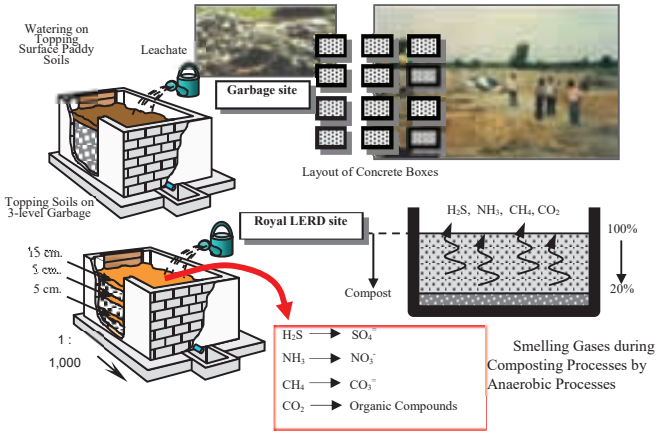


Fig. 2 the characteristics of concrete box technology and randomized complete block design

The watering operation worked every 7 days at 0, 60, 120, and 180 L at 7am until throughout 45 days of experiment. The temperature was measured every day at all 3 levels of paddy soils at 7 am, 1 pm, and 5 pm. In consequence, measuring temperature in-concrete boxes took place every hour a day when the composting period was completely on 7, 14, 21, and 28 days. Moreover, watering time was at 7 am, and temperature measurement began before and after 12 hours on every 7 days of composting time.

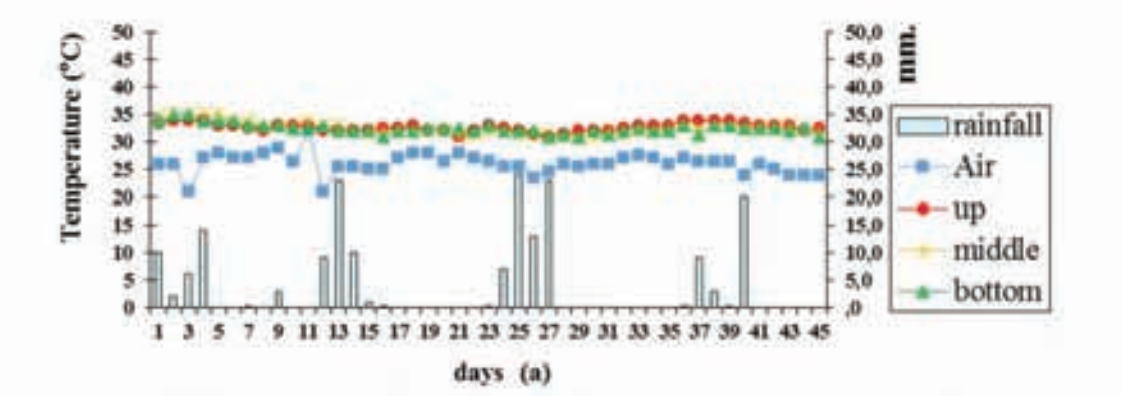
III. Results and Discussion

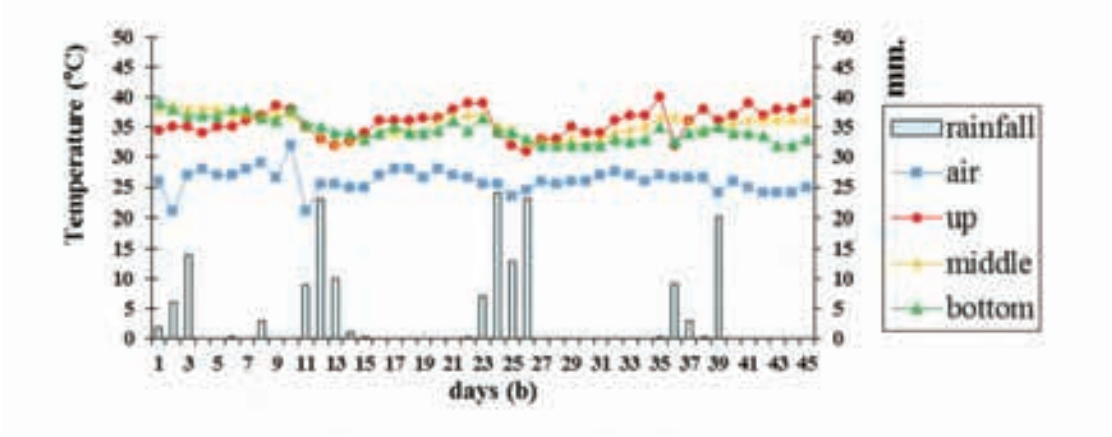
According to the community garbage disposal technology belonged to the King's initiation under the nature-by-nature processes alongside to simple technology in order to take local materials to fulfill prior principles. Thus, this paper intended to describe how to dispose the community garbage by the concrete box technology with willingness. In addition, the contents sequence had to arrange in the following orders:

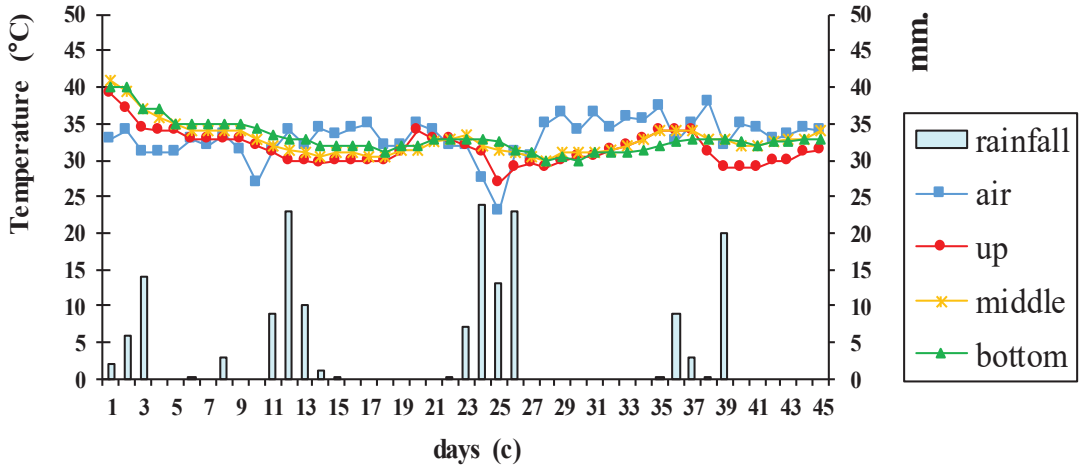
A. Amount of Water for Making Moisture in Garbage Disposal

The experiment was conducted at the Royal LERD project site by using water amount of 0, 60, 120, and 180 liters on every 7 days for 4 treatments and 3 replications (3 boxes of each treatment) for the whole period of 45 days as shown in Fig. 3. After statistical analysis, there were not different temperature (surface, middle and the bottom of concrete boxes) among of amount watering at 0, 60, 120, and 180 liters every 7 days. For convenience of working, the 60-L watering every 7 days has been selected for moistening garbage inside the concrete boxes in order to accelerating the organic garbage digestion to obtain the completed compost.

Owing to open lid of the concrete box was preferable technology for garbage composting, rain falling in the concrete boxes could not avoid. Unlimited water to wet the organic garbage might affect to the bacterial digesting processes one way or another [6]-[7]. Throughout 45-day experiment found that there were not effects of amount and duration of rainfall to garbage temperature on surface, middle and bottom of concrete boxes. Only 4 to 5 hours during and after rain falling showed the decrease of garbage temperature, then it gradually increases together with releasing heat due to the bacterial organic process occurring after adjusting them to wet condition as shown in Fig. 3. Normally, rainwater is cooler than 15 °C in the central part of Thailand, and also released heat according to both aerobic and anaerobic processes were above 60 °C. When they mixed together, the garbage temperature had to down below 50 °C as seen in Fig. 3.




Temperature (°C)
mm.
rainfall
air
up
middle
bottom
days (b)


Temperature (°C)
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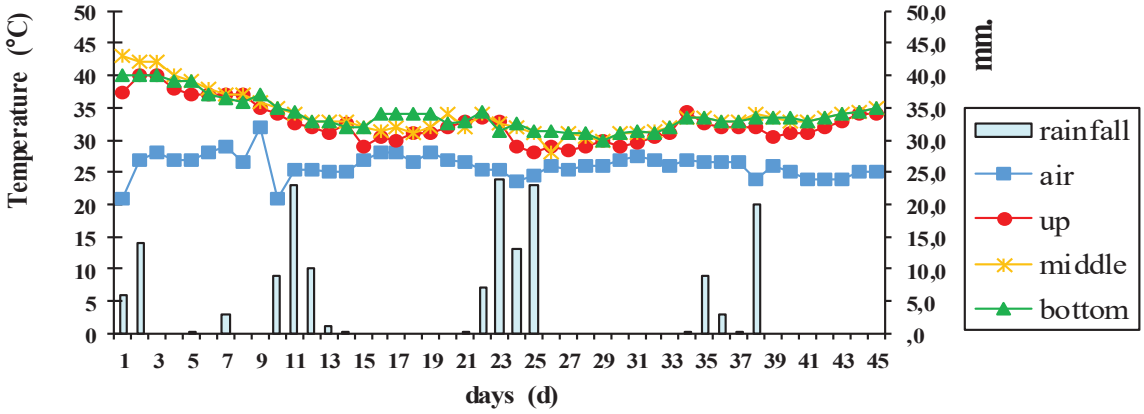
Temperature (°C)
mm.
rainfall
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up
middle
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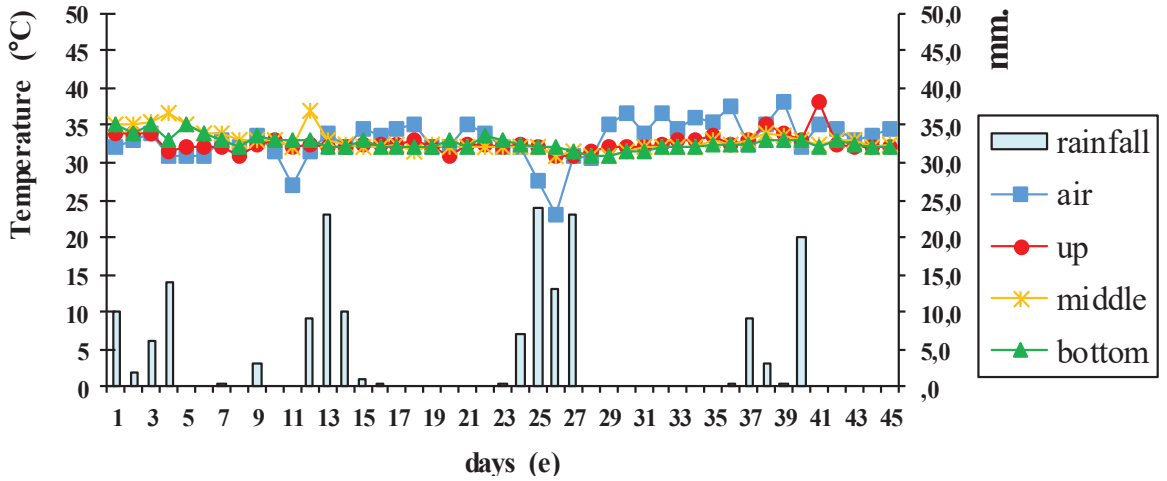
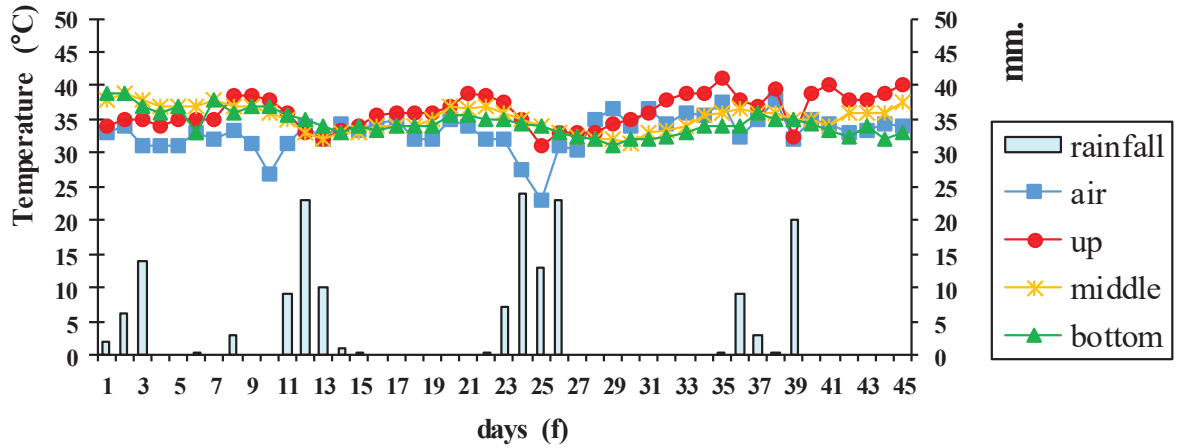
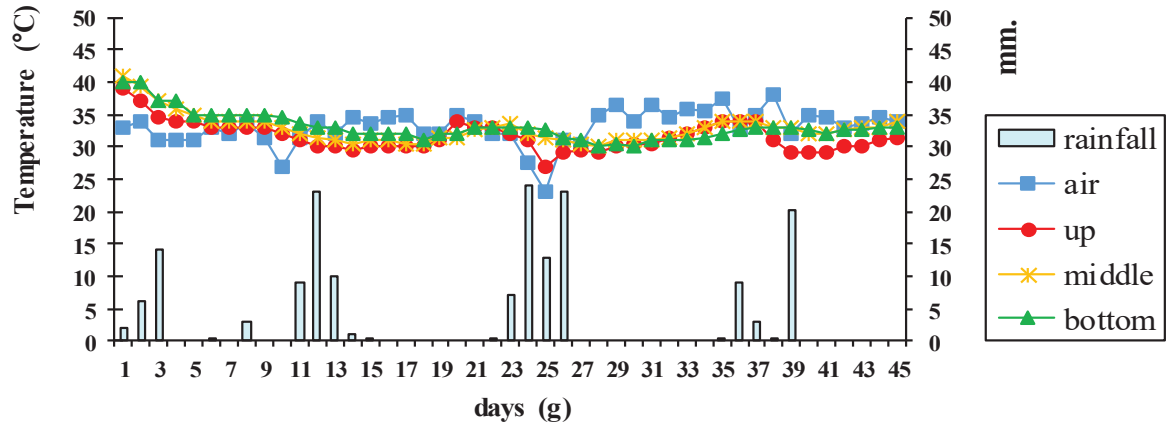
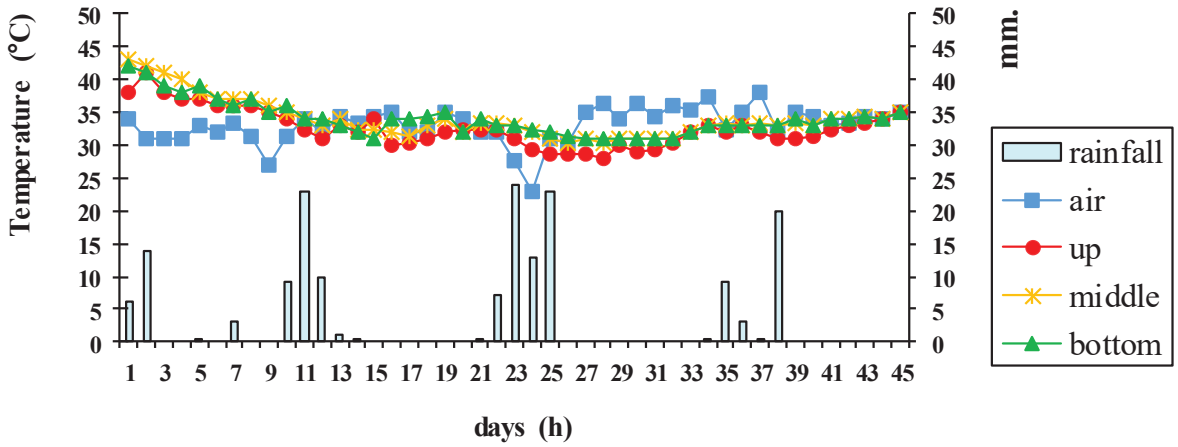
days (d)


Temperature (°C)
mm.
rainfall
air
up
middle
bottom
days (e)


Temperature (°C)
mm.
rainfall
air
up
middle
bottom
days (f)


Temperature (°C)
mm.
rainfall
air
up
middle
bottom
days (g)



Remarks 1: Measuring time 7am;
a = watering 0 L/7d, b = watering 60 L/7d,
c = watering 120 L/7d, d = water 180 L/7d
Measuring time 1 pm;
e = watering 0 L/7d, f = watering 60 L/7d,
g = watering 120 L/7d, h = water 180 L/7d
Remark 2: Rainfall during 45-day experiment 19 storms: d1 = 1.02 mm, d2 = 9.05 mm, d3 = 10.04 mm, d4 = 2.00 mm, d5 = 6.06 mm, d6 = 14.00 mm, d7=9.04 mm, d8 = 23.04 mm, d9 = 10.00 mm, d10= 1.02 mm, d11 = 0.08 mm, d12 = 0.04 mm, d13 = 7.08 mm, d14 = 24.04 mm, d15 = 13.00 mm, d16 = 0.04 mm, d17 = 9.08 mm, d18 = 3.02 mm

Fig. 3 Amount of watering relating to in-box garbage temperature and also impacts of rainfall to bacterial digesting process of fresh garbage fermentation as conducting research at the Royal LERD project site in Phetchaburi province

B. Nutrients and Heavy Metals in Long-Period Fermented Garbage

The open lid of concrete boxes for the community organic waste fermentation for enhances aerobic digestion processes by bacteria. At the same time, the experiment was taken the alternating between fresh-food market wastes and finer paddy soils. This process needs electron acceptors to increase the organic garbage digestion in order to get good quality of compost as well. Owing to electron acceptors were some constituents of finer paddy soils such as oxygen, ions, manganese, nitrate, etc. It was the matter of fact that red soil and paddy soil have been originated from the same source. Red soil converted from brown paddy soil or top soil of any places by oxidizing process of ion compounds becoming to red soil. In conclusion, garbage plus red soil or paddy soil would be better electron acceptor materials for producing the organic or garbage compost. The amount of compost played a significant role of electron acceptors in searching the materials to encourage the digesting processes.

Qualities of compost were shown in form of percentage of nutrients (nitrogen, phosphorus and potassium), C/N ratio and moisture content as seen the data in Table I. The compost quality was up to the garbage composition. The average percentages of nutrients from all conditions were show below the standard value of compost set by the Land Development Department, Thailand. The analyzed results found no significantly differences in statistical test. Although, nutrients were lower than standard but these compost can be used to improve soil porosity and soil structure which was full of tiny air channels and pores that hold air, moisture and nutrients.

TABLE I
AMOUNT OF NUTRIENT ELEMENTS

Treatment	Rep.	Moisture (%)	Total-N (%)	Total-P (%)	Total-K (%)	C/N ratio
no water	1	67.00	0.42	0.05	1.50	11.33
0 L	2	55.32	0.35	0.03	1.25	7.86
	3	56.18	0.35	0.07	1.25	13.40
	Avg.	59.50	0.37	0.05	1.33	10.86
	1	60.03	0.21	0.06	1.25	17.62
60 L	2	56.89	0.28	0.05	1.38	14.00
	3	47.33	0.21	0.02	1.25	12.95
	Avg.	54.75	0.23	0.04	1.29	14.86
	1	59.89	0.35	0.08	1.50	10.49
120 L	2	54.35	0.21	0.05	1.50	16.29
	3	60.84	0.35	0.05	1.25	11.89
	Avg.	58.36	0.30	0.06	1.42	12.89
	1	52.05	0.28	0.02	1.50	11.21
180 L	2	57.78	0.32	0.05	1.50	13.78
	3	69.11	0.31	0.06	1.38	16.97
	Avg.	59.64	0.30	0.04	1.46	13.99

Moreover, the important reasons would be small exist of organic wastes that were converted into inorganic materials as garbage compost with normal producing nutrient and heavy metal contents. In this study, heavy metals in compost have shown values of Cd 0.002 ppm and Pb 0.001-0.003 ppm, which was lower than Biowaste Ordinance (BioAbfV), Germany standard [11]. Although the heavy metal contaminant in compost was still found at low level, the elimination of such contaminants can be removing by

plant or phytoremediation technique. For safely use, compost should be used for grow crop product and should be keep in dry storage room about a week before using for any purposes.

IV. Conclusion

The alternate laying community garbage and paddy soils are surely provided the watering technique to enhance an aerobic process by vertical movement of water to take dissolved oxygen to the bottom of layer as same as electron acceptors which absorbed heat release for complete the composting processes. The result confirmed that the 60-L watering every 7 days has been selected for moistening garbage inside the concrete boxes in order to accelerating the organic garbage digestion to obtain the completed compost and convenience for working. Owing to open lid of the concrete box was preferable technology for garbage composting. Unlimited water was wet the organic garbage might affect to the bacterial digesting processes. Throughout 45-day experiment found that there were not effects to amount and duration of rainfall to garbage temperature and after rain falling showed the decrease of garbage temperature, then it gradually increases together with releasing heat due to the bacterial organic process occurring after adjusting them to wet condition. The compost obtained was found normal quality likewise the other experiments in Thailand and also low heavy metals in the community garbage compost as well.

Acknowledgment

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Urban Boundary Profile and Its Effects on Haze Episode in Thailand

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***Abstract*—**Heights of atmospheric boundary layer and air pollution concentrations of Bangkok, ChiangMai, and HatYai were used to identify air pollution episode in Thailand. The study areas were located in central, southern, and northern of Thailand, respectively. They have been suffering from suspended particulate matter caused by biomass burning especially on ChiangMai. The worst period was occurred at the end of February through April of each year, the northern part of Thailand always faces haze problems. The particulate matter less than 10 micrometer (PM10) concentrations were higher than Thai's ambient air quality standard (120 micrograms per cubic meter) more than two times. There are many factors which caused the haze episode. Biomass burning and uncontrolled forest fire are the reasons that were used to explain the episode. However, there is another reason to associate the episode. The reason is the air parcel movement because air always moves as a "wind" or "lift up" as a result of adiabatic cooling process. In case of northern haze episode, both of them were interrupted by the environmental factors. Wind was blocked by mountain and the adiabatic cooling process was blocked by the warmer air above northern of Thailand. The episode was compared to a "box" contained four sides surround the area (same as mountain) which could block horizontal wind. Furthermore, "the box" might have upper lid due to warmer air on the box. It was called "temperature inversion" condition which could limit convective movement of air parcel via adiabatic cooling process. The measurement of temperature profile in the north of Thailand during haze episode showed that daytime temperature inversion on 27 February 2009 caused two times higher than normal concentrations of CO and particulate matter.

***Keywords*—**Haze episode, Micrometeorology, Temperature Inversion

I. Introduction

THE atmosphere play important role towards the things on the surface world. To change the characteristics of the atmosphere will effect on environment to another. Thailand locates in the tropical area with a distinctive atmosphere and style is different from other areas due to the location near the Equator. As a result, Thailand can receive solar energy more than other zones. Therefore, The height of the atmosphere layer is capable of combining the elements of air, known as Mixing depth or Mixing height [1], [2], but nevertheless, in the case of the hot air mass floating above or was called "temperature inversion", the phenomenon that has resulted in air mass trap or cannot float up including air pollutant caused by human activity [3], that caused air pollutant accumulation in the ambient air. [4]-[6].

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II. Procedure

A. Methodology

1) Collected upper meteorological data from Thai meteorological department which measured by Radiosonde technique at Chiang Mai during the years 2002-2008 [7].

2) Measured the upper meteorological parameters by using radiosonde technique at Chiang Mai, three times a day [8].

3) Measured PM10, CO, NOx concentrations at three levels of height of three study areas (Bangkok, HatYai and ChiangMai)

4) Calculated mixing height and analyze the relation to air pollutant concentrations.

B. Study area

Bangkok is the capital city of Thailand located in the central of country. Bangkok is classified as a megacity with urbanized area. It was coved Characteristic of the city is urban area. Land is covered by building and concrete. The air pollutant sampling site was on the roof top of the highest building (328 meters above ground), Biyok Sky building in Thailand. The upper meteorology was measured at BangNa meteorological station. The sampling period was on 17-21 February 2008.

HatYai is a major city of southern Thailand. Land use is classified as urban and business area. The sampling site was on the roof top of the highest building in HatYai (125 meters above ground), Lee Garden building. The upper meteorology was measured at SongKlar meteorological station. The sampling period was on 17-21 December 2007.

Chiang Mai province is northern capital city of Thailand. The province is a land of forest and mountain similar to basin pan. The sampling site was on the roof top of Centara Duangtawan building (152 meters above ground). The upper meteorological station was located at 494749.38 m E and 2075601.98 m N. The sampling period was on 25-29 February 2008.

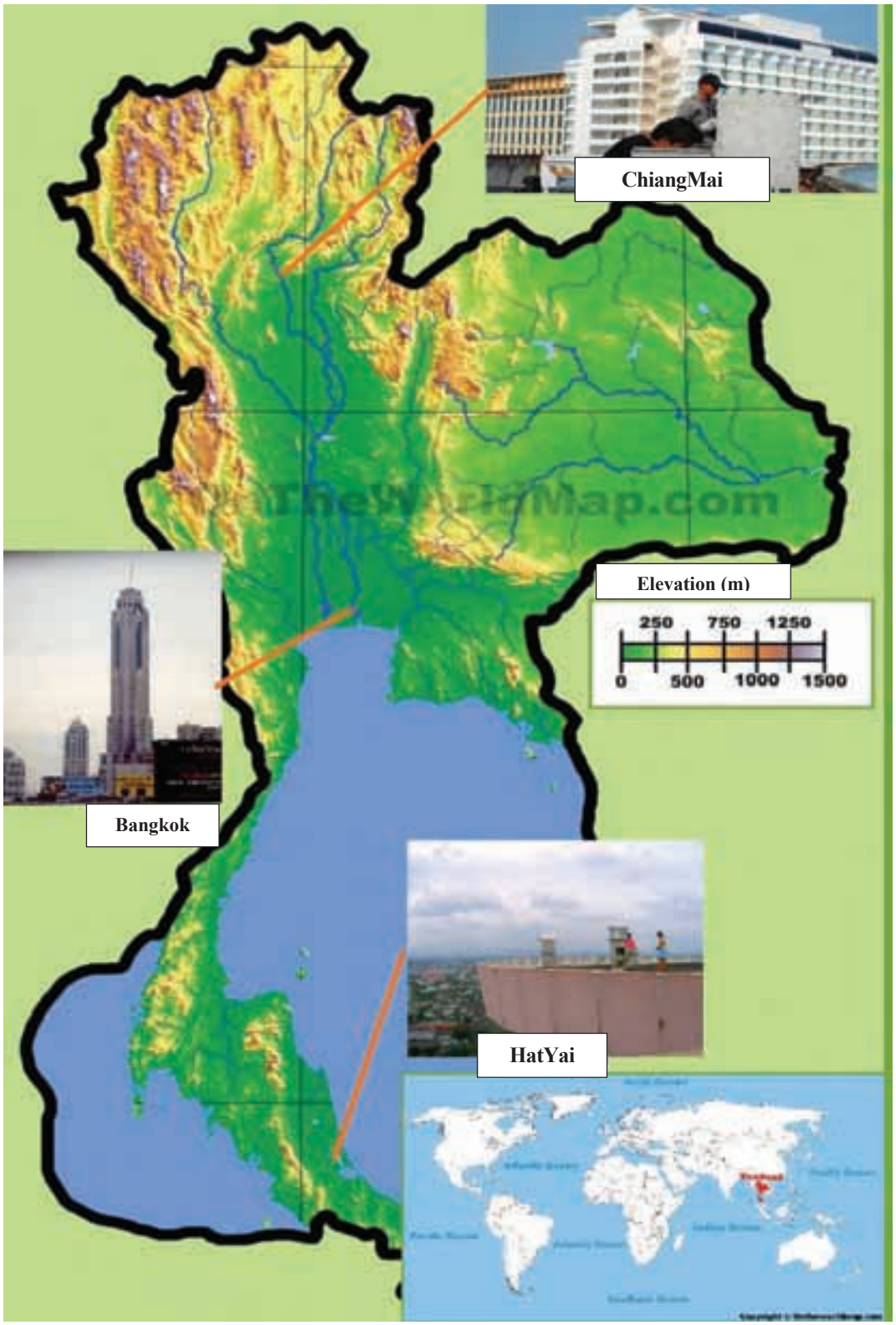

Fig. 1 Study areas and locations

C. Calculation method

To calculate mixing height based on Holzworth by using surface meteorological data meteorological and upper meteorological data of the study area. They will be used to calculate the mixing height twice a day. Temperature and atmospheric pressure of upper meteorological data will be used to calculate Dry Adiabatic Lapse Rate in order to intercept the plot. The surface temperature will be used to find the minimum and maximum values. The minimum temperature defines as

temperature at 02.00-04.00 a.m., local time plus 5 degrees Celsius in order to compensate for the difference in temperature between the cities and the rural areas (Heat Island), based on the principle of Holzworth, value added and then taken to calculate height in the morning (GMT 0) (as show in equation (1)).

The maximum temperature defines as the temperature at 2.00 – 4.00 p.m., local time was used calculated mixing height in the afternoon (12 GMT) as in equation (2).

$$SFCTH_{0GMT} = T_{min5} \times (1000/PR) \times 0.286 \quad (1)$$

$$SFCTH_{12GMT} = T_{max} \times (1000/PR) \times 0.286 \quad (2)$$

SFCTH= Potential temperature (^{o}C)

T_{min5}= Minimum temperature, plus 5 degree Celsius (^{o}C)
T_{max} = Maximum temperature (^{o}C)
PR = Atmospheric pressure (mb)
0.286 = Constant

III. Results and Discussion

A. Urban boundary Profiles

Radiosonde gave temperature profiles of the urban areas (Bangkok, HatYai and ChiangMai) as show in Fig. 2, 3 and 4. The temperature profile showed the atmospheric stability of the areas. There is a significant point on the ChiangMai's temperature profile on 27 February 2008. There is an daytime (12LST) inversion at 536 meters above ground.

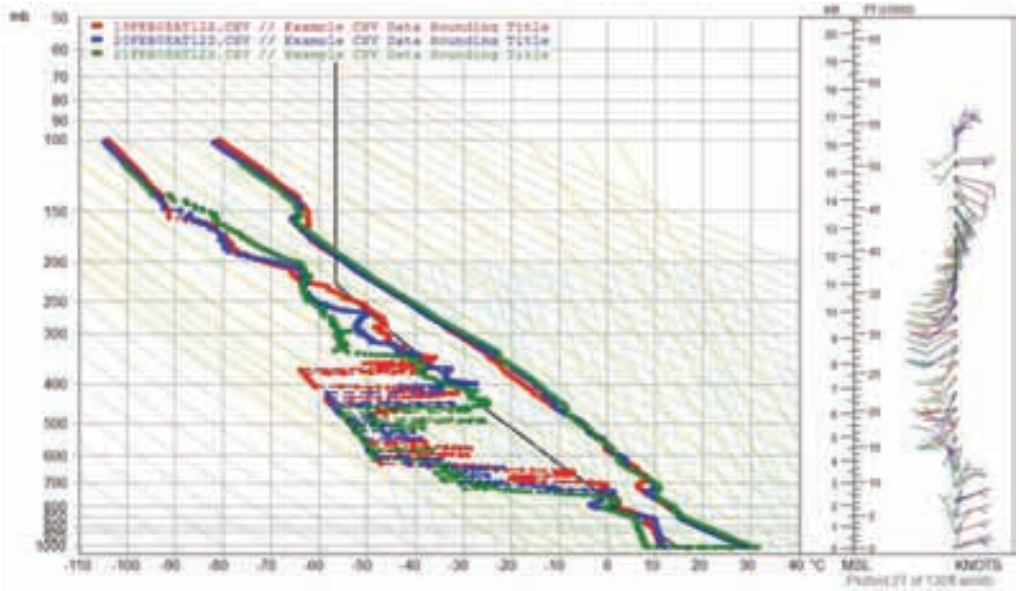

Fig. 2 Bangkok's Temperature profile during 17-21 February 2008

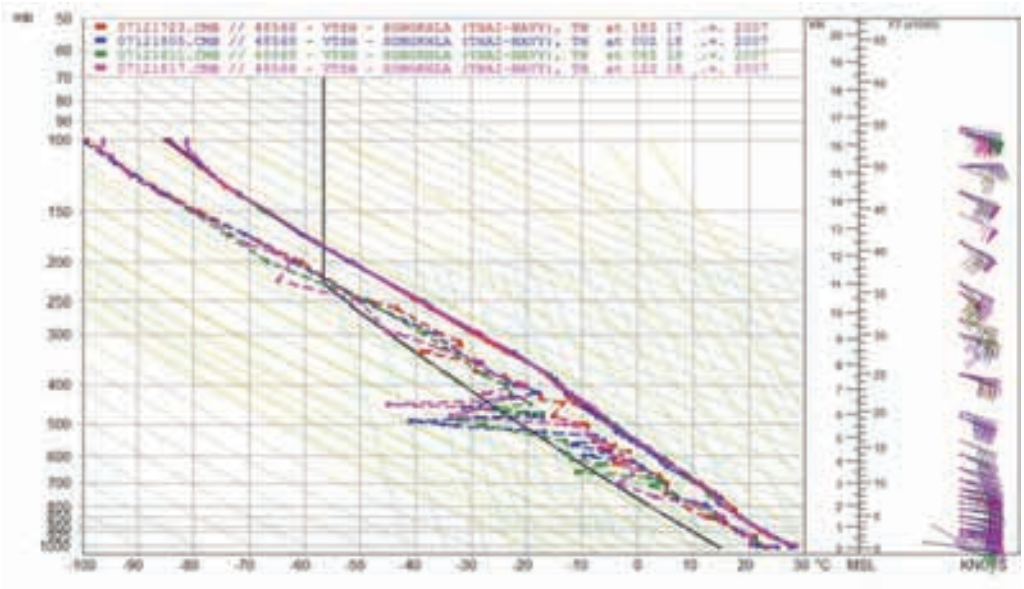

Fig. 3 HatYai's Temperature profile during 17-21 December 2007

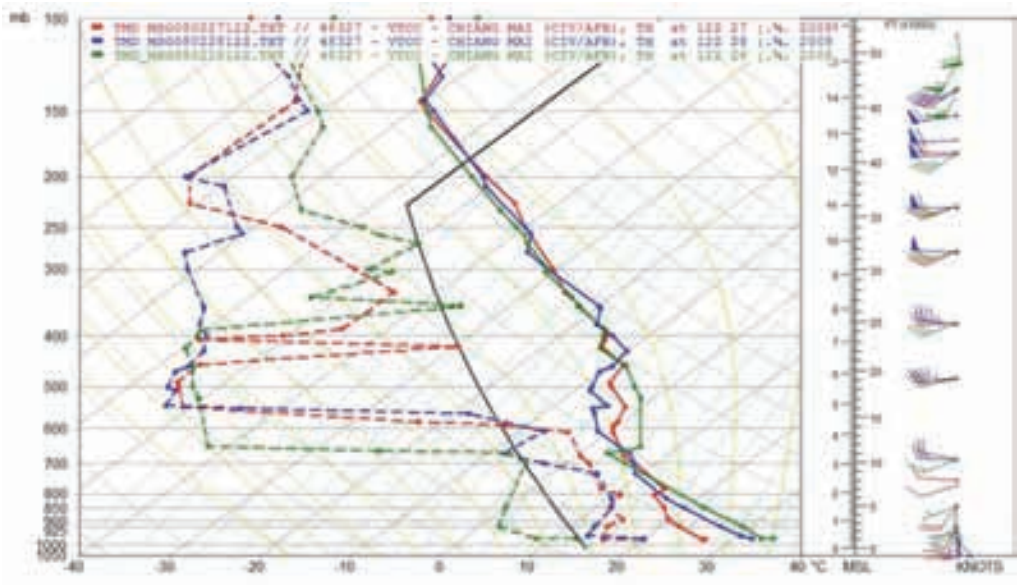

Fig. 4 ChiangMai's Temperature profile during 25-29 February 2008

B. Mixing Height

Surface and upper meteorological data during 2002-2006 [7] were used to calculate mixing height. It found that monthly average mixing height was increased in February and decreased in April and may (as shown in Fig. 4). The lowest mixing height at 0 GMT was 12 meters in January and the highest was 259 meters. The lowest 12GMT Mixing height was 96 meters in August and the highest was 3,732 meters in April (table 1). Furthermore, diurnal variation of the mixing height was decreased in the night time and increased in the morning as a result of solar radiation [8].

TABLE I
MIXING HEIGHT (METERS) AT 0GMT AND 12 GMT, CHIANG MAI DURING 2002-2006

	Mixing height 0 GMT (meters)	Mixing height 12 GMT (meters)
January	12-1,288	107-2,713
February	30-1,472	881-3,629
March	24-2,099	766-3,645
April	27-1,295	555-3,732
May	45-3,259	244-4,231
June	713-1,942	108-2,804
July	510-1,518	177-3,152
August	559-2,009	96-2,334
September	737-1,343	413-2,251
October	288-2,433	170-2,957
November	17-1,404	170-2,214
December	49-1,501	640-2,237

C. Relationship to Particulate Concentration

Chiang Mai is a province located in the north of Thailand. It is a land of forest and mountain similar to basin pan. By the end of February to April, there are many fire spots in the north of Thailand including neighbor country due to agriculture preparing process. It caused smog with high particulate matter concentration. The local people suffered from eye irritation, sore throat, nose burning sensation due to particles and gases. PM10 was higher Thai's air quality ambient standard, 120 microgram per cubic meter.

It also found that micrometeorological conditions were associated the problem at the time of firing. Thai Meteorological Department has been used radiosonde to collect upper meteorological data such as atmospheric pressure, temperature, wind speed and direction. The upper meteorological data was used to calculate the mixing height. In year 2008, the averaged mixing height in the morning (0 GMT) was 359 meters and it was highest in June (560 meters). The lowest was 160 meters in December. The averaged afternoon mixing height (12 GMT) was 1,377 meters and the heighted was 3650 meters in May. The lowest was 530 meters in November (winter). (as shown in Fig. 5) In year 2009, the averaged mixing height at 0 GMT was 352 meters higher than year 2008 and the averaged mixing height at 12 GMT was 1,272 meters and lowest in May (410 meters) (as shown in Fig. 6).

The haze episode in March 2007, PM10 concentrations were higher than Thai air quality ambient standard, 120 microgram per cubic meter for a period of up to 23 days when the level of mixing height affected on PM10 concentrations. The low mixing height levels (on 4, 5, 6, 14, and 17 of March 2007) came with high PM10 concentrations. Especially on 14th March, the PM10 concentration was nearly 300 microgram per cubic meter. The averaged mixing heights in the afternoon in year 2008 and 2009 were 1,377 and 1,272 meters respectively. Therefore, the mixing height which is lower than the average level risks the episode occurring. According to above reason, there were high PM10 concentrations during 1st - 31th of March 2007 because the mixing heights were lower than 600 meters (as shown in Fig. 7).

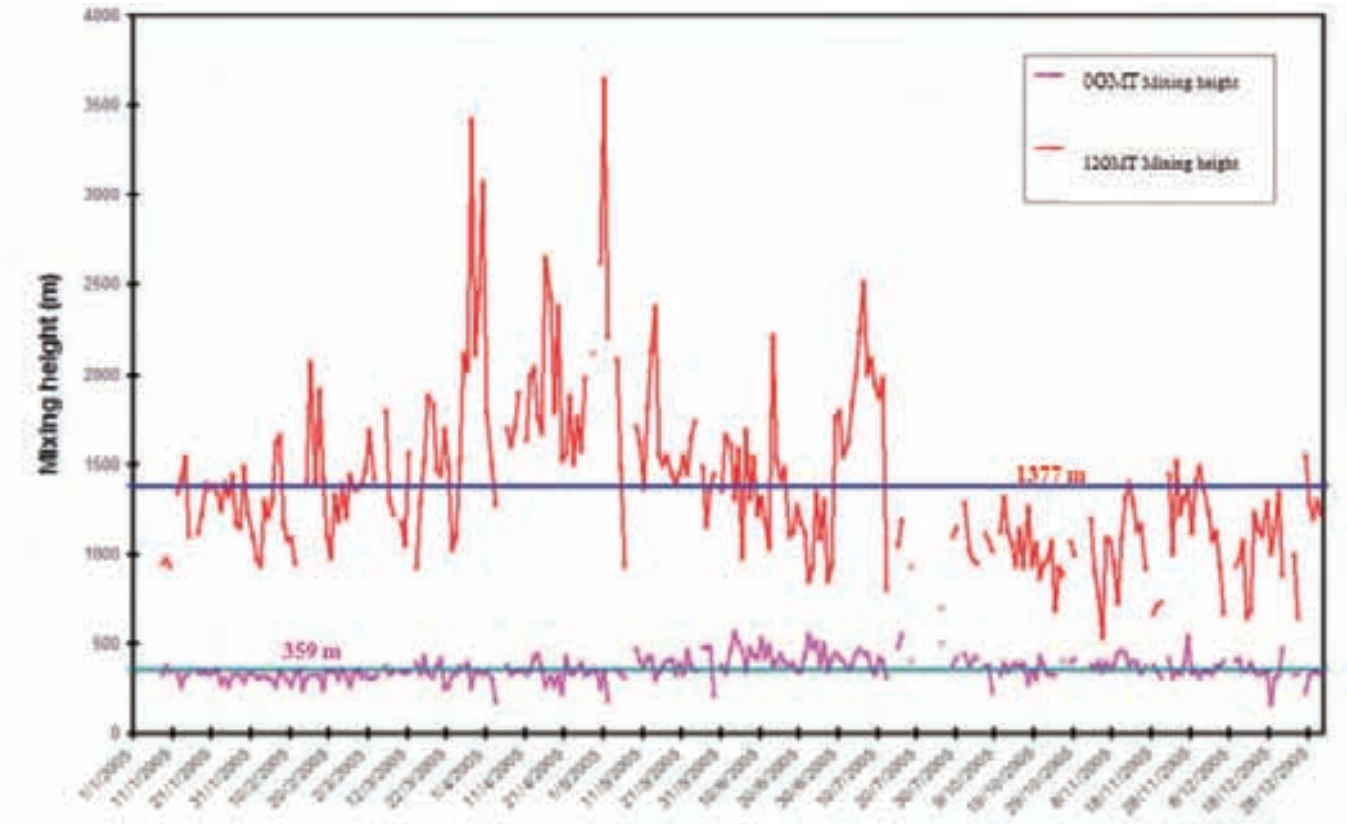

Fig. 5 Mixing height at Chiang Mai in year 2008.

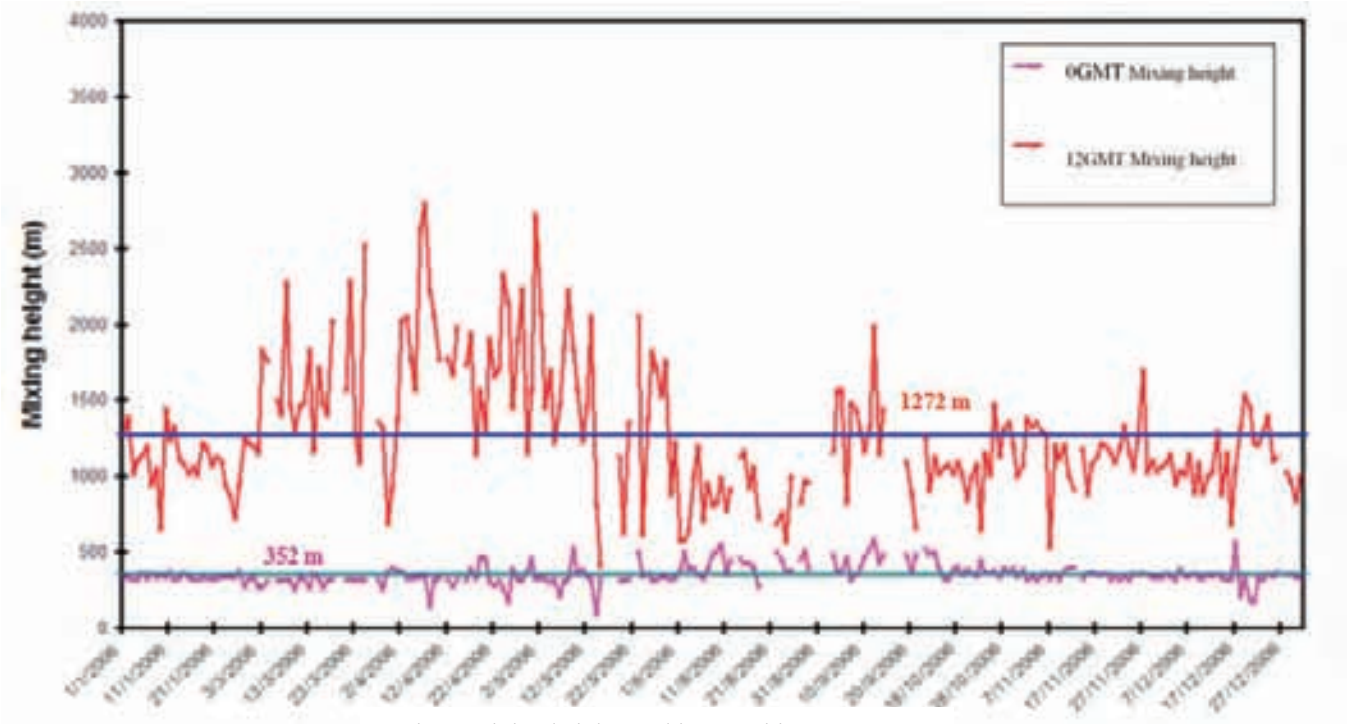

Fig. 6 Mixing height at Chiang Mai in year 2009.

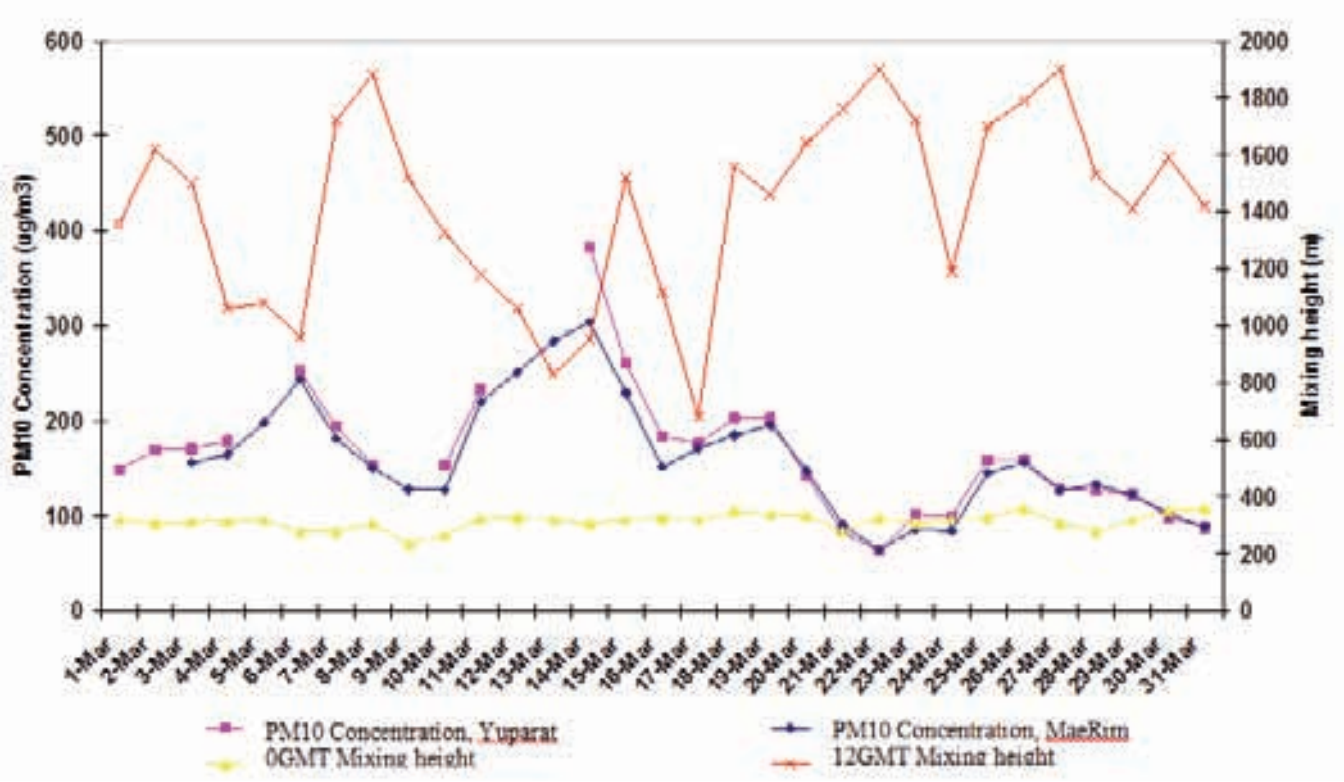

Fig. 7 PM10 concentrations and Mixing heights during March 2007.

Meanwhile the measured temperature profile (by radiosonde technique) on 28 February 2008 at ChiangMai province also show a significantly relationship on primary pollutant concentrations such as particulate (PM), carbon monoxide (CO). The 500 meters above ground inversion on temperature profile at noon found a double concentration of PM10, TSP CO and NOx concentrations compared to non-inversion profile (Table II).

TABLE II
AIR POLLUTANT CONCENTRATIONS AT CHIANGMAI DURING 25-29 FEBRUARY 2008

Period/Time	Air Pollutant concentrations			
	PM10 (ug/m^3)	TSP (ug/m^3)	CO (ppm)	NOx(ppb)
25-26 February 2008				
21.00-00.00	124.3	96.3	0.743	34.57
00.00-03.00	140.3		0.545	22.44
03.00-06.00	89.7	81.9	0.028	11.19
06.00-09.00	85.6		0.171	16.38
09.00-12.00	129.5	104.5	0.184	19.21
12.00-15.00	84.3		ND	16.46
15.00-18.00	68.7	70.0	0.048	17.66
18.00-21.00	82.6		0.696	20.95
26-27 February 2008				
21.00-00.00	93.2	84.4	0.900	17.74
00.00-03.00	86.1		0.477	10.53
03.00-06.00	86.6	90.3	0.512	11.12
06.00-09.00	109.1		0.663	17.84
09.00-12.00	154.4	133.8	0.874	28.21
12.00-15.00	124.3		0.642	16.59
15.00-18.00	141.6	156.5	0.734	16.44
18.00-21.00	53.6		0.951	20.33
27-28 February 2008				
21.00-00.00	64.8	61.9	1.025	20.56
00.00-03.00	64.6		0.806	14.59
03.00-06.00	60.5	79.8	0.842	15.24
06.00-09.00	104.4		1.107	24.85
09.00-12.00	64.5	69.0	0.833	18.73
12.00-15.00	49.4		0.556	14.45
15.00-18.00	72.8	82.9	0.514	18.51
18.00-21.00	70.7		0.731	15.49

IV. Conclusion

Decreasing mixing height means limited vertical movement of air parcel. It causes changes on atmosphere condition and air pollution concentration. Especially on closed areas such as basin pan area because natural dilution process was limited by physical geography including limitation of vertical movement in case of low mixing height.

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Applicable Eco-Engineering Techniques through Watershed Management over Phetchaburi Rain Shadow Zone in Providing Sustainable Water Yield to Downstream Community in Thailand

K. Chunkao, K. Duangmal, O. Phewnil, P. Wichittrakarn, P. Rollap, S. Mokatip, J. Phermsin

***Abstract*—**The research aimed to study the role of ecological engineering technique (EET) in managing community water yield shortage during very dry period in 2013-2015 over Phusawan watershed as located in the rain shadow zone inside Phetchaburi province, Thailand. The watershed is characterized as hilly and high sloping area in the headwater, medium slope and rugged terrain in the middle, and small pierce of flatland and good for growing crops in downstream site. In accordance with EET concept, the storage reservoir was constructed at the upper part of river mount, while rock, earth, and mulching dams as well as a few detention storage reservoirs were additionally made along the riverfront in upstream watershed. However, the research results found annual rainfall 773.62 mm and becoming to streamflow approximately 0.32 MCM/km^2 which was lower than another watershed in Phetchaburi province. The surface water quality was analyzed in 3 groups i.e. physical-chemical water quality, ions, heavy metals and pesticides in water as collected from headwater to Phusawan storage dam. All indicators of physical-chemical water quality were under standards, only alkalinity being high to itch the human skin after taking a shower. Anyhow, heavy metals and pesticides in water were found under standards at all measuring stations but they were contaminated very high content in sediment as taken from the bottom of reservoir. Summarily speaking, the EET measures as constructed on upstream has to be improved for keeping in place of the excess pesticides as applied for pineapple growing and heavy metals from wash-off soil erosion process in order to make storage water in reservoir sustainably clean for providing to downstream Phusawan communities.

***Keywords*—**Eco-Engineering technique, watershed, rain shadow, water yield.

I. Introduction

NATURALLY, the tropical zone of the world is located in a region of the earth surrounding the equator by limiting in latitude 23*26'14.1" N and 23*26'14.1" S where average rainfall in wet month is 60 mm (2.4 inches) or more and average annual temperature 18 ºC (64 ºF) [1]-[3]. The location of Thailand is one of among the other country where the sun angle on climate most areas within the tropics are hot year-round but it is somewhat clearly existed in summer (February-April), rainy season (May-November), and winter (December-January). Such climatic periods are able to provide the unlimited condition for growing agricultural crops in everywhere of the whole Kingdom of Thailand. Unavoidably, the people which are landless for doing agriculture were illegally moved in some potential areas in order to take over lands for serving their purposes. Phetchaburi provincial area located nearby well-known Hua Hin beach for tourism, plenty rain shadow areas available but they are lacked of fertile soils and rainwater for the villager s not for growing crops but also for everyday lifestyle consumption. Phusawan watershed is the said community locating inside the rain shadow which has been confronted with long-period drought in every year due to the coverage area in the leeward side of Tenasserim Hills range, the divided border line between Thailand and Myanmar. In other words, the Phusawan watershed ecosystems are eligible to functions likewise windward side of the mountain and another ecosystem in Phetchaburi province or neighboring ecosystems.

A rain shadow is a dry area on the leeward of the mountainous areas which block the passage of rain-producing weather systems, causing wet condition in windward zone rather than leeward side. Consequently, the long-period drought is naturally taken over more than 7 months that has been influenced to run dry becoming to ephemeral and/or intermittent stream rather than perennial stream [1]-[4]. After toughing the earth surface, the lag time was too short to let some penetration and storing in soil as soil water for feeding to stream all year-round but it was opposite and causing water for consumption of the natives. There are a lot EET which is the engineering tools or instruments, are capable to construct for maintaining ecosystems function to support itself and another neighboring ecosystems [5]-[7]. In general, the EET construction for ecosystems function has been seen in all parts of the world in various systematic environments such as storage and diversion dams, irrigated drainage systems, for bridge across the rivers, reforestation in waste lands, storm wetlands along rivers, riverbank controlling construction, terracing and soil erosion control measures, landslide control walls, fire-lines, excess stream-water reservoirs, rock check

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dam and earth check dam, etc. Each one has their own function for regulating their ecosystems [2]-[8]. However, there are no need EET construction with the virgin ecosystems rather than deteriorated ones because of its long-time adjustment becoming existing ecosystems. While windward side of mountain range has no needs the EET construction storage reservoir rather than the leeward side (rain shadow areas) which is the dry land due to less rain falling.

Phusawan watershed is the unit area for water management under rain shadow zone which is naturally confronted with water shortages up to drought. The said phenomena have caused the bug problems on lack if water supply for in-house consumption, growing crops, livestock operation, etc. This research paper is aimed to study in the role of Eco-Engineering technique in watershed management for sustainable water yield of quantity, quality and timing for wellbeing of native people.

II. Methodology

A. Area Description

Phusawan watershed is located on the area under the rain shadow of Tenasserim Hills range, the boundary between westerly Thailand and Myanmar along Prachuab Khirikhan province throughout Tak province as shown in Fig. 1. Accordance to mountainous area of rain shadow, Phusawan watershed is characterized mostly steep sloping and rugged terrain, especially small size of flatland for growing paddy rice but only orchards are available on rugged upland areas

Fig. 1 Geological map of Phusawan watershed as located in Phetchaburi province under rain shadow of Tenasserim Hills range between Thailand and Myanmar

The covering area of rain-shadow Phusawan watershed is classified as low rainfall and dry climate together with shallow depth of infertile soils and high content of gravel and stone which were weathered from limestone, granite and shale rock types. Moreover, the climatic condition is identified 3 seasons: winter during late October to early February, summer during late February to early April and rainy season during late April to early October. Naturally, amount of annual rainfall is approximated less than 1,000 mm (700-1,000 mm), warm temperature 20-40 ^{o}C, pan evaporation 1,200-1,400 mm, sunshine period 6-7 hours, and wind speed 5-30 m/s.

B. Rainfall Measurement

The 8-inch standard rain gage was installed at the middle of Phusawan watershed in 2014 round-year and measuring immediately after rain stop falling. Also, monthly stream flow was measured at outlet of Phusawan watershed (above Phusawan storage dam) by floating technique. Besides, water quality samples were collected at four stations as localized in Fig. 1. Those water samples were analyzed to determine their quality in physical-chemical water quality, ions and heavy metal contaminants, pesticides in stream water and water storage dam through standard methods for the Examination of Water and Wastewater [9].

III. Results and Discussion

C. Annual Rainfall

The results of 2014-year rainfall measurement were 724.76 mm, monthly ranging between 1.6 m to 0.29 m, no rainfall in June and July. The annual rainfall over Phusawan watershed was about a half of country's mean annual rainfall (approximately 1,700 mm) as shown in Table I. It showed that the area under the rain shadow zone like Phusawan watershed has not been wet enough to grow any kinds of economic crops. Pineapple seems to fit in rain shadow zone that shows high potentiality in producing high yields even growing on gravel soils (Table I).

TABLE I
Monthly Rainfall and Stream Flow of Phusawan Watershed as Measured in August 2014 to July 2015

No.	Month	Rain Water (mm)	Discharge (m^3/km^2)
1	August	154.63	47,720
2	September	97.64	6,040
3	October	288.95	199,550
4	November	71.0	8.2
5	December	2.0	0.23
6	January	9.4	1.1
7	February	1.6	0.2
8	March	0.4	none
9	April	3.4	39
10	May	49.8	573
11	June	46.0	529
12	July	29.9	344
	Total	724.76	253,932

D. Stream Water

The discharge for five-time measurements was obtained 4.18 cms (11 Oct 2014), 2.82 cms (31 Oct 2014), 2.72 cms (15 Nov 2015), 3.56 cms (13 Dec 2015) and non-measurable flow (10 Jan 2015) floating methods which applied from the hydrological works of [1]-[3] and [10]. The stream flow calculation was found the average water yields in quantity of 253,932 m^3/kg^2 (approximately 0.25 MCM/km^2) that very low amount when it compared with flow from hill-evergreen forest watershed about 1 MCM/km^2, and somewhat low when it compared with dry-dipterocarp forest watershed about 0.56 MCM/km^2. In fact, the forest cover was about 79% (Table II) which should be provided water absorption before gradually feeding the

stream but it was mostly located on steep slope and mountain ridge. Anyhow, the cultivated land actually was taken over small flatland and rugged terrain. The shifting dwelling areas were not distributed around the watershed area due to soils was not fertile enough to grow crops as shown in Fig. 2.

TABLE II
TYPES OF LAND USE AREAS OF PHUSAWAN WATERSHED IN 2014

Land use	Area (Rai)
Forest	3,476
Cultivated Land	700.35
Shifting Area/Waste Land	105
Dwelling Area	96
Water Sources	2.3
Total	4379.56

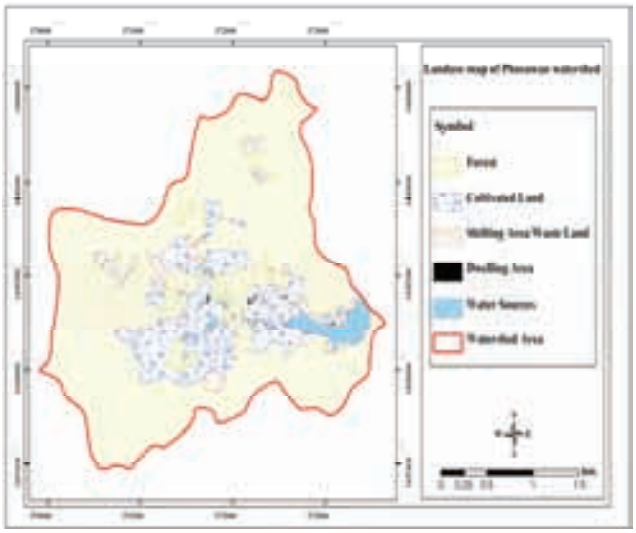

Fig. 2 Land use map of Phusawan watershed

E. Water Quality

The results of water quality analysis were presented in Table III by dividing into 3 groups, i.e., physical-chemical water quality, ions and heavy metal contaminants, and contaminating pesticide residues as presented in Table III.

TABLE III
PHYSICAL-CHEMICAL, IONS AND HEAVY METALS, AND PESTICIDE WATER QUALITY OF PHUSAWAN AS COLLECTED DURING AUGUST 2014 TO JULY 2015

Water Quality Indicators	Water Quality Sampling Station					Standards
	Station 1	Station 2	Station 3	Station 4	Station 5	
Water Quality: Physical and Chemical						
Temperature (°C)	23.9	25.2	30.3	32.3	31.8	n* / 23-32**
pH (-)	6.9	7.7	7.3	8.4	8.6	5 – 9
Salinity (ppt)	0	0.4	0.1	0.1	0.1	-
EC (µS/cm)	282	945	454	382	372	-
Color (Color unit)	10	<5	14	<5	<5	n*
Turbidity (NTU)	6.1	8.6	6.5	15.3	12.3	30**
Dissolved solids (mg/L)	188	617	305	256	250	-
Suspended solids (mg/L)	3.3	19	13	16	15	-
Total solids (mg/L)	191.3	636	438	272	246	-
Dissolved oxygen (mg/L)	2.1	2.1	3.2	7.1	8	4.0* / 3.0**
COD (mg/L)	37	16	33	26	31	-
BOD (mg/L)	5.4	1.5	4.4	4	3.6	2.0*
Acidity (mg/L)	123	469	194	180	169	-
Alkalinity (mg/L)	78	370	185	125	130	-
NH_3 (mg/L)	0.16	0.12	0.2	0.14	0.1	0.5*
Nitrate (mg/L)	1.01	2.19	0.55	0.63	0.85	5.0*
TKN (mg/L)	0	0.84	1.4	1.5	1.16	-
Phosphate (mg/L)	0.06	0.08	0.19	0.07	0.08	-
H_2S (mg/L)	0.32	0.23	0.23	0.05	0.02	-
Sulfate (mg/L)	5.8	18	31	10.6	11	-
Oil and Grease (mg/L)	0	0	0.4	0.2	0	-

Remarks: n* natural values * standard values in third class surface water quality ** water quality being suitable for aquatic animal surviving.

Totally, all water quality indicators are under officially obligated standards as marked in Table IV. The non-identified standards should be accepted the same resolution, except the indicators with non-normal values that might be harm done to human health. Acidity, alkalinity and pesticides are among those water quality indicators with no standards available in which their chemical properties can make human displeasure and irritation, especially itching skin after showering and even touching it (Fig. 3).

As stated above, almost water quality indicators have shown in harmless water for general consumption but the concentration of alkalinity seem higher than water quality of

Phetchaburi River due to limestone wash-off water containing more bicarbonate form in water. In consequence, the water quality of Phusawan dam followed the upstream water quality. The lesson learnt that the water quality in stream was influenced by rock types in upstream and headwater rather than anything else. However, the water quality of Phusawan storage dam is really needed to find means for safety utilizing by in-depth analyzing the chemical that cause of poising the skin and another health problems.

TABLE IV
PHYSICAL-CHEMICAL, IONS AND HEAVY METALS, AND PESTICIDE WATER QUALITY OF PHUSAWAN AS COLLECTED DURING AUGUST 2014 TO JULY 2015

Water Quality Indicators	Water Quality Sampling Station					Standards
	Station 1	Station 2	Station 3	Station 4	Station 5	
Water Quality : Ions and Heavy Metals						
K (mg/L)	8.3	8.8	9.7	9.1	9.50	-
Ca (mg/L)	54	210	90	55	56	-
Mg (mg/L)	24	160	95	70	74	-
Fe (mg/L)	0.454	0.613	0.254	0.543	0.374	-
Mu (mg/L)	0.068	0.063	0.435	0.085	0.062	1.0*
As (mg/L)	0.013	0.014	0.065	0.02	0.078	0.01*
Cd (mg/L)	0.003	0.004	0.005	0.004	0.001	0.05*
Pb (mg/L)	0	0.01	0.037	0	0.016	0.05*
Cu (mg/L)	0.015	0.011	0.012	0.008	0.007	0.1*
Zn (mg/L)	0.052	0.054	0.06	0.043	0.078	1.0*
Hg (mg/L)	0.0004	0.001	0.0007	0.0005	0.001	0.002*
Water Quality : Herbicide						
Abamectin	None	None	None	None	None	-
Ametryn	None	None	None	None	None	-
Atrazine	None	None	None	None	None	-
Glyphosate	None	None	None	None	None	-
AMPA	None	None	None	None	None	-
Paraquat	None	None	None	None	None	-
Water Quality : Microbiology						
Coliform bacteria MPN/100 ml	-	-	-	-	1,300	20,000*
Fecal coliform bacteria MPN/100 ml	-	-	-	-	220	4,000*

Remarks: n* natural values * standard values in third class surface water quality ** water quality being suitable for aquatic animal surviving.

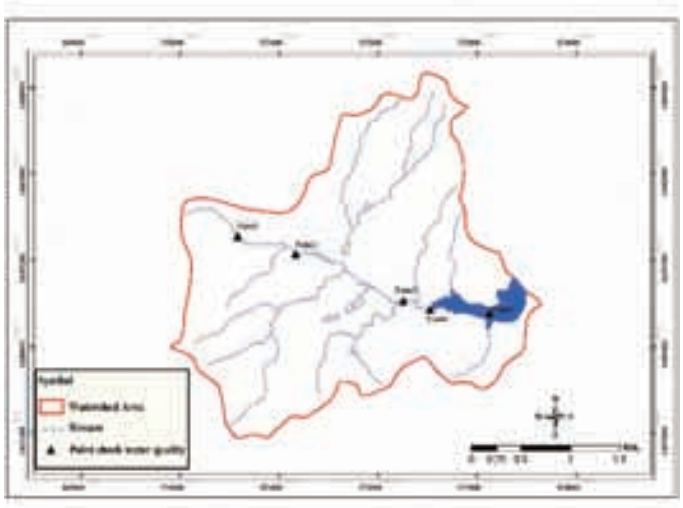

Fig. 3 Water quality sampling station in Phusawan watershed

F. Impact of EET

In principles, watershed refers to unit area for water management in terms of occurrence, distribution and contamination in order to provide the sustainable water yields to the other ecosystems consumption. The said statement implies that the watershed scientists have to manage sustainable water yields with optimum quantity, desirable quality and flow regime [1]-[3], [11]. If the water supply is shortage, EET measure has to be applied in order to have water available for serving any purposes of request [1], [4]-[8], [12]-13]. As mentioned before, the hydrological characteristics of rain-shadow Phusawan watershed could not produce water quantity round-year and avoidably to receive surface water contaminating somewhat high content of acidity due to fermenting litter fall along small marsh as well as in storage dam, and also alkalinity from limestone rock type in headwater. Nevertheless, soil erosion has been found in the whole parts of Phusawan watershed that needs the control measures for keeping soil in place. Accordance to those water conditions and watershed status, the EET techniques have been applicable by constructing storage dam, sectional storage areas on every watercourse, engineering and vegetative works for soil erosion control such as terracing, concrete walls and revegetation [1], [6]-[8], [13]-[14]. So far, the water quality of storage dam has to be treat alkalinity before using it in order to run away from skin itching.

IV. Conclusions

Phusawan watershed is located under rain shadow zone that is the main cause of water shortage in dry period. So, the EET has been proposed to keep water in soils and in the storage dams. Unfortunately, the stored water has been contaminated high concentrated of alkalinity but the others were under standards. Decreasing alkalinity is really needed to keep away from skin itching.

Acknowledgment

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Utilization of Beverage Sludge for Compensate Chemical Fertilizer for Growing Vegetables on Garden nearby Beverage Production Plant at Bangkok Urban Fringe

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***Abstract*—**Approximately 60-70 t/day of sludge from the Thai Asia Pacific Beverage (TAPB) brewery factory is disposed of as waste to landfill. This sludge is comprised of plant nutrients which can be used to grow economic agricultural crops. Sludge contained a high content of N (4.11%), P (2.24%), and K (0.50%). Three step experiments were investigated. The results of first phase confirmed that application of sludge for growing of vegetables such as Chinese cabbage and Chinese kale, applied sludge rate at 25 t/ha was obtain the highest products. The second phase; ratio of TAPB sludge to soil of 1 : 1 is recommended for growing flowers and vegetables in pots. The final step involved five volunteer farmers, which lived nearby beverage production plant at Bangkok urban fringe, who applied sludge for growing their crops of sweet basil, cucumber, long bean, eggplant and marigold. The experiment resulted in increased net income for sweet basil, cucumber and marigold crop. The results indicated that the crop yields of cucumber and marigold were not significantly different between the TAPB sludge plot and the control plot with chemical fertilizer but the sweet basil, long bean and eggplant crops were highly significant different due to nutrient balancing of each plant being different for optimal growth. The research results suggest that sludge can be applied for better condition of soil texture and also to increase nutrient supply to encourage plant growth and replace chemical fertilizer.

***Keywords*—** sludge utilization, beverage production plant, agricultural community

I. Introduction

THE industrialization is defined as conversion processes from raw material inputs through the technical units to become ready product outputs and waste/pollutants in which they have to decrease until within standard values before releasing to the public environment. Unfortunately, there is no technology to eliminate all contaminants as produced from the industrialization processes that can be indicated by monitoring program. This is why the industrialists try hard to minimize waste and pollutants together with higher efficiency of control units [1].

Sludge from wastewater treatment system of the Thai Asia Pacific Beverage (TAPB) brewery factory were huge numbers approximately 60-70 t/day. Fresh sludge was presumed to replace the organic compost which could be used for stimulating to crop growth rate. There were plenty of plant nutrients, especially N, P and K, the key nutrients for plant growth, which can utilize as organic compost for growing vegetables and agricultural cropping. Phosphorus sources are being exhausted and soil quality is degrading, treated sludge is used as fertilizer and soil conditioner [2]. Due to the TAPB factory is located at Bangkok urban fringe near agricultural community and most of villagers near the TAPB factory were farmers. So, the surrounding villages are focused on promoting the community service responsibility (CSR) by taking sludge from wastewater treatment system for agricultural apply. The research results will be used for promote CSR program. This research aimed to study utilizing of sludge as the organic compost to grow economic vegetables crops in surrounding community cultivating areas.

II. Locations of Experimental Areas

TAPB is located at housing number 11, village1, Saiyai sub-district, Sainoi district, Nontaburi province, Bang Bua Tong-Suphan road, at 6-km stake, the distance about 35 km from Bangkok, between latitude 14°06'22.22" N and longitude 100°17'46.42". The TAPB area is covered about 1500 hectares surrounding by croplands inner surrounding and the outer belonging to villages, as shown in Fig.1. The surrounding villages are focused on promoting the community service responsibility (CSR) by support sludge for growing their vegetables and another purposive.

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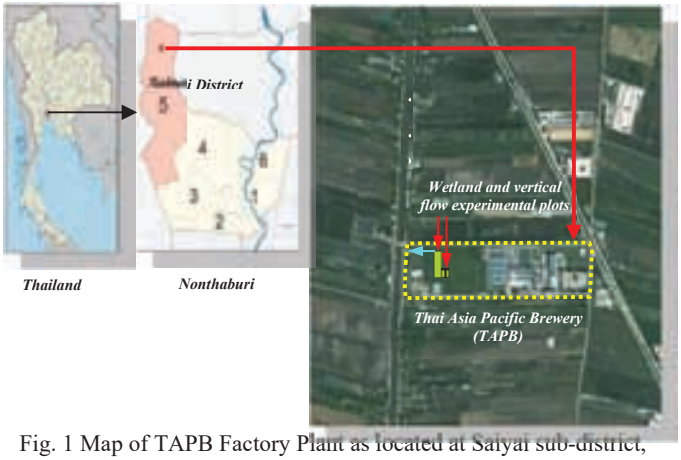

Fig. 1 Map of TAPB Factory Plant as located at Saiyai sub-district, Sainoi district, Nontaburi province, in northernly about 35 km from Bangkok, Thailand

III. Experiments of Growing Vegetables

A. On-site Experimental Areas

In regard to demonstration on how to use fresh sludge as organic compost, the on-site experimental areas has been required to grow vegetables which are occupation of the villagers surrounding the TAPB factory boundary in western north direction. Fortunately, the research teams made the site visit and social surveying for getting to know each other to the villagers that brought about in-place experimental areas for growing vegetables plus fresh sludge as organic compost utilization instead of chemical fertilizers. Luckily, the head villager has provided his land, approximately 20 hectares for growing vegetables for demonstration to the native people.

Due to the experiment was taken Split Plot Design in which land was divided into one part for growing Chinese kale and the one for growing Chinese cabbage. Each part was taken in Completely Randomized Design (CRD) that including 2 main plots, 5 sub-plots and 3 replications (2 x 4 m plot each), each main plot which contains 15 plots (plus 35-cm gap) between plots which could make the total 50-m length, 10-m width and 1-m ditches between main plots and surrounding area which is connected with the irrigated sub-canal by manual water gate. The vegetable garden was heightened at 15 - 20 cm from the ground surface by digging down ditches deeper and taking submerged soils overtopping on the surface before turning up soil surface for air dry about 3 weeks. After that, the soil lumps were loosened to the small-sized soil particles without compactness by any heavy equipment. Afterwards, it is the time for putting wet AS sludge 0, 1, 2, 4 and 6 tons/rai on growing sub-plots (3 replications each) S0, S1, S2, S4 and S6 respectively; following activity setting for mixing between surface soil and wet AS sludge; then after, leveling ground surface had to be completed before growing vegetables. According to the growing space is given to 25 x 25 cm for both Chinese Kale and Chinese cabbage, all together 40 soil pits for each replication (sub-sub-plots) that being marked and digging down to the zone of dense roots at depth of 25 cm.

Actually, soil samples were taken before growing Chinese kale and Chinese cabbage by collecting soils in V-shaped form at depth of 15 cm for randomly 20 sampling points plus surface soil sheet 2 to 3-cm thickness together 1 kg from each replication. Then, soil samples were spread out in laboratory in order to make air dry for few days before 2-mm sieving and grinding that could be enough to keep in containers for analyzing nutrients and another parameters.

Chinese kale and Chinese cabbage were grown in mix soils between fresh sludge and in-place soils. The research result indicators were weight per unit area of Chinese kale and Chinese cabbage including the growth of height and biomass in relation to the chemical component constituents. The variation of those indicators is depended on the independent indicators from sludge chemical elements and compounds such as N, P, K and others.

B. Proportion Between Soil : Sludge Ratio for Crop-Growing Pots

Due to the first step of experiment, the result showed 4 tons/rai (10 tons/acre) for sludge application could be only point out its applicability for on-site growing vegetables but it was not employed for co-growing pots. So, it must be reconsidered in case of determining the proportion between sludge (C:N ratio equivalent to 5:1) and soil by employing the crop-growing pots. Anyway, five ratios between soil and sludge were taken in 4:0, 3:1, 1:1, 1:3 and 0:4 in which they were mixed together before transferring into experimental pots (25-cm diameter at the upper frame, 12-cm diameter at bottom frame, and 19-cm height).

C. Application of Sludge for Economic Crop Growing

In reality, the Thai-Asia Pacific Beverage Company has created the unit of community social responsibility (CSR) for sustainability of producing together with villagers' life quality improvement. To achieve such goal, the villagers' needs to updated social-economy information in relations to self-providing without any exploitation to TAPB Company. At this moment, sludge seems possible to distribute for organic compost for growing vegetable which would be the villagers' occupation promotion. Therefore, the social surveying has to be done by studying the secondary data, intensive interviewing (personal interviewing), and questionnaire answering as action research. The project requested five volunteer farmers from the village near production plant for applied sludge in their cultivation crops at rate of 4 tons/rai to compensate chemical fertilizer. The economic crops included sweet basil, eggplant, cucumber, long bean, and marigold. The experiment was set up in each farm area in 3 replications. The growth rate was measured every week and crop yield were determined after harvesting period.

IV. Results and Discussions

Accordance with sludge has been produced about 60 tons/day, whenever Heineken beer is needed to serve the markets. It makes a big pile of total sludge that accumulates quite a lot day by day with useless in landfill. In principles, sludge from food factory is abundantly comprised of plant nutrients for growing economic crops such as vegetables,

flowerers, ornamental and fruit trees and farm plants. The details of study as follows:-

A. Feasibility Study on Sludge Utilization for Vegetable Cultivation

The preliminary study was conducted by utilizing sludge like fertilizer for growing Chinese Kale and Chinese cabbage on each main plot of 50 m x 10 m for 5 sub-plots (treatments) and 3 replications (2 x 4 m plot each), that making them altogether 15 plots (plus 35-cm gap) between plots with 1-m ditches and surrounding area with the irrigated sub-canal by manual water gate. The dewatered sludge was applied approximately 4 tons/rai by mixing them around each plot before planting both Chinese kale and Chinese cabbage. The results found the Chinese Kale 1,162.7 kg/rai (standard 1,500 kg/rai), Chinese cabbage 5,146.7 kg/rai (standard 3,000 kg/rai)

Implication of research results have brought to stress that sludge is applicable for using not only for better conditioning soil texture but also for increasing nutrient to encourage plant growth, especially for growing Chinese cabbage. Limiting sludge application 4 tons/rai (10 tons/acre) would bring so many questions, why not less or more than this amount. The amount changing of sludge in relation to soil properties and crop product should be focused on the future research in order to attract to make use of research results for extend the knowledge to people around the TAPB factory. For clear understanding this experiment, the sludge (60 tons/day UASB sludge plus 10 tons/day AS sludge) is composed of moisture 84.38 %by weight, pH 6.6, EC (Electrical Conductivity) 7.51 dS/m, Organic Matter 34.13 %, N 4.11 %, P 2.24 %, K 0.5 %. While soils as used for experiment were composed of clay texture, pH 4.8, organic matter 2.7 %, total N 0.16 %, available P 14 mg/L, exchangeable K 300 mg/L, exchangeable Ca 2,720 mg/L, exchangeable Mg 440 mg/L. Exchangeable cations (K^+, Ca^{2+}, Mg^{2+}) also increased with application rate. Cation exchange capacity (CEC) was slightly increased by beverage sludge application and induced to increase organic matter [3]-[4]. During 1–45 days of incubation, the KCl exchangeable acidity decreased and increased thereafter comparing with the control.

Growth of Chinese Kale after 45-day harvesting found no statistical differences for applying sludge 2, 4, and 6 tons/rai but gaining wet weight was not more than applying sludge and 1 tons/rai applying sludge, and remarkably the highest product (1,162.7 kg/rai) found from applying sludge for 4 tons/rai. In the same manner, the 45-day harvesting Chinese Cabbage found exactly the same results and the highest product (5,146.7 kg/rai) from applying sludge 4 tons/rai as shown Table 2. It had some remarks from the above experiment that a great number of sludge applying decreased the products at the beginning due to affecting factors according to heat releasing and intermediate products as obtained from rapid organic digestion process to interrupt the growth rate as shown in Table 1. The results were consistent with the findings of Saviozzi et al [5] reported that higher rates apply of winery sludge caused the rise in salinity. This increased in the soluble salts could threaten the yield performance of sensitive crops.

However, the results showed the highlight of this experiment in case of applying TAPB sludge for on-site growing vegetables that should be 4 tons/rai (10 tons/acre) in order to obtain the highest products. In other words, the sludge as produced by TAPB factory is feasible to utilize for growing vegetables that presumably showed positive effect on the nitrogen and organic matter contents of the soil and better conditioning of soil texture and also increasing some available nutrients.

TABLE 1
PRODUCTS OF CHINESE KALE AND CHINESE CABBAGE FOR 45-DAY HARVESTING IN RELATED TO RATE OF APPLYING SLUDGE

Sludge rate (tons/rai)	Production (kg/rai)			
	Chinese Kale		Chinese Cabbage	
	Wet weight	Dry weight	Wet weight	Dry weight
0	773.1[a]	51.2[a]	2,256.8[a]	96.3[a]
1	899.7[a]	52.3[a]	3,837.9[b]	143.7[b]
2	1,061.1[b]	69.1[b]	3,920.0[b]	140.8[b]
4	1,162.7[b]	74.1[b]	5,146.7[b]	141.1[b]
6	1,087.2[b]	73.9[b]	4,130.1[b]	118.8[a]

B. Proportion Between Soil : Sludge Ratio for Crop-Growing Pots

The proportion between sludge (C:N ratio equivalent to 5:1) and soil by employing the crop-growing pots. Five ratios between soil and sludge were taken in 4:0, 3:1, 1:1, 1:3 and 0:4 in which they were mixed together before transferring into experimental pots (25-cm diameter at the upper frame, 12-cm diameter at bottom frame and 19-cm height). The findings of experiments by growing plants such as tolerant vegetables and flowerers as follows:-

Determination of suitable soil: sludge ratio was investigated by experimenting in growing tolerant flowerers and vegetable with experimental pots. The selected tolerant flowerers were comprised of Flowerers: Everlasting (*Gomphrena globosa* Linn.) (Thai name: Barn Mai Roo Roiy), Partulaca Rose (*Partulaca grandiflora* Hook.f) (Thai name: Prae Siang Hai), Znnia (*Zinnia violacea* Cav.) (Thai name: Bansuen) and Marigold (*Tagaetes* spp.) (Thai name: Daorueng); and The selected tolerant vegetables were comprise of Chinese Radish (*Raphanus sativus* Linn.) (Thai name: Phak Kad Hua), Eggplant (*Solanum melongena* Linn.) (Thai name: Makhue Proa) and Water Convolvulus (*Ipomoea aguatica* Forsk), (Thai name: Phak Bungchin). The experiments were conducted for 8-week periods and shown the results in Figure 5 which indicated the suitable soil:sludge ratio equivalent 1:1 (2:2) and 1:1-1:3 for vegetables in which they can be named as tolerant for growing well except Portulaca Rose and Chinese Kale that cannot survival in small experimental units (Fig. 2 and Fig. 3).

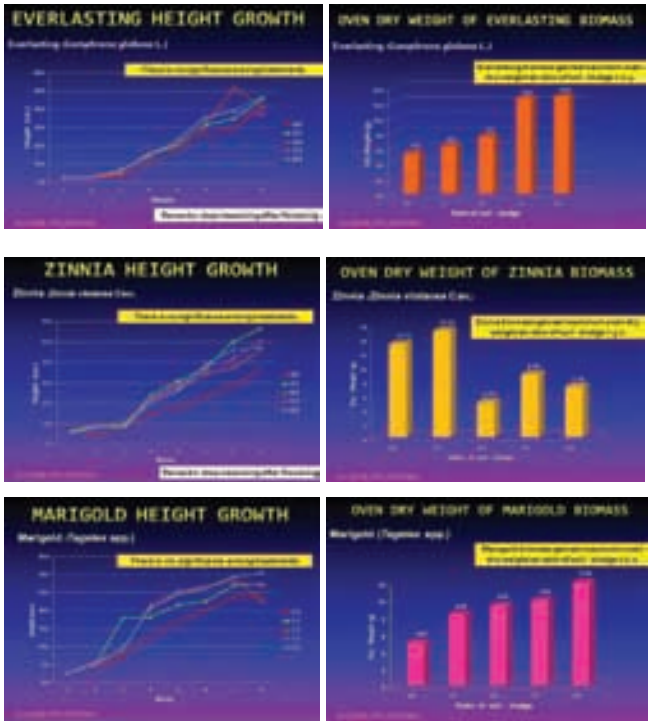

Fig. 2 Height growth and oven-dry weight (biomass) of flowers as grown condition of sludge:soil ratios (4:0, 3:1, 2:2, 1:3 and 0:4)

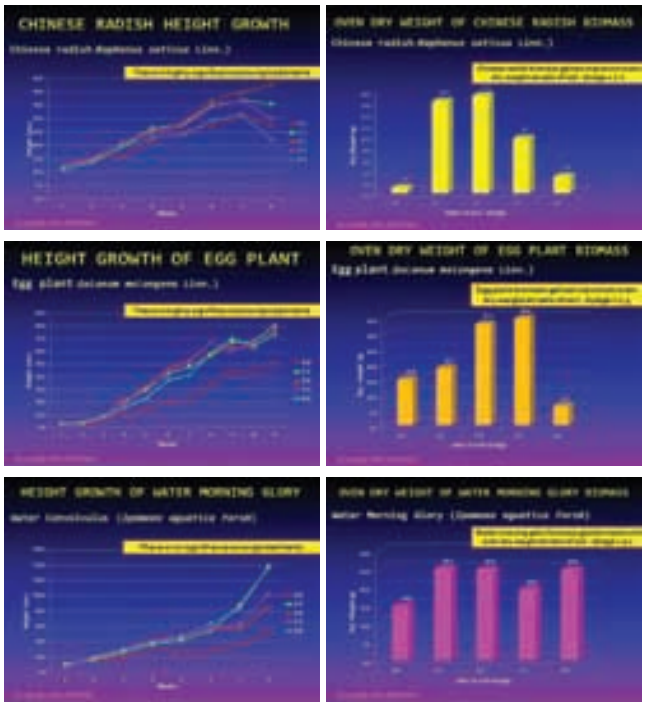

Fig 3. Height growth and oven-dry weight (biomass) of vegetables as grown condition of sludge: soil ratios (4:0, 3:1, 2:2, 1:3 and 0:4)

C. Application of Sludge for Economic Crop Growing

There are 5 volunteer farmers who tried to applied TAPB sludge at rate of 4 tons/rai (10 tons/acre) for growing their crops, i.e. sweet basil, cucumber, long bean, eggplant and marigold. The results demonstrated that crop yields of cucumber and marigold were not significantly different between TAPB sludge plot and control plot (chemical fertilizer) but sweet basil, long bean and eggplant were highly significant difference, the details were illustrated in Fig. 4. Because of nutrient balancing each plant was different requirement for its growing. Hence, it should be tested before apply TAPB sludge on any economic crops.

Fig. 4 Crop yield of sweet basil, cucumber, long bean, eggplant and marigold grown with TAPB sludge (treatment plots) and chemical fertilizer (control plots)

However, the cost and benefit analysis of TAPB sludge utilization included variable cost, total revenue, and net profit margin (net income) found that net income of sweet basil, cucumber and marigold were decrease about 20.31%, 16.62% and 1.23%, respectively, but long bean and eggplant were decline.

V. Conclusions

Sludge from brewery factory contained high content of Nitrogen (4.11%), Phosphorus (2.24%) and Potassium (0.50%), respectively. In case of applying sludge for on-site growing vegetables that should be applied for 4 tons/rai (10 tons/acre) in order to obtain the highest products. Flowers and vegetables as named tolerance could be grow any proportions of soils and TAPB sludge. Flowers and vegetables as named

sensitiveness could be grow at ratio of soils to sludge equal 1:1 ratio. It concluded the ratio of soils to sludge at 1:1 is strongly recommended for crop growing and economic plants growing. Sludge could use for improving soil texture, supplying nutrient to encourage plant growth and compensate chemical fertilizer utilization [6-8].

Net income was investigated in five volunteer farmers who grow their crops with TAPB sludge instead of chemical fertilizer. The findings showed their net incomes were increasing in sweet basil, cucumber and marigold. On the other hand, it was decreasing in long bean and eggplant because of nutrient in sludge were unbalancing. In other words, the free-of-charge sludge from Thai-Asia Beverage Production Plant would be called as the excellent CSR program in order to attach environmental education activities that was the best way to befriend with people around the TAPB factory.

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Environmental Impact Assessment in Developing Countries: Case Study of Thailand

K. Duangmal, K. Chunkao, S. Bualert

***Abstract*—**Environmental impact assessment (EIA) is an important tool for decision making of development projects since 1970 in Thailand. It was used to create mitigation plan for impact controlling. In Thailand, there are seven categories of development project, community services, mining, energy, transportation, petrochemical industry, industry and water resources. During 2011-2015, there were 2,418 projects approved by Office of Natural Resources and Environmental Policy and Planning (ONEP). It was averaged 466-596 projects per year. The succession rate of the approved projects trended to decrease especially on energy, transportation, petrochemical industry, industry and water resource sections. The sections are important parts for developing the country such as infrastructure, energy for foreign investment project. The decreasing of approved project was caused by committee. There were many times of committee changing and individual perception of the committee members including corruption phobia of the members on developer. Furthermore, the committee prefer amount of data more than specialist. The delay of considerate project made a delay of country development and lost chance to economic competition in 2015, the year of ASEAN

***Keywords*—**EIA, developing countries, economic, ONEP.

I. Introduction

BEFORE 1975, Thailand as developing country was faced on the environmental deterioration according to socio-economic development for better living of the people in the whole kingdom. Unbelievably, the country has been identified as developing country until the present time, and expecting to be on this identification level in the future. Actually, the development projects have been grouped as industry, transportation, communication, residences, public heath, arts and culture, education, national security, tourism, and also including water resources, energy, electronics and machinery, fisheries, forestry, agriculture, import and export, and so on. The mentioned development projects usually go hand in hand with negative impacts rather than positive impact, only if careless implementation before, during, and after construction. Therefore, careful project implantation is really needed to accomplish the project with less negative impact. In doing so, EIA was proposed to the Office of Natural Resource and Environmental Policy and Planning (ONEP) to study before starting up the development project in order to obtain the mitigation measures and plant to control all concerned activities. According to other countries EIA requirement world-wide [1], in central and eastern Europe [1], in Latin America and SE Asia [1]-[4], and in Africa [5].

EIA is a tool for making decision in implementing development project to determine the impacts on changing in the processes of environment and ecosystems for some period of time. However, EIA is the way of doing to find out the normal structure and function of environment and ecosystems as caused by proposed activities of such development project. Thailand has followed this principle since the year of 1978 which was the start-up time to study before constructing the development project but it seemed blur because of no cooperation of steak-holders, EIA reviewers, NGO, and project owners. Notably, the EIA reviewing is somewhat strict but the development project with EIA did not show any effectiveness in environmental protection. For general benefits, this paper will promisingly present the Thailand EIA experiences to someone who is interested in EIA study in Thailand as a case study for sharing this know to apply in their own country.

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II. Obligated-EIA Project List

No matters take in good care or careless for controlling the project activities (technology, energy, how, when, and where), the negative impacts are normally occurred one way or another. Since, there are various factors involving, especially the project activities, and also the lack of integrated management for construction. In other words, project activities play vital role in the causes of negative impacts, particularly projects with polluting the severe toxicants but they are ignored to study EIA before starting up the construction. In preventing so, the government of Thailand by ONEP has announced the development projects into 7 groups and one area (class 1 watershed) as present the details in Table I.

III. Project Activity Identification and Impacted Environment

Theoretically, each project had its own various activities which is very necessary to someone that concerns with development such project. The following items are the process on how to identify the project activities:

1. Understanding the development project and its functions,
2. Divide working period in three phases: before, during, and after construction the projects,
3. Find out the threats and its changeable power of each activity in each phase (before, during, and after construction)

4. Evaluate each threat power to impact on either one environment as belonged to physical, biological, human use value, and life quality environment.
5. Find environmental indicators as impacted by specific threats of each activity in each phase of project working period,
6. Grouping the environment concerning with impacts of each phase and the holistic project.

IV. Working EIA Process

References [3], [7]-[12] concluded EIA process, including the consideration of alternative, project design, screening, scoping, report preparation, review, consultation of participation, mitigation, decision-making and monitoring of project impact. UNEP has suggested that EIA must be integrated into the process of designing and implementing project or into project cycle is showed in Fig. 1. [13].

TABLE I
List of Development Projects as Required EIA Report under The Obligation of ONEP [6]

No.	Group of Development Project	Example	Number of Project
1	Community service	condominium, hospital, real estate	4
2	Mining	mining	1
3	Energy	electrical power plant	1
4	Transportation	expressway, highway, train, airport, port	8
5	Petrochemical industry	petrochemical industry	6
6	Industry	industrial estate, cement, steel	10
7	Water resource	dam, irrigation project	3
8	class 1 watershed area	all project	
	sum		33

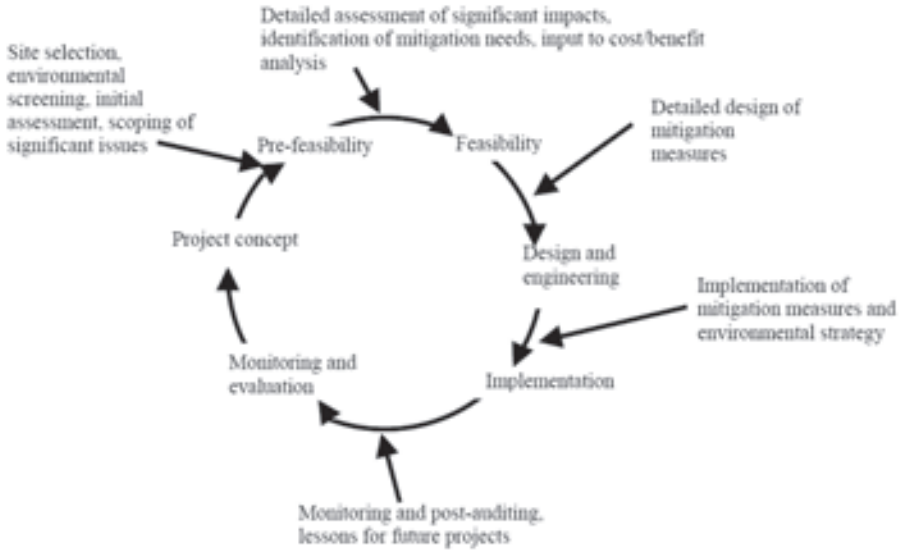

Fig. 1 United Nation Environment Programme [13]

Practically, the working EIA process begins from the project creation by the project owner in which the owner needs to develop in terms of socio-economic development. The end of working EIA process is exactly placed on the EIA report (mostly called as EIA statement) which should be drafted making people participation before to submit the "environmental specialist committee" for academic reviewing. For easy understanding, the working EIA process is presented in Fig. 2.

V. Approval EIA Process

The process of EIA approval in Thailand is very complicated and consumed time from the submitted day until the Environmental Board consideration due to the no-trustfulness of steak-holders and the people at the project site as come from the past learning. Moreover, the steak-holders do not believe in the environmental specialist committee who come from various fields of study and only one way consideration. Nevertheless, people still believe that whenever the existence of EIA, that project is surely constructed without any doubt. For more comfortable understandings, the approval EIA process is presented in Fig. 3.

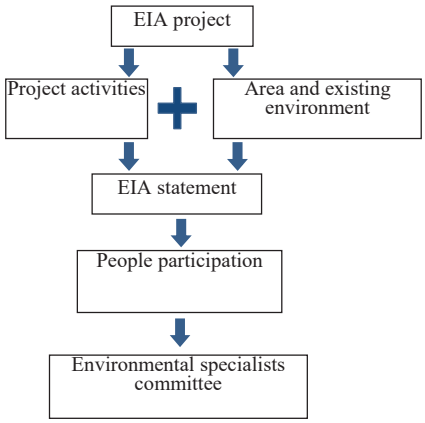

Fig. 2 Working EIA process for obtaining the drafted EIA report before reviewing by the environmental specialist committee

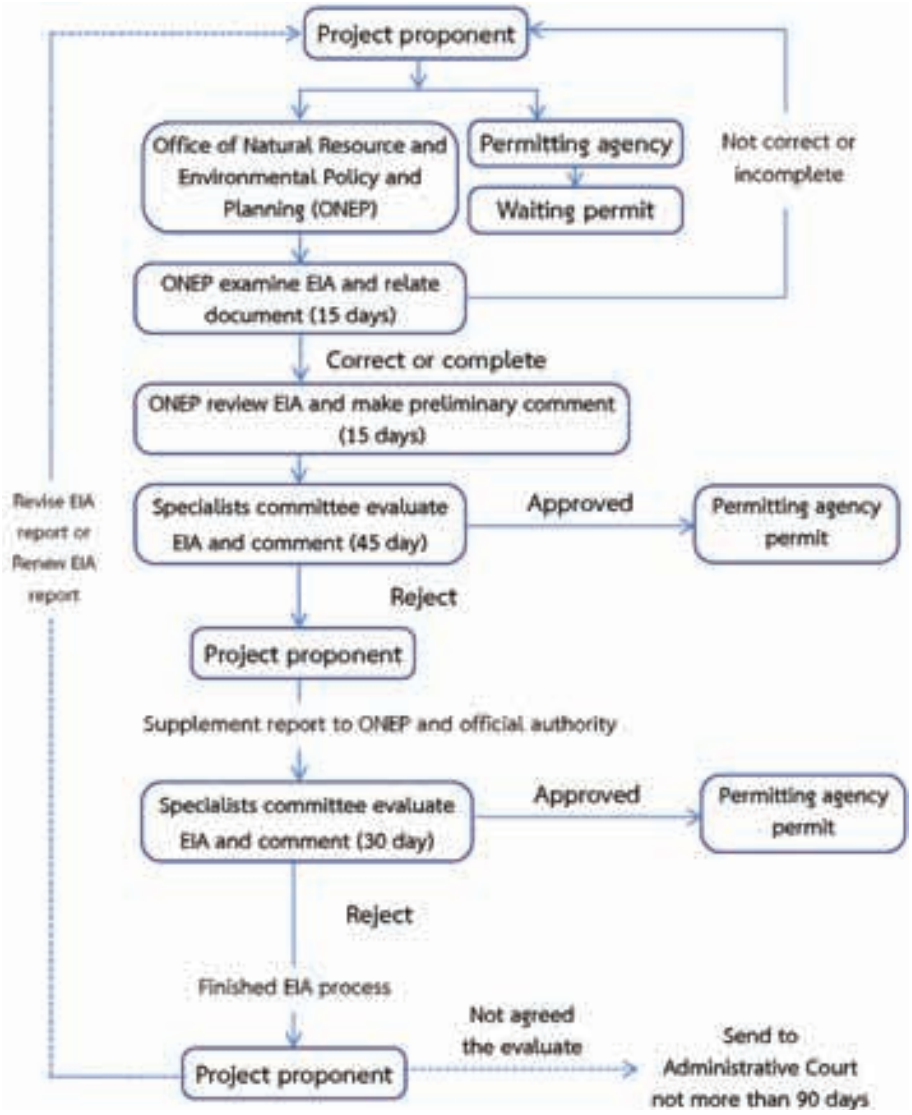

Fig. 3 Approval EIA process for private sector project in Thailand [14]

As stated before, EIA approval in Thailand is complicated and consumed time for consideration. Incase if private sector, the approval process can be applicable in Fig. 2. If the project belonged to the government, the approval EIA process has to end at the Cabinet Meeting because of such project not belonging to only government but it belongs to every Thai citizen. For clearly understanding, the EIA approval process is presented in Fig. 4.

VI. Lesson Learnt EIA Study in Thailand

Thailand is called as developing country due to rapid growth of population, 18 million in 1952, 22 million in 1957, 40 million in 1975, 67 million in 2015 [13]. In consequence, the poverty has been spread out to every part of the country, particularly low income and less saving money as the same as large number of uneducated population that causes on difficulty to develop them not catching the fast development on technology. Actually, EIA is soft technology that needs education as background to understand the holistic issued of EIA study. Some case, the development project was against to construct from the steak-holders and the local people but no logical reason to feedback to the government. Besides, corruption would be excused to against for any development project that why the approved EIA projects were approximately 6,800 projects from the total of 8,000 project during 1992 to 2015.The 105-day limitation for completed consideration could be brought another cause of delay for approval EIA project, because of more details to be reviewed and looking carefully an integration mitigation setting. Agent accident from any point sources at anywhere in Thailand, the new mitigation might be set up for environmental conservation, and causing the delay of EIA consideration.

Seeking new way of EIA study and approving is still kept in mind of EIA study agencies but it seems very difficult to achieve the target. However, the government has proposed

SIA, and HIA that bring the good sign to achieve the requirement.

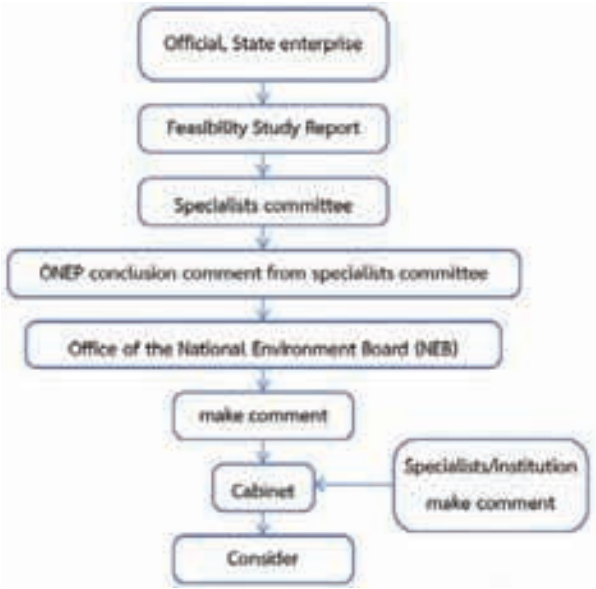

Fig. 4 Approval EIA Process for Government Project in Thailand [14]

ACKNOWLEDGMENT

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Effluent from Royal LERD Wastewater Treatment Systems to Furnish Nutrients for Phytoplankton to Generate the Abundance of Hard Clam (*Meretrix* spp.) on Muddy Beach

O. Phewnil, S. Khowhit, W. Inkapatanakul, A. Boutson, K. Chunkao, O. Chueawong, T. Pattamapitoon, N. Chanwong, C. Nimpee

***Abstract*—**The King's Royally Initiated Laem Phak Bia Environmental Research and Development Project ("the Royal LERD Project") is located in Laem Phak Bia Sub-District, Ban Laem District, Phetchaburi Province, Thailand. Phetchaburi municipal wastewater was treated with a simple technology by using aquatic plants, constructed wetland, oxidation ponds through a nature-by-nature process. The effluent from the Royal LERD Project was discharged into Laem Phak Bia muddy beach. The soil sediment samples were collected from two zones (200 and 600 meters from the coast of the beach), and tested for cation-exchange capacity (CEC), pH and organic matter and soil particles content. The marine water samples were also collected from the beach in wet and dry seasons and analyzed for its quality and compositions, including but not limited to, biochemical oxygen demand (BOD), dissolved oxygen (DO), suspended solids (SS), nutrients, heavy metals (As, Cd, Cr, Hg, and Pb), and phytoplankton at high and low tides. The soil texture was sandy loam with high concentration of calcium and magnesium which showed a property of base (pH 8). The marine water was qualified with the standard limits of coastal water quality. A dominant species was *Coscinodiscus* sp. It was found approximately 70.46% of total phytoplankton species in *Meretrix casta* gastrointestinal tract. The concentration of the heavy metals (As, Cd, Cr, Hg, Ni and Pb) in the tissues and water content of two species of hard clams indicated that heavy metals in *Meretrix casta* were higher than those in *Meretrix meretrix*. However, the heavy metals in both species were under the standard limits and safe for consumption. It can be concluded that nutrients in effluent from the wastewater treatment systems play important role in promoting the growth of phytoplankton and generating abundance of hard clams on muddy beach.

***Keywords*—**Wastewater, phytoplankton, hard clam, *Meretrix* spp., muddy beach.

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I. Introduction

STREAM pollution has been one of the biggest problems in most big cities. The engineering devices were mandatorily implemented to treat wastewater which is produced from municipalities, industrial factories, hotels and livestock farms. Beside the wastewater sources aforementioned, traditional settlement of Thai people alongside riverbanks have also contributed to community wastewater pollution. This is the reason why stream pollution gradually increases day by day while the problem seems to last long. Phetchaburi municipality is considered as a big municipality covering the area of 5.4 km^2 and total population living there is approximately 44,000 people [1].

Phetchaburi River is a main river which flows through the center of Phetchaburi municipal area. The stream pollution of Phetchaburi River occurred over years until the Royal LERD Project was established by the Chaipattana Foundation. The Royal LERD Project wastewater treatment systems are simple technology with natural process. Under the Royal LERD Project, wastewater in Phetchaburi municipal area has been stored and pumped through HDPE underground pipe to the Royal LERD Project's area which is located approximately 18.5 km far from Phetchaburi municipal area. Then, the wastewater has been treated by four wastewater treatment systems. The systems consisted of oxidation pond, constructed wetlands, grass filtration system, and mangrove forest filtration system. Effluent from the wastewater treatment systems were discharged through natural mangrove forest area before flowing into the sea. BOD concentration of wastewater from Phetchaburi municipal was declined from 200 mg/L to 60 mg/L due to anaerobic process in HDPE underground pipe. The effluent from the treatment systems was found BOD concentration less than 20 mg/L. BOD concentration was found about 10 mg/L at natural mangrove forest, 4 mg/L at coastal zone and 4 mg/L in sea water [2]-[5]. It was proven that oxidation ponds and wetlands can be the most suitable technology for treatment of wastewater produced from households, villages, communities, and municipalities. In principle, the nature-by-nature process consists of photosynthesis, thermo-siphon, and thermo-osmosis processes that support organic digestion of bacteria in wastewater [6]-[8]. The benefit of effluent is marine animal abundance increasing

due to the fertility of nutrients in the effluent and plenty of mangrove forest. Hard clam is the dominant species on the Laem Phak Bia muddy beach due to deposited suspended solids (SS) into the coastal area would enhanced the growth of hard calm. This study focused on the influence of ecological factor and habilitation on hard clam (*Meretrix* spp.) living on Laem Phak Bia muddy beach where the effluent from wastewater treatment system of the Royal LERD Project was released into.

II. Methodology

A. Location of Study Area

The King's Royally Initiated Laem Phak Bia Environmental Research and Development Project is located at Moo 1, Laem Phak Bia Sub-District, Ban Laem District, Phetchaburi Province, Thailand. The Royal LERD Project is in the lower central part of Thailand, approximately 150 km far from Bangkok, the capital city. The study area is in the Royal LERD Project's coastal area as illustrated in Fig. 1.

Fig. 1 Localization of the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project (the Royal LERD Project), Phetchaburi Province, Thailand

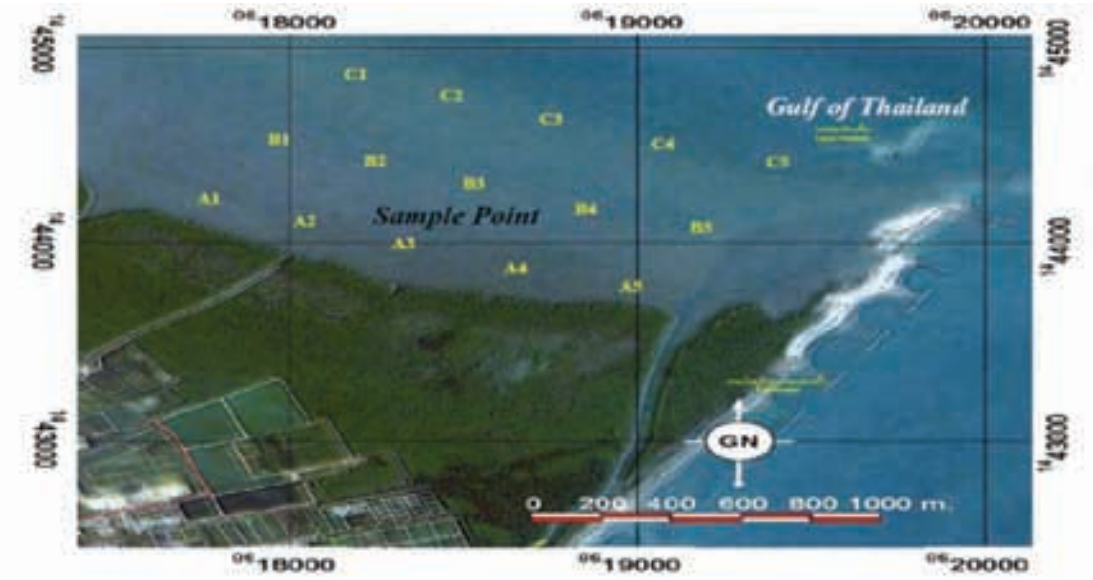


Fig. 2 Water and Soil Sediment Sampling Stations at the Royal LERD Project's Coastal Area

B. Experimental Design and Sampling Stations

15 marine water samples were randomly collected from three zones of coastal line in wet period (August 2012) and dry period (March 2013); zone A: 200 meters far from the coastal line (A1-A5), zone B: 600 meters far from the coastal line (B1-B5), and zone C: 1,000 meters far from the coastal line (C1-

C5) as illustrated in Fig. 2. The water samples were analyzed according to the standard methods for the examination of water and wastewater [8]. The water samples were taken in the highest tide period. In addition, 1-liter bottles of samples were stored at 4°C. Twelve parameters of water quality were determined including temperature, DO, pH, BOD, phosphate, nitrate, ammonia and heavy metals (Table I).

15 soil sediment samples were collected from the same point as water sampling stations in the lowest tide period. The samples were determined for soil texture, organic matter, pH, CEC. The soil samples and soil sediment samples were dried, ground, passed through two sieve (Ø 2 mm and 0.5 mm). Physical and chemical properties of soil were analyzed for soil texture, organic matter content, moisture, bulk density, pH, CEC, calcium, magnesium, and potassium [9]. Furthermore, water samples, sediments, and hard clams were collected from four zones (zone A, B, C, and D) as shown in Fig. 3. The concentration of heavy metals was analyzed. Arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), and nickel (Ni) were determined as shown in Table I.

TABLE I
PARAMETERS AND ANALYTICAL METHOD

Parameters	Material and Method
Temperature (°C)	Thermometer
pH	pH Meter
DO (mg/L)	DO Meter
Phosphate (mg/L)	Ascorbic Acid Method
Nitrate (mg/L)	Cadmium Reduction Method
Ammonia (mg/L)	Phenate Method
Arsenic (As)(mg/kg)	ICP-MS
Cadmium (Cd) (mg/kg)	ICP-MS
Chromium (Cr) (mg/kg)	ICP-AES
Mercury (Hg) (mg/kg)	ICP-MS
Lead (Pb) (mg/kg)	ICP-MS
Nickel (Ni) (mg/kg)	ICP-MS

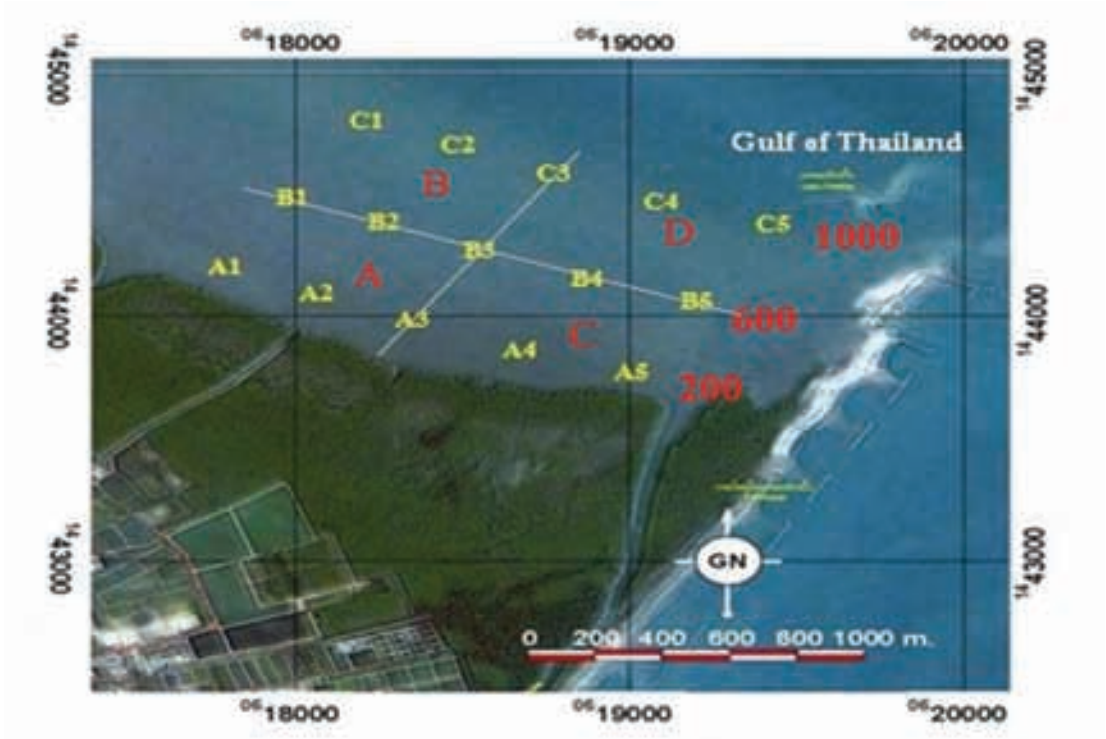


Fig. 3 Marine Water, Soil Sediment, and Hard Clams Sampling Stations at the Royal LERD Project's Coastal Area in 4 Zones (Zone A, B, C, and, D)

C. Statistical Analysis

The mean values of each point of water and sediment sampling station were calculated and then compared with effluent standard for coastal aquaculture safety [10]. The data was input for ANOVA-test, Duncan's New Multiple Range Test (DMRT) and correlation test.

III. RESULT AND DISCUSSION

A. Sediment Quality of Coastal Area

Sediment samples were considered for soil physical and chemical properties. Soil texture in zone A (200 meters far from the coastal line) and zone B (600 meters far from the coastal line) demonstrated the silty loam and the sandy loam respectively. The analytical result of soil particles which were found proved that most of soil particles were sand and silt, details as shown in Table II. The values of sediment quality showed pH as neutral and base properties. The contents of phosphorus, calcium, potassium, magnesium, and CEC were not different significantly. It could be concluded that chemical sediment quality in zone A and zone B had no difference. Nevertheless, percentage of sand particles in zone B was

higher than zone A, but percentage of silt particles in zone B was lesser than zone A. Sediments at 0-15 cm from top soil were habitats of hard clams (*Meretrix meretrix* and *Meretrix casta*). It was found that hard clams were more abundant in zone B than zone A due to sand particles that were high macro-pore, having air inside the pore. Furthermore, the components of sand were consisted of SiO_2, a main component of phytoplankton cells.

TABLE II
SEDIMENT QUALITY IN ZONE A AND ZONE B; 200 AND 600 M FAR FROM LAEM PHAK BIA COASTAL LINE

Parameter	Distance from Coastal	
	Zone A (200 m.)	Zone B (600 m.)
Soil Texture	Silty Loam	Sandy Loam
Sand (%)	44.4	59.4
Silt (%)	50.0	34.8
Clay (%)	5.6	5.8
pH	7.93[a]	8.05[a]
Organic Matter (%)	0.55[a]	0.49[a]
Phosphorus (mg/kg)	2.00[a]	2.80[a]
Calcium (mg/kg)	5,803[a]	5,653[a]
Potassium (mg/kg)	363[a]	310[b]
Magnesium (mg/kg)	688[a]	618[b]
CEC	3.60[a]	3.36[a]

B. Water Quality in Coastal Area

Table III demonstrated that the average temperatures in zone A, B, and C were 32.08±1.77, 31.84±1.70, and 31.59±1.72 °C respectively. The average pH value of water in coastal area was 8.18±0.12, indicating a base property. Dissolved oxygen (DO) in the coastal area had an average value of 6.14±0.82 mg/L, close to the findings in Laem Klat (6.1 mg/L) and Palian Estuary (4.81-7.31 mg/L) [11]. The average phosphate value of the water was 0.25±0.21mg/L, nitrate 0.12±0.05 mg/L, and ammonia 0.04±0.03 mg/L respectively. Even though the values of ammonia did not exceed the standard limits (less than 0.1 mg/L), phosphate and nitrate were above the standard limits (less than 0.045 and 0.06 mg/L respectively).

The record showed that the phosphate values of the water in coastal area of the Royal LERD Project varied due to the fact that Phetchaburi municipality has many purification processing activities, i.e. washing, and cleaning; especially cleaning markets, which involve the use of power detergent that causes a high concentration of phosphate in effluent [12]. The nitrate values of the water in the coastal area were higher than those of normal coastal area due to human waste emitted from various activities in Phetchaburi municipal area and storm water [13]. Additionally, as it was densely populated in Phetchaburi Municipal area, therefore, reflecting ammonia values in effluent. After comparing the results of zone A (200 m), zone B (600 m), and zone C (1,000 m) which were tested in both seasons (wet period and dry period), it could be concluded that there were no significant difference ($p > 0.05$) among the values of the water.

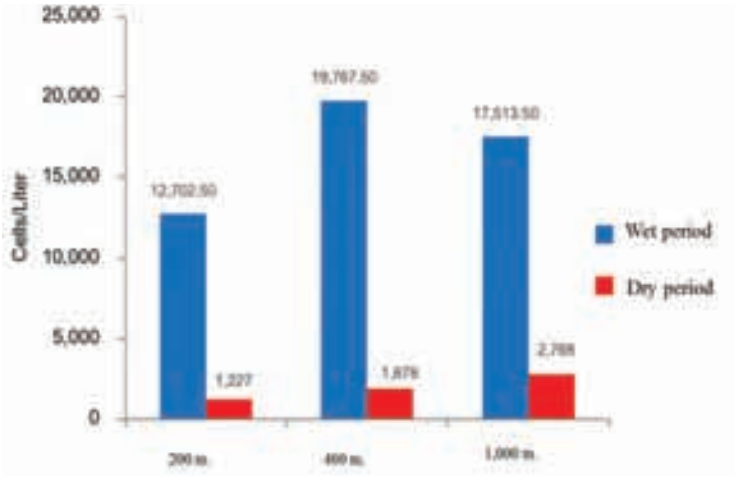
TABLE III
WATER QUALITY; TEMPERATURE, PH, DISSOLVED OXYGEN, PHOSPHATE, NITRATE, AND AMMONIA COMPARED WITH STANDARD VALUES

Parameters	Period	Zone A (200 m.)	Zone B (600 m.)	Zone C (1,000 m.)	Average
Temperature (°C)	Wet Period	33.36±0.03[b]	32.18±0.59[b]	32.34±0.68[b]	32.63±0.52[b]
	Dry Period	33.16±0.30[b]	33.40±1.40[b]	33.08±0.47[c]	33.21±0.77[c]
	Average	32.08±1.77	31.84±1.70	31.59±1.72	31.84±1.73
	Standard	Less than 28	Less than 28	Less than 28	Less than 28
pH	Wet Period	8.26±0.02[b]	8.29±0.06[a]	8.26±0.02[c]	8.27±0.03[c]
	Dry Period	8.06±0.17[a]	8.10±0.21[a]	8.09±0.01[a]	8.08±0.13[a]
	Average	8.18±0.28	8.17±0.16	8.18±0.07	8.18±0.12
	Standard	7.0-8.5	7.0-8.5	7.0-8.5	7.0-8.5
Dissolved Oxygen (DO)(mg/L)	Wet Period	6.58±0.79[b]	6.61±0.11[b]	6.68±0.15[b]	6.62±0.35[b]
	Dry Period	5.14±0.47[a]	5.19±1.41[a]	5.66±0.46[a]	5.33±0.78[a]
	Average	5.89±0.80	6.13±1.03	6.39±0.65	6.14±0.82
	Standard	More than 4	More than 4	More than 4	More than 4
Phosphate (mg/L)	Wet Period	0.54±0.29[b]	0.40±0.09[b]	0.33±0.11[c]	0.42±0.16[c]
	Dry Period	0.12±0.03[a]	0.08±0.01[a]	0.03±0.01[a]	0.08±0.02[a]
	Average	0.34±0.25	0.24±0.23	0.17±0.14	0.25±0.21
	Standard	Less than 0.045	Less than 0.045	Less than 0.045	Less than 0.045
Nitrate (mg/L)	Wet Period	0.23±0.07[b]	0.18±0.07[c]	0.11±0.01[b]	0.17±0.05[c]
	Dry Period	0.09±0.01[a]	0.07±0.02[a]	0.06±0.01[a]	0.07±0.01[b]
	Average	0.15±0.07	0.12±0.06	0.09±0.02	0.12±0.05
	Standard	Less than 0.06	Less than 0.06	Less than 0.06	Less than 0.06
Ammonia (mg/L)	Wet Period	0.07±0.03[b]	0.06±0.01[b]	0.07±0.01[c]	0.07±0.02[c]
	Dry Period	0.02±0.02[a]	0.01±0.01[a]	0.02±0.01[a]	0.02±0.01[a]
	Average	0.04±0.03	0.03±0.02	0.04±0.03	0.04±0.03
	Standard	Less than 0.1	Less than 0.1	Less than 0.1	Less than 0.1

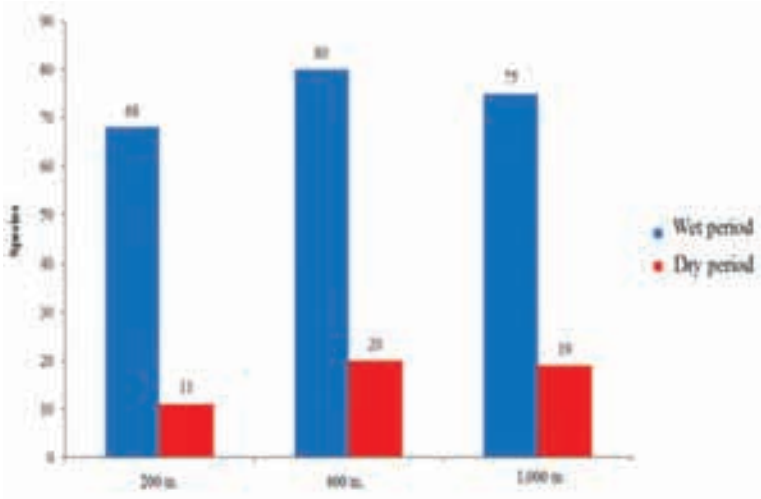
Remarks: The Standard of Effluent Standard for Coastal Aquaculture from Ministry of Natural Resources and Environment, Thailand. ($p > 0.05$).

C. Type and Amount of Phytoplankton in Coastal Area

Phytoplankton were found in 52 genus of 2 division, 104 species, and 55,856.5 cells/L. The findings found 5 genus in division Cyanophyta (5 Species) and 47 genus in division Chromophyta (99 Species). The amount of phytoplankton in wet period and dry period were significant different ($p<0.05$), found 88 species and 49,983.5 cells/L in wet period, as well as 26 species and 5,873 cells/L in dry period. Furthermore, the amount of phytoplankton in Zone A (200 meters from coastal line), Zone B (600 meters from coastal line), and Zone C (1,000 meter from coastal line) were significant difference ($p <0.05$) as shown in Fig. 4. The dominant species belonged to *Coscinodiscus* sp., *Chaetoceros* sp., *Oscillatoria* sp., and *Protoperidinium* sp., respectively.



(a)



(b)

Fig. 4 (a) Species of Phytoplankton at 200 m, 600 m and 1,000 m from Laem Phak Bia Coastal Line, (b) Amount of Phytoplankton at 200 m, 600 m and 1,000 m from Laem Phak Bia Coastal Line

D. Species of Phytoplankton in Gastrointestinal Tract of Meretrix casta

The results in Table IV showed the most values of phytoplankton in gastrointestinal tract of Hard Clams (*Meretrix casta*) in wet period were *Coscinodiscus* sp. (72.33%), *Paralia sulcata* (8.51%), *Oscillatoria* sp. (8.51%), *Protoperidinium* sp. (4.25%), respectively. In dry period, the most values of phytoplankton were *Coscinodiscus sp.* (71.04%), *Cyclotella* sp. (22.36%), *Paralia sulcata* (2.63%), *Thalassiosira* sp. (1.32%), *Synedra* sp. (1.32%), and *Skeletonema* sp. (1.32%), respectively. Although, the results demonstrated that various species of phytoplankton were found in gastrointestinal tract of Hard Clam (*Meretrix casta*), but *Coscinodiscus* sp. was only one species which incomplete cell. It might be concluded that *Coscinodiscus* sp. play vital role in main food of Hard Clams. On the other hand, phytoplankton in gastrointestinal tract of *Phylloda foliacea* (Freshwater Hard Clams) found incomplete cells of *Synedra ulna*, *Pediastrum simplex*, *Peridinium* sp., *Aulacoseira granulate*, and *Ceratium hirundinella* [14]. Diatom, especially *Coscinodiscus* sp. have been high values of carbohydrate (29%) and glucose would become to glycogen, which were

essential for Growth and reproductive of Hard Clams in juvenile stage. Carbohydrate would be accumulated in glycogen form which major carbohydrate sources were diatom [15]-[19].

TABLE IV
SPECIES COMPOSITION OF PHYTOPLANKTON IN GASTROINTESTINAL TRACT OF MERETRIX CASTA IN THE COASTAL AREA OF LAEM PHAK BIA

Phytoplankton	Wet Period			Dry Period		
	Cell/L/Kg	%	Feature	Cell/L/Kg	%	Feature
Coscinodiscus sp.	1,400	29.78	Complete	1,000	13.15	Complete
Coscinodiscus sp.	2,000	42.55	Incomplete	4,400	57.89	Incomplete
Cyclotella sp.	400	8.51	Complete	1,700	22.36	Complete
Oscillatoria sp.	300	6.38	Complete	-	-	-
Paralia sulcata	400	8.51	Complete	200	2.63	Complete
Protoperidinium sp.	200	4.25	Complete	-	-	-
Skeletonema sp.	-	-	-	100	1.32	Complete
Synedra sp.	-	-	-	100	1.32	Complete
Thalassiosira sp.	-	-	-	100	1.32	Complete
Total	783	100		1,086	100	

E. Ecological Factors of Hard Clams Habitation

The relationship between phytoplankton-water quality, phytoplankton-Hard Clams, and soil sediment-Hard Clams were tested by Pearson correlation test. Although, the statistical results showed significant relation between phytoplankton and water quality in Zone 200 meters, 600 meters, and 1,000 meters from coastal line, wet period and dry period, including the values of temperature, pH, salinity, dissolved oxygen, and nitrate as shown in Table V and Table VI, but relation between phytoplankton-Hard Clams and sediment-Hard Clams (*Meretrix casta*) were not significant as shown in Table VII and Table VIII. It could be implied that ecological factors of Hard Clams habitation related to phytoplankton which play important role in food sources of Hard Calms. However, water quality also related to growth of phytoplankton due to phytoplankton need nutrients from sea water for their growth, especially carbohydrate, nitrogen, and phosphate.

TABLE V
CORRELATION ANALYSIS OF MARINE WATER QUALITY AND AMOUNT OF PHYTOPLANKTON IN WET AND DRY PERIOD OF LAEM PHAK BIA COASTAL AREA

Parameters	Phytoplankton		Diatom	
	Wet Period	Dry Period	Wet Period	Dry Period
Temperature	0.623*	0.771**	-0.618*	0.573*
pH	0.434	0.663**	0.429	0.504
Salinity	0.524	0.554*	0.170	-0.388
DO	0.628*	0.284	0.632*	-0.055
BOD	0.466	0.038	0.453	0.334
Phosphate	-0.672**	0.253	0.057	-0.111
Nitrate	-0.176	-0.668*	-0.164	-0.316
Ammonia	-0.022	0.019	-0.010	0.114
Chlorophyll a	-0488	0.288	-0.486	0.365

TABLE VI
CORRELATION OF MARINE WATER QUALITY AND AMOUNT OF PHYTOPLANKTON ON LAEM PHAK BIA COASTAL ZONE

Parameters	Correlation Coefficient (r)		
	200 m.	600 m.	Average
Temperature (°C)	0.9647	0.8245	0.8343**
Total Dissolved Solids	0.1906	0.8018	0.4075
Salinity	0.8312	0.9759	0.8424**
pH	0.8335	0.9265	0.8754**
Dissolved Oxygen	0.9420	0.9823	0.9248**
BOD	0.7295	0.5360	0.5683
TKN	0.5236	0.5981	0.1937
Nitrate	0.7930	0.8173	0.8745**
Ammonia	0.4469	0.4698	0.3217
Orthophosphate	0.4491	0.7385	0.4639
Chlorophyll a	0.4627	0.4660	0.4615

TABLE VII
CORRELATION OF *PHYTOPLANKTON AND MERETRIX CASTA* IN LAEM PHAK BIA COASTAL AREA

Parameter	Correlation Coefficient
Species of Phytoplankton	
- Wet Period	-0.081
- Dry Period	-0.309
Amount of Phytoplankton	
- Wet Period	0.097
- Dry Period	-0.222

TABLE VIII
CORRELATION OF *MERETRIX CASTA AND SOIL SEDIMENT QUALITY* IN LAEM PHAK BIA COASTAL AREA

Parameter	Correlation Coefficient (r)
pH	0.419
Organic matter	0.384
Phosphorus	0.347
Calcium	0.438
Potassium	0.026
Magnesium	0.182
CEC	0.034

F. Accumulation Concentration of Heavy Metals in Marine Water, Sediment, and Hard Clams

According to human activities produced wastewater and it were collected to LERD project for treatment before discharge to sea water. The activities in Phetchaburi Municipality, i.e. washing and cleaning from household, cleaning from small industries which may contaminated some heavy metals in wastewater. The statement of LERD project demonstrated that heavy metals (Pb, Ni, As, Cr, Cd, and Hg) contaminated in wastewater as shown in Table IX. The findings indicated that heavy metals came from municipal activities, would be detected in sedimentation pond, stabilization pond, mangrove forest, and coastal area, respectively. The detected values would be decline from first pond of treatment system (sedimentation pond) to coastal area due to heavy metals also adsorbed with sediment and deposited into bottom of the ponds. The comparing of the values of heavy metals in marine water at Leam Phak Bia coastal area and other places found that mercury (Hg), Lead (Pb), and cadmium (Cd) in marine water at Laem Phak Bia coastal area were similar to other place, except arsenic (As) were less than other places and chromium (Cr) higher than other places as shown in Table X. The values of heavy metals accumulation in sediment at Laem Phak Bia coastal area found arsenic 3.280 mg/kg, cadmium (Cd) 0.023 mg/kg, chromium (Cr) 10.454 mg/kg, mercury (Hg) 0.015 mg/kg, nickel (Ni) 2.963 mg/kg, and lead (Pb) 18.831 mg/L, respectively. The comparing at values of heavy metals in wet period and dry period at Zone A, B, C, and D were not significant different as shown in Table XI. In addition, the detected values of heavy metals in Laem Phak Bia coastal area were also less than the detected values in other places as shown in Table XII caused of community activities and storm water in Phetchaburi town were not pretty much heavy metal contamination from factory activities.

TABLE IX
HEAVY METAL CONCENTRATION IN LAGOON TREATMENT OF PHETCHABURI MUNICIPAL WASTEWATER TREATMENT PLANT

Stations	Pb (mg/kg)	Ni (mg/kg)	As (mg/kg)	Cr (mg/kg)	Cd (mg/kg)	Hg (mg/kg)
Sedimentation Pond	48.10	18.40	0.09	37.60	1.70	2.30
Stabilization Pond	40.57	22.13	18.27	13.20	1.67	0.011
Mangrove Forest	22.13	16.80	4.56	16.79	0.76	0.20
Coastal Area	21.59	2.69	3.04	1.17	1.03	0.05

TABLE X
AVERAGE CONCENTRATION OF HEAVY METAL IN MARINE WATER OF LAEM PHAK BIA COASTAL AND OTHER COUNTRIES

Location	Hg	As	Pb	Cr	Cd	Reference
The King's Royal LERD Project	0.01	0.02	0.03	0.18	0.01	Present study
Port Phillip Bay, Victoria, Australia	nd	2.24-3.16	0.02-0.13	nd	<0.005-0.070	[20]
Open coastal Indian Ocean, Australia	nd	1.00	nd	0.03	0.01	[21]
Al-Khor, Gulf of Suez, Egypt	nd	nd	0.22-2.07	0.06-0.25	0.06-0.60	[22]
North Australian coast, Australia	nd	0.394-1.35	<0.002-0.057	nd	0.002-0.034	[23]
Mauritius Coastal, Mauritius	nd	nd	0.074	0.067	0.234	[24]
Gulf of Suez, Egypt	nd	nd	0.78	0.19	1.99	[25]
North of Borneo Estuaries, Malaysia	nd	nd	0.055	0.009	0.005	[26]
North-western Mediterranean, Italy	nd	nd	0.06	0.35	0.01	[27]
El-Mex Bay, Egypt	nd	nd	14.19	3.68	3.12	[28]
Singapore coastal, Singapore	nd	0.335-2.035	0.013-0.109	0.072-0.350	0.02-0.08	[29]
Tianjin Bohai Bay, China	0.04	1.26	7.18	0.40	0.12	[30]
Eastern Aegean coastal, Turkey	0.01	nd	1.45	0.99	0.21	[31]
Coastal of San Jorge Bay, Chile	nd	nd	0.04	1.33	0.03	[32]

TABLE XI
CONCENTRATION OF HEAVY METAL CONCENTRATION IN SEDIMENT (DRY WEIGHT)

Season	Zone	Concentration of Heavy Metal (mg/kg)					
		As	Cd	Cr	Hg	Ni	Pb
Wet Period	A	3.274	0.025	11.192	0.022	3.054	17.307
	B	3.605	0.025	10.436	0.010	2.937	18.088
	C	3.715	0.025	11.201	0.010	3.262	16.225
	D	3.257	0.025	9.690	0.010	2.830	21.271
	Average (1)	3.463	0.025	10.630	0.013	3.021	18.223
Dry Period	A	3.530	0.020	10.725	0.010	2.995	18.180
	B	2.946	0.020	10.089	0.021	2.788	19.821
	C	2.896	0.020	10.354	0.026	2.790	19.415
	D	3.014	0.020	9.940	0.010	3.047	20.338
	Average (1)	3.097	0.020	10.277	0.017	2.905	19.439
Annual Average		3.280	0.023	10.454	0.015	2.963	18.831

TABLE XII
AVERAGE CONCENTRATION OF HEAVY METAL IN SEDIMENTS OF LAEM PHAK BIA COASTAL AND STANDARD LIMITS

Location	Concentration of Heavy Metal (mg/kg)						Reference
	As	Cd	Cr	Hg	Ni	Pb	
Laem Phak Bia	3.280	0.023	10.454	0.015	2.963	18.831	Study Area
EPA96	11.00	0.58	36.00	-	20.00	37.00	[33]
Florida	9.79	0.99	43.40	0.18	22.70	35.80	[34]
New York	6.00	0.60	26.00	0.15	16.00	31.00	[35]
Wisconsin	9.80	0.99	43.00	0.18	23.00	36.00	[36]
Canada	5.90	0.60	37.30	0.17	-	35.00	[37]
EC&MENVIQ	7.00	0.90	55.00	0.20	35.00	42.00	[38]
AUS/NZ	20.00	1.50	80.00	0.15	21.00	50.00	[39]
HongKong	12.00	1.50	80.00	0.50	40.00	75.00	[40]
EPA96	48.00	3.2	120.00	-	33.00	82.00	[33]
Florida	33.00	4.98	110.00	1.06	48.60	128.00	[34]
New York	33.00	9.00	110.00	1.30	50.00	110.00	[35]
Wisconsin	33.00	5.00	110.00	1.10	49.00	130.00	[36]
Canada	17.00	3.50	90.00	0.49	-	91.30	[37]
EC&MENVIQ	17.00	3.00	100.00	1.00	61.00	170.00	[38]
AUS/NZ	70.00	10.00	370.00	1.00	52.00	220.00	[39]
Hong Kong	42.00	4.00	160.00	1.00	40.00	110.00	[40]

TABLE XIII
AVERAGE ACCUMULATED CONCENTRATION OF HEAVY METAL IN TISSUE AND WATER IN SHELL OF *MERETRIX MERETRIX* AND *MERRETRIX CASTA* IN LAEM PHAK BIA COASTAL AREA

Hard Clams	Accumulated Concentration (mg/kg)					
	As	Cd	Cr	Hg	Pb	Ni
Meretrix meretrix						
Tissue	0.01153	0.00136	0.00095	0.00014	0.00372	0.00231
Water in Shell	0.00107	0.00073	0.00028	0.00004	0.00213	0.00014
Meretrix casta						
Tissue	0.01331	0.00148	0.00161	0.00033	0.00669	0.00248
Water in Shell	0.00122	0.00005	0.00015	0.00005	0.00274	0.00008
Standard Limits						
[41]		2		0.5	1	
[42]		3		1	4	
[38]		1		0.5	1.5	
[43]		2			2	
[44]		2		0.2	10	
[45]		2			6	
[46]		2				
[47]		4			1.7	

Both species of Hard Clams samples from 4 Zone (Zone A, B, C, and D) were determined for heavy metals in tissue and water in shell. The results found Arsenic (As), Cadmium (Cd), Chromium (Cr), Mercury (Hg), Lead (Pb), and Nickel (Ni) were not exceed standard limits for food according to Ministry of Public Health, Thailand, and other standard limits as shown in Table XIII. The values of heavy metals in Hard Clams tissue were greater than water in shell, it could be said that heavy metals were accumulated in tissue because of its bioavailability. However, the detected values indicated that both species of Hard Clams could be safety for consumption.

IV. Conclusion

The marine samples were collected inform various areas; 200 m, 600 m, and 1,000 m far from Laem Phak Bia coastal line while soil sediment and hard clam samples were collected from four zones (zone A, B, C, and D) in vertical and horizontal lines of coastal area. The results demonstrated that water quality was significantly related to plankton, but water quality and soil sediment were not related directly to hard clams. The heavy metals (As, Cd, Cr, Hg, Pb, and Ni) in marine water, sediment, and hard clams (*Meretrix meretrix* and *Meretrix casta*) were also did not exceed the standard limits stipulated by the Ministry of Natural Resource and Environment and the Ministry of Public Health of Thailand. The findings indicated that marine quality played an important role as being an ecological factor of hard calm abundance as the water quality was closely related to the growth of phytoplankton, and diatom (*Coscinodiscus* sp.), while soil sediment might help support for hard clam's habitation, especially in zone 600 meters far from coastal line.

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Potential Impact of UV on Blooming Cyanobacteria toward Herbivore Fishes in Phetchaburi Municipal Wastewater Treatment System, Thailand

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***Abstract*—**Applying herbivore fishes for controlling the algae blooming is very necessary in order to obtain high efficiency for community wastewater treatment and edible fish product by oxidation pond technology. Unfortunately, some species of cyanobacteria (*Spirulina plantensis*, *Microcystis aeruginosa* and *Oscillatoria* sp.) were blooming in oxidation pond especially in the winter season (December to February 2014) such as *Microcystis aeruginosa* produced microcystin as biotoxin that can kill herbivore fishes in oxidation pond about 800 kilogram/pond/day. As a result, the proportion of herbivore fishes growth for controlling algae changed balance was less than 3 fishes/m^2 that turned the wastewater color in oxidation pond from light green to dark green. Moreover, the algae and cyanobacteria blocked up solar radiation in the day time and consume more free oxygen for respiration process in the night time led to ecological balance change in oxidation pond. Cyanobacteria toxin was diluted by high speed of clean water flowing from reservoir and storing flowing water in the empty ponds was another technique that could help herbivore fish survival.

***Keywords*—**Cyanobacteria, ultraviolet, herbivore fishes, wastewater.

I. Introduction

MUNICIPAL wastewater treatment technology through nature-by-nature process is oxidation pond technology. The oxidation pond technology consisted of 5 consecutive ponds, including 1 sedimentation pond and 4 oxidation ponds. The oxidation pond treatment system feed herbivore fishes, ratio of 3-4 fishes/m^2 in order to control the amount of algae in balancing status [1]. On the other hand, oxidation pond treatment system was open system which influenced by the external environmental factor, such as solar radiation, rainfall and wind, etc., especially in winter season. The algae and cyanobacteria were bloom caused of eutrophication. As a result, the herbivore fishes in the pond were die by some kind of cyanobacteria, such as *Microcystis aeruginosa*, and *Oscillatoria* sp., etc. They can produce toxin, for example: Microcystin from *Microcystis aeruginosa* can damage liver of fish then fishes were die at a later time. [2], [3]

This study aimed to find out the potential impact of UV on blooming cyanobacteria toward herbivore fishes in Phetchaburi municipal wastewater treatment system, Thailand.

II. Procedure

A. Location of the Royal LERD Project Site

H. M. The King's initiative nature by nature process on Laem Phak Bia Environmental Research and Development project (LERD Project) at Laem Phak Bia sub distract, Ban Laem district, Phetchaburi province, Thailand which located at latitude 130* 02'40" to 130* 03'20'N and longitude 1000* 05'10" to 1000* 05'10"E, or UTM at 1442240 N to 1443480 N and 0617780 to 0619271 E, approximately 122 km from Bangkok to the south. Actually, the project site is far from the city of Phetchaburi (Phetchaburi municipal) about 12 km, which is composed of local people 40,000 people plus tourists and illegal workers about 10,000 people. The coverage area is about 260 hectares which localizes inside the natural mangrove forest as laid down from Phetchaburi province to Bangkok (Phetchaburi-Bangkok mangrove forest) with about 140 km length and 2 km width (seashore to inland side of mangrove forest).

In fact, the study areas are not only the LERD project site at Laem Phak Bia but also included the whole area of Phetchaburi municipal as point sources of community wastewater which produced approximately 7,000 m^3/day. However, the modification of sewage drainage system in town was taken care for holding back wastewater instead of direct flowing to Phetchaburi River, but turning back by lifting up the pipe ends to collect via culverts to the four sub pumping stations then pumping wastewater to the main collection pumping station (Klongyang collection station). For implementation, Phetchaburi municipal wastewater at Klongyang collection wastewater station had to pump continuously through 18.5 km HPDE pipe to the LERD project site about 3,600 m^3/day to treat at the first pond (sedimentation pond 1), to the second pond (oxidation pond 2), the third pond (oxidation pond 3), the fourth pond (oxidation pond 4) and the fifth pond (oxidation pond 5) for community wastewater treatment as illustrated in Fig. 1. In addition, all five wastewater treatment ponds was laid down in the open air without interruption of sunshine and blow wind in order to promote algae photosynthesis and pond evaporation.

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Free oxygen was added up in wastewater by thermo siphon and thermo osmosis processes for bacterial organic digestion.

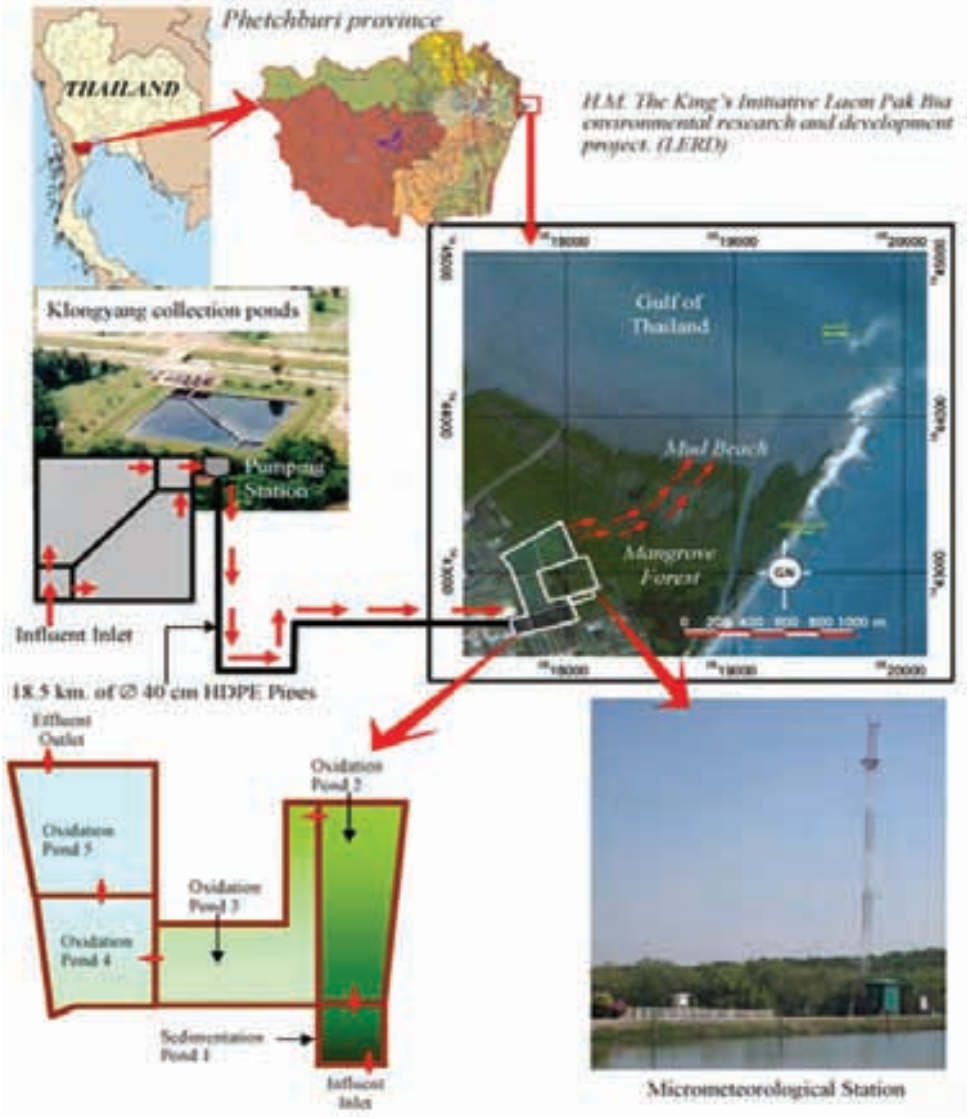

Fig. 1 Municipal Wastewater Treatment System at The King's Royally Initiated Laem Phak Bia Environmental Research and Development Project site, Phetchaburi Province, Thailand

B. Solar Radiation Measurement

The 40 meter micrometeorological tower has been settling down at the east corner of the LERD Project site for measuring the ultraviolet radiation-B (UV-B), ultraviolet radiation-A (UV-A) and visible light (VIBGYOR). Data collection would be daily analyzed to serve needs of any experiment that concerning with H.M. the King's initiative nature by nature process on community wastewater treatment.

C. Water Sampling and Analysis

Water samples were taken at the center of each pond by grab sampling techniques between January to February 2014 once a week in 11.00 am. [4] as shown in Fig. 1, and analyzed for the physical and chemical wastewater quality [5] and samples of cyanobacteria in the water samples. The samples were determined for species and volume of cyanobacteria, according method standard of Kasetsart University [6].

III. Result and Discussions

A. Quantitative of Solar Radiation

The cause of blooming cyanobacteria was hypothesized on the ultraviolet radiation (UVR) and all wavelength of spectrum. Therefore, the measurement of solar energy was conducted at the LERD Project. Unfortunately, the instrument can measure only UV-A and UV-B, not for UV-C measuring, but primary evaluation can be taken in relation to the blooming cyanobacteria after overflowing the oxidation pond before moving into mangrove forest. However, the measuring solar radiation on the spectrum (violet, indigo, blue, green, yellow, orange and red), UV-B and UV-A were shown in Fig. 2.

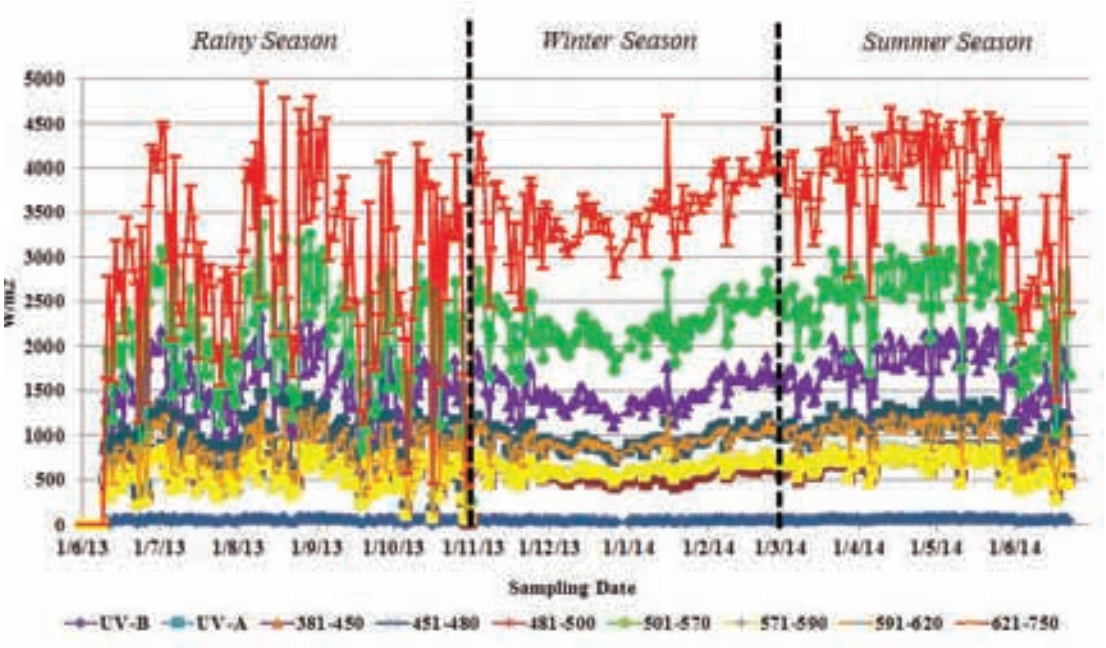

Fig. 2 Incoming Solar Radiation Between 300-750 nm in rainy season, winter season and summer season

From Fig. 2, the solar radiation in winter season were quite regularly than rainy season and summer season because in winter season was low humidity. As a result, the solar radiations can exposure through atmosphere to the surface of wastewater and wastewater body.

Results of measuring spectrum (violet, indigo, blue, green, yellow, orange and red) were shown in Fig. 3 in which the UV-A, UV-B and the high quality spectrum that can exposure to the surface of the wastewater was red light (621-750 nm; avg. 3,499.8 W/m^2), green light (501-570 nm; avg. 2,234.9 W/m^2) and violet light (381-450 nm; avg. 1,469.0 W/m^2), respectively. The high quality spectrum were beneficial for photosynthesis process of algae [7]-[8], especially cyanobacteria because they have a lot of pigment in them cell, i.e. chlorophyll, carotenoids, phycobilin, phycoerythrin and phycocyanin. These pigments have ability to absorb radiation differently. The chlorophyll can absorb violet light and red light, carotenoids can absorb green light and phycobilin/ phycoerythrin/ phycocyanin can absorb green light and yellow light. This corresponds to radiation that exposure to the surface of wastewater in winter season [9]-[11].

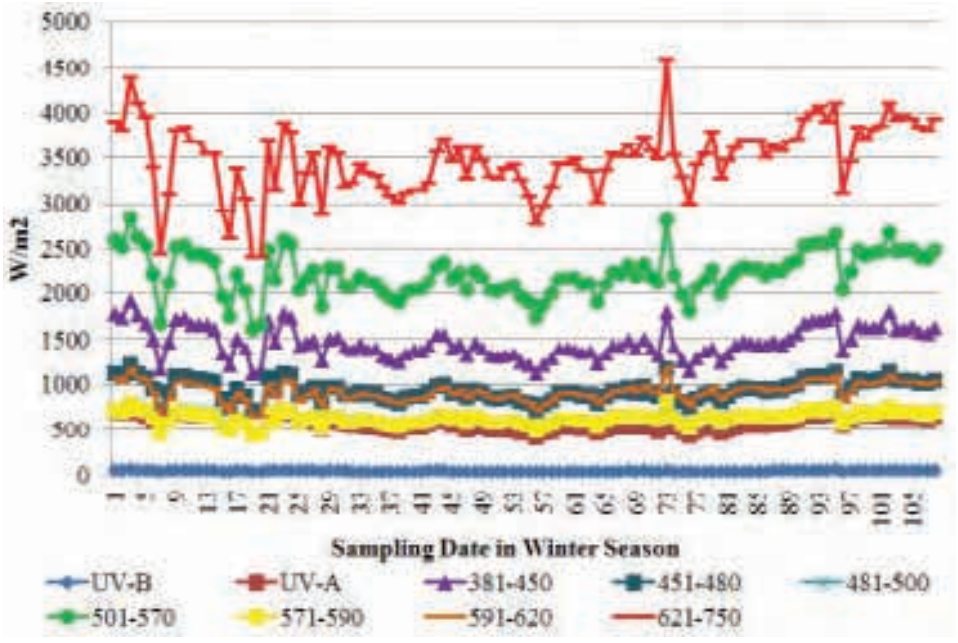

Fig. 3 Incoming Solar Radiation Between 300-750 nm in winter season

B. Wastewater Quality of Oxidation Pond Technology

The amount of cyanobacteria in oxidation pond often occur in winter season, The results found that wastewater temperature 27.5-29.0 °C was suitable for cyanobacteria growth condition [12] and effected to water quality change as show in Table I.

TABLE I
WATER QUALITY OF OXIDATION POND IN WINTER SEASON (JANUARY-FEBRUARY 2014)

Parameter	WW1	WW2	WW3	WW4	WW5	Standard
Temp. (°C)	29.0^{a}	28.5^{ab}	28.5^{ab}	27.5^{c}	28.0^{bc}	-
pH	7.5^{c}	8.5^{b}	8.5^{b}	9.0^{a}	9.1^{a}	5.5-9.0
BOD (mg/L)	61.5^{a}	26.8^{b}	26.5^{b}	24.3^{c}	19.3^{d}	≤20
TKN (mg/L)	12.2^{a}	5.4^{c}	6.6^{b}	4.9^{d}	4.0^{e}	≤20
NH_4^+ (mg/L)	4.2^{a}	1.1^{c}	1.7^{b}	0.8^{d}	0.2^{e}	-
NO_3^- (mg/L)	0.4^{a}	0.3^{a}	0.2^{a}	0.3^{a}	0.3^{a}	-
TP (mg/L)	3.3^{a}	1.2^{b}	1.2^{b}	0.6^{c}	0.3^{d}	≤2
PO_4^{3-} (mg/L)	3.8^{a}	2.1^{b}	1.1^{c}	0.5^{d}	0.3^{e}	-
SS (mg/L)	48.6^{e}	57.5^{c}	55.7^{b}	60.5^{a}	57.2^{d}	≤30

Remark:-Standard = standard effluent quality from community wastewater treatment plant by Pollution Control Department, Thailand. WW1 = Sedimentation pond 1, WW2 = Oxidation pond2, WW3 = Oxidation pond 3, WW4 = Oxidation pond 4, WW5 = Oxidation pond 5

From Table I, The BOD, TKN, NH_4^+, NO_3^-, TP and PO_4^{3-} were significantly decrease that showed the treatment efficiency as 68.6, 67.2, 95.2, 25.0 90.9 and 92.1%, respectively. However, the treatment system decreased SS, because the aerobic bacteria were degraded organic substance to inorganic substance and algae used inorganic substance as energy source for photosynthesis process together with winter season, the sky was very clear and wastewater temperature was down to 27.5-2 9∘C which were suitable for cyanobacteria blooming [12] and made pH content increase.

C. Cyanobacteria Species in Oxidation Pond Technology

The study found phytoplankton in three Divisions: (1) Division Cyanophyta (Class Cyanophyceae; blue green algae 11 Species); (2) Division Chlorophyta (Class Chlorophyceae 27 Species and Class Euglenophyta (euglenoids) 16 Species; (3) Division Chromophyta (Class Bacillariophyceae; diatom 7 Species, and Class Dinophyceae; dinoflagellate 2 Species. Cyanobacteria in oxidation pond often occurred in winter season as shown in Table II.

TABLE II
SPECIES AND CYANOBACTERIA PERCENTAGE OF OXIDATION POND IN WINTER SEASON (JANUARY-FEBRUARY 2014)

Cyanobacteria	%	WW1 (cell/m^3)	WW2 (cell/m^3)	WW3 (cell/m^3)	WW4 (cell/m^3)	WW5 (cell/m^3)
Spirulina plantensis	82.7	1.1×10^{6}	6.7×10^{8}	7.4×10^{8}	3.7×10^{8}	2.8×10^{6}
Microcystis aeruginosa	11.1	3.5×10^{4}	7.3×10^{5}	3.8×10^{7}	1.7×10^{8}	2.6×10^{7}
Oscillatoria sp.	3.5	1.3×10^{6}	5.8×10^{7}	1.1×10^{7}	4.7×10^{6}	1.9×10^{5}
Cylindrospermopsis philipinensis	1.2	-	-	-	4.6×10^{5}	2.7×10^{7}
Rhaphidiopsis sp.	1.2	2.2×10^{7}	4.9×10^{6}	1.6×10^{4}	6.5×10^{4}	3.4×10^{5}
Chroococcus minutes	0.06	1.1×10^{6}	6.5×10^{4}	7.7×10^{4}	3.2×10^{4}	5.8×10^{4}
Gloeocapsa sp.	0.01	1.4×10^{5}	5.5×10^{4}	4.1×10^{4}	-	-
Anabaenopsis sp.	0.01	-	-	-	-	2.2×10^{5}
Microcystis incerta	0.01	6.6×10^{3}	-	-	-	1.1×10^{5}
Spirulina subsalsa	0.01	-	-	-	-	1.2×10^{6}
Merismopedia minima	0.001	-	9.5×10^{3}	5.4×10^{3}	6.6×10^{3}	4.1×10^{3}

Remark:- WW1 = Sedimentation pond 1, WW2 = Oxidation pond, WW3 = Oxidation pond 3, WW4 = Oxidation pond 4,WW5 = Oxidation pond 5

Normally, eutrophication in open surface water usually occur in eutrophic lake (high nutrient content NO_3^- and PO_4^{3-}) but the wastewater quality in oxidation pond, which is the Royal LERD Project's technology were significantly decrease from WW1 to WW5 and low nutrient. (Table I). But found cyanobacteria (i.e. *Microcystis aeruginosa* and *Oscillatoria* sp.) were significantly increase in winter season and significantly decrease in the beginning of summer season. (Fig. 3) because they have five pigments in their cells. As a result, they could absorb high quality of red light, green light and violet light in winter season by photosynthesis process then increase cells up. Amount of *Microcystis aeruginosa* and *Oscillatoria* sp. decreased in summer season.

Throughout the blooming cyanobacteria duration in winter season (January-February 2014). Herbivore fishes, control balance of algae were died at most 800 kg/pond/day. In order to keep balancing of ecological process. Cyanobacteria were diluted by high speed of clean water flowing from reservoir and storing flowing water in the empty ponds were another techniques that could help herbivore fish survival [8].

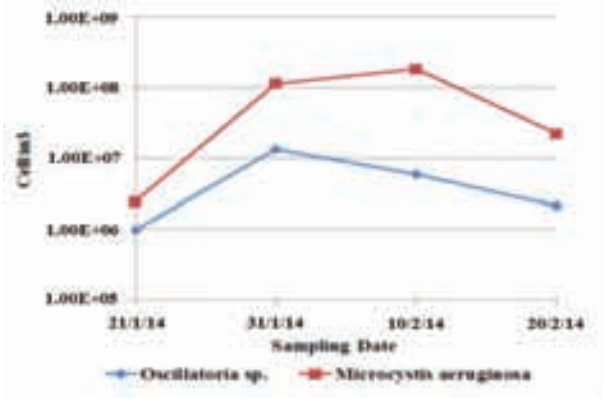

Fig. 3 The average amount of *Microcystis aeruginosa* and *Oscillatoria* sp. in oxidation pond technology (winter season)

IV. Conclusion

Potential impact of solar radiation in winter season generated eutrophication in oxidation pond technology at the LERD Project, Phetchaburi Province, Thailand because the solar radiation in winter season was highest intensity when compared with rainy season and summer season. The cyanobacteria in wastewater, they had five pigments in their cells, which absorbed red light, green light and violet light from solar spectrum by photosynthesis process and increased cells. In addition, The Cyanobacteria could produce biotoxin "microcystin" which could damage the liver of fish and made it dead at later time.

Acknowledgment

We are thankful to staffs of The King's Royally Initiative Laem Phak Bia Environmental Research and Development Project for their supporting. We are also in debt to The Chaipattana Foundation for conducting and funding this study. We would like to give thanks to the Eco-Science Community Research Group (ESCRG), Department Environmental Science, Faculty of Environment, Kasetsart University for facilitating the personnel and laboratories.

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Environmental Training on Home Garbage Separation for Small Fisheries Village, Phetchaburi, Thailand

T. Boonprakong, P. Sotong, S. Boonmang, S. Sawangjit, J. Phermsin

***Abstract*—**The research was focused on environmental education (EE) for garbage separation at home by knocking-door technique in order to sustain long term management as selected Laem Phak Bia fishery village to be a case study. The study was divided into three phases: Firstly, EE knocking-door technique being applied for training villagers; Secondly, villagers self-implementation by dropping their home garbage into the separate bins which were organic waste, recycle, hazardous waste and shell bins. Then, the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project supported to collect only organic waste every day and made composting for 3 years and without supporting from the King's project for 4 years; and Thirdly, monitoring knowledge and knowing how to separate home garbage. The research results found that the villagers were satisfied in knowledge, attitude, awareness, sensitivity and skills. In consequence, self-implementation was acceptable in behavior for separating home garbage in which the village has been found somewhat clean without garbage in forms of plastic bags, paper, wooden boxes, remnants of iron, cloth, wood, paper and others. Expectedly, the knocking-door technique for environmental education training on home garbage separation in small fishery village is applicable for long term management.

***Keywords*—**Training, garbage separation, village, environmental education.

I. Introduction

THAILAND has been confronted with over excess for more than 30 years, especially the big dense-populated cities as well as tourist areas such as Bangkok, Chiangmai, Chiangrai, Phuket, Pattaya, Samui Island and Khon Kaen. Actually, a man can produce the garbage between 0.5-1.0 kg/day that should be distributed all over the country with lower concentration, but they moved in high population density along with bunchy garbage of them. Consequently, the physical, chemical, and biological problems have generated to air and water pollution including visual pollution and losses of good lands for agriculture according to use constructing sanitary landfills for in fertile paddy areas. In doing so, those good lands for agriculture can never be back to grow any agricultural crops because of chemical toxicant contamination and still being long-period toxic soils. The dumping garbage and active sanitary landfills might pollute some solid wastes and toxic leachate into the neighboring crop growing areas and draw into public water sources such as creeks, streams, rivers and reservoirs [1]. It has been known that the main cause was on by means of unseparated organic, recycled and hazardous wastes before landfilling. Because of previous reason, government has promoted the environmental education (EE) program not only by Department of Environmental Quality Promotion, Ministry of Natural Resources and Environment (DEQP-MNRE), but also by Ministry of Education (MOE) for formal education curriculum from kindergartens to high schools and for non-formal education program through extension program, radio, TV, brochure, newspaper and others. Unfortunately, the EE implementation seemed not to serve the DEQP-MNRE policy in which the community garbage problems have found not only existing but also quantitative increasing as well as its toxicity [2]. Long-period experiences could be pointed out that the formal and informal EE education were followed traditionally and collectively day-to-day lifestyle rather than specification to take EE knowledge through transferring technology to the target learners whom are perceptible the EE knowledge up to skills how to do accomplish such things [3]-[5]. Generally, environmental conservation is all-time ethics in everyone's mind in order to do things with self-protection. Life-long learning environment plays vital role in culturing the levels of environmental education (in-depth knowledge, attitude, awareness, sensitivity and skills) in which the formal education ought to learn not only in classrooms, but also field works from kindergarten to high school graduation and throughout the college [3],[6]-[8]. For non-formal education process, the learning by doing at home and also at working sites as well as during participating group activities has been agreeable to have the habit of environmental performance with automatically carefulness which due to the environmental ethics in mind. Besides the non-formal education as learning by doing, another performance of environmental activities should be included by radio, TV, movies, posters, camping, etc. in terms of additional skills for inborn train in environment. If so, it is believed that his or her behaviors in environmental issues are ready to become good environmental conservationists of the country. In other words, learning by doing environment at home plus some experiences from outside activities could be

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appropriated for EE program [2]-[4], [9]. Looking back on Thai culture, the young kids have to obey their parents without any argument in which the way of cultural transferring from generation to the next generation. Besides, the people are familiar with mouth to mouth communication rather than acknowledging from someone else in order to make clear understanding the mentioned story. This is the reason why Thai people prefer to follow from mouth to mouth rather than group meeting and mass communication, especially people in remote areas. By referring aforesaid Thai culture, this research took the fisheries village around the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project (the Royal LERD project) sited at Laem Phak Bia sub-district, Ban Laem district, Phetchaburi province as the case study for knocking door technique for one by one training on garbage separation before taking away to dispose by responsible municipality. Hopefully, the knocking door technique can break through the obstacles of home garbage separation in order to take its results to propaganda in another parts of the country [2], [4]-[5], [7], [10].

II. Methodology

According to long-time consecutive research, the method are divided into 3 phases in order to follow the home garbage separation of Phaneun village (small fisheries village with population 741 persons from 154 household) which is located around the Royal LERD project site and taken as the study area. The details of study on home garbage separation by knocking door technique as followed [11]: -

A. Phase 1: Training on Home Garbage Separation

Training on home garbage separation was taken in the year 2006 by surveying the area of study site, home garbage dumping site, observing home garbage discarding and managing, and home garbage structure determining. Then after, the garbage structure inventory, field observation, interviewing questionnaire and process of knocking door technique were constructed and validly examined by experts in garbage management. Twenty years of EE background and experience in solid waste management were trained on home garbage disposal for a week before starting up interviewing to sampling fisherman villagers. Consequently, the EE program on home garbage separation was started up by knocking door technique together with trainers (interviewers), questionnaire, posters, and brochure as the trading materials. The pre-test was made before interviewing about 10 minutes, then the home garbage separation began to teach for 30 minutes in each family by using the content from prepared questionnaires as the interviewing questions. After finished teaching, the post-test was conducted to evaluate each interviewee on home garbage separation. Lastly, the past knowledge on home garbage separation was re-tested after the first transferring about 3 months, along with studying on garbage structure.

B. Phase 2: Evaluating on Behaviors of Home Garbage Separation

The EE study toward evaluating on behaviors of home garbage separation was conducted during the years of 2007-2014 (7-year period) under the volunteer to collect the daily organic wastes for 3-year consecutive period from Phaneun village and to ferment by concrete box technology of the Royal LERD project to produce the organic compost. Every garbage-collection day, the knowledge of home garbage repetitiously propagated through local cable radio in order to reach the 5-level EE knowledge such as knowledge, attitude, awareness, sensitivity and skills. Since then, the livelihood of the Phaneun village natives have been kept in normal lifestyle of fishermen without any more assistance in collection of home organic garbage to ferment in concrete box technology to make the compost. In other words, the Royal LERD project has had nothing to do with home organic garbage to make the organic compost because the people of Phaneun fisherman village have plenty of knowledge, right attitude, knowing how home garbage coming from and its destiny, quick responsibility of sensitivity, and high skills for doing with home garbage separation.

C. Phase 3: Testing on Knowledge of Garbage Disposal

The year of 2015 is the time for testing how to manage solid wastes of fisherman-community Phaneun village which surrounds the Royal LERD project site by knocking-door technique that have done in 2007.

III. Results and Discussions

A. First Phase Results: Achievement of Home Garbage Separation

1. Background Information

From random selecting sample houses by knocking door technique for interviewing about home garbage separation of 128 volunteers, it included men 85.90% and women 14.10% with the age ranging 31-45 years (36.70%), 46-60 years (28.10%) and less than 31 years plus above 60 years (35.80%) along with education background of primary school level (6-year schooling period). Their occupation were identified as fisheries 50%, salt industry 37.50% and miscellaneous occupation 12.5% of the total population 741 person of Laem Phak Bia county, Laem Phak Bia sub-district, Ban Laem district, Phetchaburi province.

2. Home Garbage Structure

The results of field inventory is found that the fisherman people of Phaneun village, only Laem Phak Bia county (total population 741 persons from 154 household) produced the home garbage 0.43 kg/person/day with the garbage structure of organic wastes, recycle and hazardous waste for: (1) before 2006- year training 10,950 g (46.2%), 12,160 g (51.4%) and 2.4%), respectively; (2) after 1-month training 3,510.30 g (76.1%), 989.20 g (21.4%) and 115.30 g (2.5%), respectively; after 2-month training 2,438.90 g (73.2%), 836.30 g (25.1%) and 57.1 g (1.7%), respectively; after 3-month training

2,112.20 g (68.9%), 898.70 g (29.3%) and 56.5 g (1.8%); year-2013 training 3,550 g (53.6%), 10,680 g (67.7%) and 1,540 g (9.8%), respectively; year-2015 training 5,500 g (53.6%), 4,610 g (44.9%) and 150 g (1.5%) as shown in Table I and garbage structure is detailed in Table II.

In fact, the home garbage data as shown in Table I and II obtained from randomly quarterly sampling from household bins. The results of structure evaluation have been shown very narrow variation for long period intervals, especially organic wastes [11]-[15]. Even if, the training program has been accomplished for three times, they are still be produced home garbage very closed to each other. It is noticeable in hazardous wastes which is not only small quantity, but also hardly change its quantity no matter before neither after training, due to the villagers deal with marine animals as producers for selling out to another parts of the country [3],[8],[16].

TABLE I
QUANTITY AND STRUCTURE OF HOME GARBAGE AS RANDOMLY TAKEN FROM COLLECTED BINS UNDER TRAINING PROGRAM IN HOME GARBAGE SEPARATION TRANSFERRING THROUGH KNOCKING DOOR TECHNIQUE TO PHANEUN COUNTY PEOPLE

Time	Quantity and Structure of Home Garbage (gm)			
	Organic Wastes	Dry Wastes	Hazardous Wastes	Total
Before 2006 year training	10,950 (46.2%)	12,160 (51.4%)	570 (2.4%)	23,680
After 1 month training	3,510.30 (76.1%)	989.20 (21.4%)	115.30 (2.5%)	4,614.80
After 2 month training	2,438.90 (73.2%)	836.30 (25.1%)	57.10 (1.7%)	3,332.30
After 3 month training	2,112.20 (68.9%)	898.70 (29.3%)	56.50 (1.8%)	3,067.40
2013 year (Testing EE)	3,550 (22.7%)	10,680 (67.7%)	1,540 (9.8%)	15,770
2015 year (Interviewing)	5,500 (53.6%)	4,610 (44.9%)	150 (1.5%)	10,260

TABLE II
DETAILS OF HOME GARBAGE AS COLLECTED QUARTERLY SAMPLING IN YEARS OF 2006, 2013 AND 2015 FOR REPRESENTING PRODUCTS OF PHANEUN COUNTY POPULATION AT LAEM PHAK BIA SUB-DISTRICT, BAN LAEM DISTRICT, PHETCHABURI PROVINCE

Garbage	Quantity and Structure of Home Garbage					
	2006		2013		2015	
	(g)	%	(g)	%	(g)	%
1.Organic wastes	10,950	46.24	3,555	22.50	5,500	53.61
2.Plastic	7,280	30.74	8,370	53	1,850	18.03
3.Glass	1,060	4.48	950	6	700	6.82
4.Paper	2,120	8.95	470	3	780	7.60
5.Scraps of wood	50	0.21	190	1.20	900	8.78
6.Foam	390	1.65	550	3.50	100	0.97
7.Piece of cloth	400	1.69	-	-	120	1.17
8.Rubber	440	1.85	150	1	-	-
9.Hazardouswastes	570	2.41	1,540	9.80	150	1.46
10.Can and other	420	1.77	-	-	160	1.56
Total	23,680	100	15,770	100	10,260	100

knowing to

3. Garbage Problem and Causes

Results from interviewed persons (100 persons) indicated that organic wastes were accused as the most important problems about 68%, recycle and non-degradable wastes 31% and hazardous wastes 1% of interviewed people of Phaneun county, Laem Phak Bia sub-district, Ban Laem district, Phetchaburi province.

4. Conventional Garbage Disposal

The results from person to person interviewing through knocking door technical process revealed that most of the Phaneun county people disposed the home garbage, firstly by burning in the open space and dumping on ground 50.63%, secondly collecting in bins 20.44%, the others 28.93%. It was delighting that 20.12% of the population of Phaneun county. Separate home garbage for getting the benefit not only for selling recycled wastes but also for feeding pets, the others 8.81% throwing on their convenience.

5. Field Garbage Contaminants

It is awful to state that the home garbage did not dispose in proper means, only dumping and burning in the open space on the ground floor of public land and throwing away in natural canals and water courses. Surprisingly, the home garbage was left over in the village that caused unpleasant visualization as well as it looked undesirable environment for well-being livelihood.

6. Training Achievement of Knocking Door Technique

After transferring home garbage separation by person to person interviewing through knocking door technique in the year of 2006, the achievement obtained the grade less 15 marks before training for 7.03% and after training 3.91% of total trainees; grade between 16-18 marks before training 60.94% and after 42.96% of total trainees; and the highest grade between 15-20 marks before training for 32.03% and after training for 53.13% of total trainees as shown in Table III.

TABLE III
ACHIEVEMENT OF TRAINING HOME GARBAGE SEPARATION THROUGH KNOCKING DOOR TECHNIQUE IN 2006 AND 2013 AT PHANEUN COUNTY, LAEM PHAK BIA SUB-DISTRICT, BAN LAEM DISTRICT, PHETCHABURI PROVINCE

Grade	Year 2006		After training 2015 (%)
	Before training (%)	After training (%)	
The grade less 15 marks	7.03	3.91	32.31
Grade between 16-18 marks	60.94	42.96	43.08
Grade between 19-20 marks	32.03	53.13	24.61

The results in Table III make sure that knocking door technique can increase the highest marks (15-20 marks) of Phaneun county population's knowledge on home garbage separation from 32.03% up to 53.13% for the amount of greater 22.1% (69%). As stated at the beginning, mouth to mouth could be accepted as person to person, if the conversation is not quite clear, the repeat questions and answers can spend more time until everything is satisfied each other. Also, teaching materials such as posters, article, brochure and others, might support the knowledge content of home garbage separation rather than only description. Another factor positively affecting on knocking door technique for increasing understanding the content might come from the training atmosphere in the trainees' home that make them high self-confidence and concentration to percept the content without worrying. Lastly, knocking door technique is the combined processes between description and practicing how to separate by doing the same time which is exactly stimulated to increase skills for home garbage separation one way or another. However, training in the year of 2013 is still shown the same trend as found in 2006-year training program. The reason would be permanent settlement of Phaneun county people as the same as its location about 2-km distance from the Royal LERD project site which influences on informational connection, especially the knowledge of home garbage separation

Nevertheless, the people of Phaneun county can make up grade after 3-month training home garbage separation on organic wastes 99.73%, recycle and non-degradable wastes 99.76%, and hazardous wastes 97.52% which is indicated the efficiency of knocking door technique beyond the other techniques such as radio and TV broadcast, cable TV, cable voice, brochure, newspaper, and printing materials. The interpretation is focused on influence on frequency of training activities which concern with frequency of repeating in hearing, doing and becoming familiar. The previous statement brings the trainees going throughout the wright tract of EE knowledge, attitude, awareness, sensitivity, and skills.

B. Second Phase Results: Behaviors of Garbage Disposal

According to obtaining high grades from person to person interviewing for training home garbage separation through knocking door technique, the organic wastes from home were brought to drop into car of the Royal LERD project for making compost through fermentation process in concrete boxes that is located inside the project site about 2 km from Phaneun county. The previous garbage disposal process was proceeded very smooth cooperation between the citizen of Phaneun county and the Royal LERD project team until the beginning of 2013 seeing the sign of failure. Therefore, the study on separation capacity of home garbage through knocking door technical process is expected to turn the disordered garbage disposal to become aware of solving home garbage separation.

For 2013-year training, there was 36.5% of Phaneun county people still separating home garbage by taking organic wastes for feeding animals, 33.5% taking recycled wastes for selling, 19% dropping into household bins for composting at the Royal LERD project site and lastly 11% burning in the open spaces as shown in Table IV which are in harmony [5], [11]-[13].

TABLE IV
CONVENTIONAL GARBAGE DISPOSAL MANAGEMENT OF PHANEUN COUNTY PEOPLE WITH 2006-YEAR TRAINING HOME GARBAGE SEPARATION AND 2013-YEAR STUDY

Garbage disposal management	Garbage Disposal Management (%)	
	2006	2013
Burning in the open spaces	50.63	11.00
Dropping into bins	20.44	19.00
Taking recycled wastes for selling	20.12	33.50
Feeding animals	8.81	36.50

The mentioned results revealed that the Phaneun county people have kept separating home garbage for feeding animals (36.5%), for selling (33.5%) and for composting (19%) combining together 89% and only 11% still burning in the open spaces. Interpretation can be stated that knocking door technique for home garbage separation is caused to train the fisherman of Phaneun county to have better skills for home garbage separation for at least 7 years (2006-2013) after training in 2006 (Table IV).

C. Third Phase Results: Knowledge of Garbage Management

1. Background Information

The research results by questionnaires and interviewing from the samples of 65 persons, which belongs to the fisherman family in the county of Laem Phak Bia of Phaneun village (total population 881 persons from 226 household)

were identified as 70.77% women and 29.23% men, belonging the age mostly between 41-45 years (32.31%), secondly including both between 31-40 years (21.54%) and 51-60 years (21.54%) and lastly less 31 years plus above 60 years (24.61%).

2. Existing Garbage Structure

Accordance with 2015-field inventory is discovered the home garbage structure in comparison with the year of 2006 and 2013 as presented in Table II, in which the uncertainty of quantitative home garbage was discovered from year 2006 to 2013 and 2015 without any obligation, but it could be the causes of fisherman home stay due to severe storms in the sea and also economic availability. However, the declined tendency is placed on organic wastes, plastic, glasses, papers, cloths, cans and hazardous wastes which are almost the whole structure of home garbage as produced by Phaneun county families.

3. Achievement of Knocking Door Technique

An application of knocking door technique for separating home garbage as produced by Phaneun county families under 3-program training in 3-months in the year of 2006. In consequence, the obtained knowledge of home garbage serration was graded in high percentage of 32.03% before training up to 53.13% after training which enhanced the Phaneun county people to separate wet organic garbage 99.73%, dry garbage 99.76% and hazardous garbage 97.52%. Unfortunately, the 7-year period without consecutive training are the main cause of EE knowledge diminishingly return that decreased the testing marks from 53.13% in 2006 down to 24.61% (Table III) by increasing the lowest mark (less 15%) in 2015 and decreasing in 2006 as shown in Table III. Although the grading mark for evaluating the knowledge of home garbage management decreased as found in 2015, the existing behavior of managing garbage disposal looks better than garbage disposal behavior before training in 2006. This would say that the skills which obtained from 2006-year training might support on how to manage the garbage disposal, particularly knowing how to separate the home garbage before disposal as followed the concept of [2]-[3], [5], , [8]-[17]. The research results from 65 villagers were showed that 62.77% knowledge, 69.69% attitude, 71.54% awareness, 60.92% sensitivity and 61.15% skills.

D.Social Benefits and EE Promotion

Aforesaid research results on promoting environmental education (EE) around the Royal LERD project site have been accomplished not only cooperation to keep the Phaneun village clean, but also to conserve the mangrove forest which is the most fruitfulness of marine animals for economic supporting. Joins together based on the Royal LERD project, Phetchaburi municipal wastewater has been transferred and treated by anaerobic organic digestion process in 18.5-km HPDE pipe during transferring to oxidation pond, vertical flow constructed wetlands, horizontal flow constructed wetlands and mangrove reforestation in order to be treated that made better water quality together with carrying dissolved organic matters as nutrient sources of phytoplankton for herbivore marine animals. This could be the reason to support why marine animals increasing in this region. Under previous statement, the villagers can earn income approximately 3,620 USD/household/year from collecting shells, mussels, hard clam, crabs and also from catching fishes and shrimps that make them better livelihood. In establishing, there have been a lot of migrated people before 1990, which was the beginning year of the Royal LERD project, gradually moving in due to more fruitfulness of economic fisheries.

It is remarkable that knocking-door EE program is one among effectively positive measure for not only making Phaneun village clean, but also persuading people joining together to conserve water quality in natural and man-made mangrove forest one way or another.

IV. Conclusions

Accordance with failure of home garbage separation has been mentioned for long period of time because of strong Thai culture being familiar with mouth to mount communication rather than using teaching media for both formal and informal education. So, the knocking door technique is proposed as the better transferring EE knowledge technology along with interviewing person to person by taking Phaneun county-fisherman village, Laem Phak Bia sub-district, Ban Laem district, Phetchaburi province as located around the Royal LERD project site. The study was divided into 3 phases: firstly, training on home garbage separation; secondly, evaluating on behaviors of home garbage separation; testing on knowledge of garbage disposal. There were 100 houses as taken from random sampling for studying on training knowledge of home garbage separation by interviewing through knocking door technique in 2006, 2013, and 2015. The results of 3-time in 3-month training in 2006 provide grading of 15-20 marks for the highest 53.13% of Phaneun county people which enhance on the people whom can be eligible for separating on wet garbage 99.73%, dry garbage 99.76%, and hazardous garbage 97.52%. After 7 years free from training, the knowledge of Phaneun county people is decreased in grading from 53.23% in 2006 down to 24.61% in 2015. The decreasing grade did not effect on behaviors of garbage disposal because of people had skills on how to do with home garbage management. Finally, knocking-door EE program is not only a tool for persuading the Phaneun villagers to know how to dispose the community garbage, but also a tool for understanding the benefit of community wastewater treatment for support their economy.

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A Conceptual Framework of Integrated Evaluation Methodology for Aquaculture Lakes

Robby Y. Tallar, Nikodemus L., Yuri S., Jian P. Suen

***Abstract*—**The ecological and hydrological system in lakes has a unique characteristics with many functions within. Aquaculture lakes are one of the most appropriate examples. This paper developed a conceptual framework of integrated evaluation methodology for aquaculture lakes. Integrated Aquaculture Lakes Index (IALI) was designed to provide an effective, low-cost assessment of aquaculture lakes for developing countries. Certain indicators used to compute the IALI include representative measures of exterior attributes (aesthetics, environmental and socio-economic variables) and interior attributes (water quality and fish types). The aesthetics, environmental and socio-economic indices are reliable metrics also used in Aesthetic Aquaculture Lake Index (AALI) and Rapid Appraisal for Fisheries Index (RAPFISH) score calculations for aquaculture lakes. Meanwhile, water quality and fish types indices are used in Aquaculture Water Quality Index (AWQI) score calculation. The evaluation index system is established according to the current condition of aquaculture lake, and the weight within each index can be calculated by Analytic Hierarchy Process (AHP) method. The offered integrated evaluation methodology for aquaculture lakes in this paper is trying to construct the evaluation index with the considerations of biotic and abiotic factors. It should also allow for prioritization of attribute needs and provide an easily understood representation of each variable to the general purpose. A comprehensive regional assessment is needed for revealing related variables within aquaculture lakes.

***Keywords*—** AHP, Aquaculture lakes, Integrated evaluation methodology, RAPFISH

I. Introduction

LAKES are standing bodies of fresh which fragile with ecological changes resulting from the hydrologic alterations [1]-[3]. In hydrological perspective, lakes can be as natural regulators of river flow, nutrients and sediments from watershed area [4]. In ecological perspective, lakes accommodated many species of both aquatic and/or non-aquatic plants and animal such as fish, birds, and amphibians rely on lakes habitat for breeding, foraging, and cover. Many people also considered lakes as a water-based tourism, leisure and recreation place and doing water sport activities such as fishing, sailing, swimming, jet skiing, boating, paddling and canoeing [5]-[6]. Therefore, functions of lakes are complex and related with ecological, water quality, aesthetic value, socio-economic, environmental and management aspects.

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Nowadays, there have been increased efforts on promoting aquaculture activity as one method to increase economic growth. Aquaculture lakes development thus has been accelerated in many areas. However, most aquaculture lakes faced an environmental crisis characterized by water quality degradation due to aquaculture activities.

This study offered the combination all aspects in evaluation methodology for aquaculture lakes with its paradigm refer to hierarchical theory and to the effects of spatial specific arrangement of an object into a space or local area. A design of integrated evaluation methodology for aquaculture lakes is presented. The method is based on the identification of a series of attributes which can be used to describe status of aquaculture lakes using certain indicators from aquaculture water quality index (AWQI), aesthetic aquaculture lake index (AALI) and rapid appraisal for fisheries index (RAPFISH). Therefore, the main objective of this study was to design an integrated evaluation index for aquaculture lakes.

II. Conceptual Framework

The general methodological procedure to develop a conceptual framework of integrated evaluation methodology for aquaculture lakes has offered in this study (Fig. 1). Several regulations such as environmental and water quality regulations from the government should be prepared to complete literature review study. In order to check the available data, several approaches have completed: collecting secondary data from related agencies, interviews and questionnaire, official reports or academic papers. Filling gaps is necessary to do if available data and literature and regulation reviews were considered inadequate. Basic method to evaluate and monitor aquaculture lakes is usually based on the simple comparison of the several parameters values with the defined range value from government regulation. In context of design and method study, it seems essential to develop and validate formulas or index for aquaculture lakes. A serious attempt was put to design an index system that can measure the overall aquaculture lake condition. Once a conceptual framework system is established, supplementary indexes for specific purposes and location can be added.

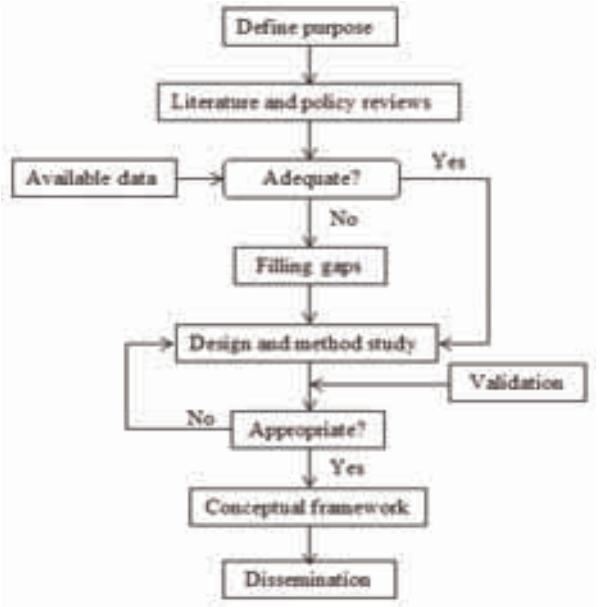

Fig. 1 General methodological procedure

As many authors have suggested in the context of a rigorous evaluation of aquaculture lakes, it is necessary to link all the attributes produced by aquaculture to three basic ideas, those of water quality, waterscape and fishery management. Waterscapes of aquaculture lakes are the appearance of the various objects within aquaculture lake area and present by bio-physical attributes (which is called exterior attributes); while water quality of aquaculture lakes can be interpreted into ixterior attributes.

In context to measure interior attributes, data from aquaculture fish types and water quality parameters will be processed by Analytical Hierarchy Process (AHP) method to analyze aquaculture water quality index (AWQI). On the other hand, bio-physical appearances will be processed by video-questionnaire method to analyze aesthetic aquaculture lake index (AALI).

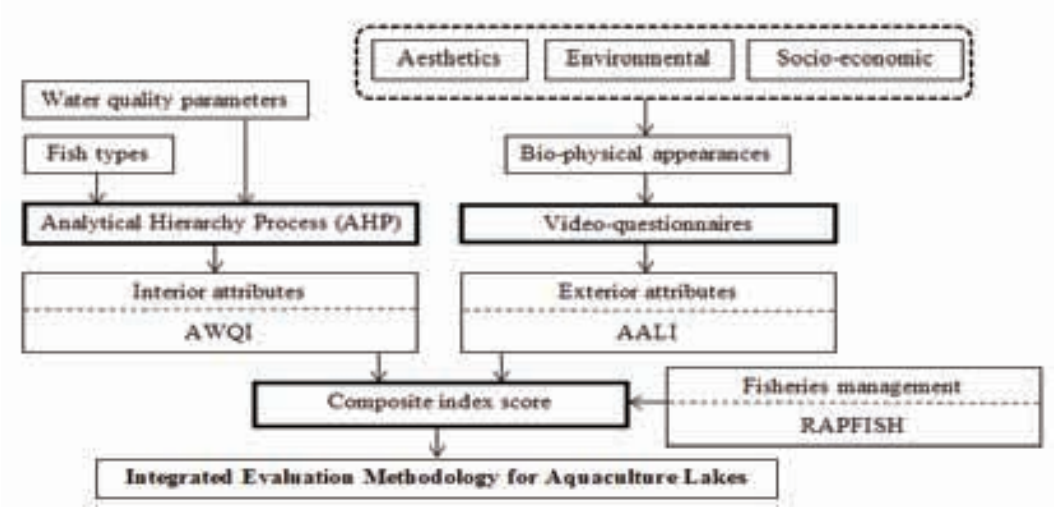

Fig. 2 The development of conceptual framework

A. Analytical Hierarchy Process (AHP) method

AHP is a multi-criteria decision making method which allows decision makers to model a complex problem in a hierarchical structure, showing the relationships of the goal, objective or criteria, and alternatives. AHP is made up of several components such as hierarchical structuring of complexity, pairwise comparisons, judgments, an eigenvector method for deriving weights, and consistency considerations. It also has different degrees of importance of their parameters and compiled with the different weighting for each parameter. AHP have been successfully implemented in many fields and study areas [7]-[10]. In general, the AHP method consists of five main steps (Fig. 3), and for solving decision problems by comparing several alternatives on the basis of the same set of attributes [11].

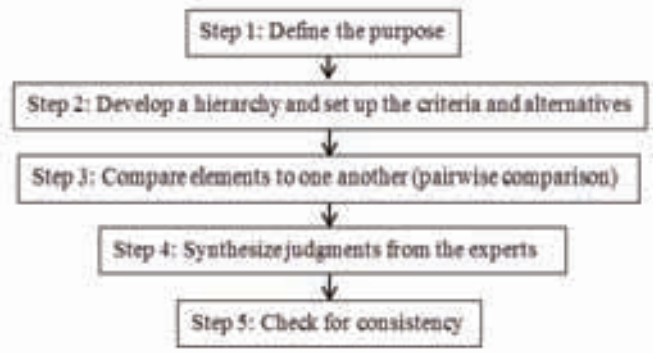

Fig. 3 The main step of the AHP method

The AHP method was used to derive ratio scales from paired comparisons, with the measurement being based on the relative importance of the water quality parameters with regard to certain types of fish. This process was performed

using Expert Choice 11.0. Therefore, the interior attributes can be measured and evaluated by using AWQI that describes the status of water quality condition for aquaculture lakes. Details about AWQI could be seen on the previous study [12].

B. Video-questionnaire method

Aesthetics, environmental, and socio-economic issues are tightly each other when sought to describe through bio-physical appearances. It is necessary to develop the criteria of aquaculture lakes, for example, visual ecology involves a critical investigation of color and clarity of water, functional morphology comprises an identification of trophic status related with percentages of water hyacinth as the important parameter to determine trophic status of aquaculture lakes and socio-economic criteria [13]-[14].

This study preferred to used video-questionnaire method to generate aesthetic aquaculture lake index (AALI) (Fig. 4) to evaluate all criteria of bio-physical appearances rather than like previous study which applied the "direct observation" by transporting the respondents to the study site [15] or using photographs [13], [16]-[18] to rate their attributes, due to its advantage in effectiveness both cost and time. These video-questionnaires method was inspired and developed from previous visual quality assessment method named the Scenic Beauty Estimation Method (SBE) [16]-[18]. Unlike the previous method, the photographs have been used to evaluate landscaping areas; in this study, video was recorded to capture the existing condition for aquaculture lakes. This advanced approach will generate a perfect combination of method to evaluate the comprehensive condition of lake from aesthetics, environmental and socio-economic aspects via people's perception. Details about video-questionnaire method could be seen on the previous study [14].

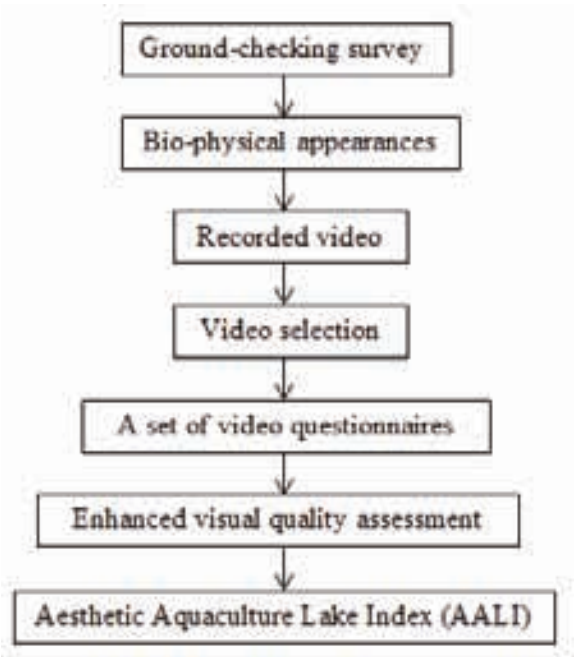

Fig. 4 Step by step method to generate AALI

C. Rapid Appraisal for fisheries (RAPFISH) method

RAPFISH method is a multi-disciplinary method for estimates the status of aquaculture [19]. It was considered as simple and easily scored attributes to provide a rapid, cost-effective, and multi-disciplinary appraisal of the status of aquaculture, in terms of comparative levels of sustainability. Work using this method covered sixth dimensions critical to the long-term viability of aquaculture: ecological, economic, institutional, ethical, social, and technological [20]. The dimensions and attributes map onto the sections and clauses of the Code of Conduct which can provide a single score for aquaculture evaluation. The example of the latest versions of attributes and scoring scale in the standard sustainability analysis of RAPFISH can be seen on Table 1. The principle advantages of RAPFISH are that additional evaluation indicators can be easily set up, the aquaculture can be analyzed with all or certain attributes within each indicator can be easily adapted to a particular condition [20].

TABLE I
THE EXAMPLE OF THE EVALUATION SCORING SCALE OF RAPFISH FOR INDONESIA

Dimension & attributes	Description	Scoring guidelines
Ecological: Farmed before maturity	Percentage of the fish caught that are smaller or younger than the size or age of maturity.	None (score 10-9); some >30% (8-6); lots >60% (5-3); a very large amount > 80% (2-0)
Economic: Marketing system	Assesses the impact of the marketing system. Open system better for sustainability, for example open auction system. Closed system, monopoly buyer not good for sustainability.	Fully open market auction system (score 10-9); partially open market auction system (8-6); semi-closed market system (5-3); monopoly or government buyer, fixed price system (2-0)
Ethical: Equity in entry to aquaculture	Assesses whether entry to the fishery is based on traditional/historical access/harvests?	Not considered (score 0-3); considered (4-7); traditional indigenous fishery (8-10)
Institutional: Governance	Assesses the overall quality or capacity of jurisdiction to provide enabling conditions for legal, regulated, reported, and protected fisheries.	1. Collaborative governance framework: management responsibilities shared by government with fishing enterprises and corporations and civil society, including scientists, NGOs, consumers and community leaders. 2. Accountability 3. Transparency 4. Trend in conflict status among resource users to management changes
Social:	Assesses the strength of social peer-group support for fisher's actions and decisions.	Score 0 (lowest sustainability) to 10 (highest sustainability), with 4/10 representing a bare pass and 7/10 a good score.
Technology: Fleet capacity in relation to resource	Assesses significant overcapacity in the catching power of this fleet/fishery.	Appropriate capacity, under good control (score 10-9); slight overcapacity, under control (8-7); overcapacity, but under good control (6-4); significant over capacity, under poor control (3-2); huge

	overcapacity (1-0).	
Institutional: Governance	Assesses the overall quality or capacity of jurisdiction (e.g., nation states) to provide enabling conditions for legal, regulated, reported, and protected fisheries. *4 attributes to score*	
	magnetic dipole moment	1 erg/G = 1 emu $\rightarrow 4\pi \times 10^{-10}$ Wb·m
J	magnetic polarization	1 erg/(G·cm^3) = 1 emu/cm^3 $\rightarrow 4\pi \times 10^{-4}$ T
χ, κ	susceptibility	$1 \rightarrow 4\pi$
χ_ρ	mass susceptibility	1 cm^3/g $\rightarrow 4\pi \times 10^{-3}$ m^3/kg
μ	permeability	$1 \rightarrow 4\pi \times 10^{-7}$ H/m $= 4\pi \times 10^{-7}$ Wb/(A·m)
μ_r	relative permeability	$\mu \rightarrow \mu_r$
w, W	energy density	1 erg/cm^3 $\rightarrow 10^{-1}$ J/m^3
N, D	demagnetizing factor	$1 \rightarrow 1/(4\pi)$

III. Results And Discussions

In order to design an integrated evaluation methodology for aquaculture lakes, it is obligated to integrate and develop rating scores system, which is called Integrated Aquaculture Lakes Index (IALI). It describes and evaluates the aptness of the lakes for aquaculture purpose and combined AALI from exterior attributes, AWQI from interior attributes and RAPFISH from fisheries management. In IALI, no specific weighting scheme was suggested. It is assumed that the responsibility in determining variables weights of index should be given to decision makers, not to the researchers [21]. However, in the first IALI application, equal weights of AWQI, AALI and RAPFISH were applied. The decision to apply the equal weighting scheme was made following recommendation from previous studies [22]-[23]. In the future, each authority is very welcomed to make changes for the weighting scheme, if justifications and further improvements can be provided. To obtain composite index score of IALI, it requires the transformation of various magnitudes and units from each supported index, so that arithmetical operation can be performed as follows:

$$\text{IALI} = \frac{\text{AWQI} + \text{AALI} + \text{RAPFISH}}{3} \quad (1)$$

Particular on value of AALI, it must be transformed first into a dimensionless quality scale of 0 – 100 to equalize all supported index.

This study offered the evaluation methodology to perform both temporal and spatial analysis. It has covered main aspects such as water quality parameters, aquaculture fish types, bio-physical appearances and management aspects. When addressing the issue of socio-ecological and environmental impacts, integrated index for aquaculture lakes is highly relevant as it is impossible to evaluate aquaculture lakes without considering those issues. More specifically, water quality parameters and fish types are considered as interior attributes meanwhile bio-physical appearances are considered as exterior attributes. The ILAI proposed in this study allowed to integrate both attributes which represented all aspects that never been combined together before. Even some previous studies tried to evaluate aquaculture lakes in many and different ways [24]-[26], none of these had incorporated the aesthetic value in their evaluation.

It is clear that, given the wide range of aquaculture types and its environment, as well as associated socio-economic and aesthetic values, a myriad of approaches will be required to manage all aquaculture lakes systems. The development of IALI are reflecting a compromise all aspects and it responds the needs of concise information about the current status of aquaculture lakes by the comprehensive approach. IALI was elaborated as a decision aid tool for stakeholders to evaluate the impact and contribution of anthropogenic activities on the aquaculture lake's environment. A credibility and applicability of subjective index such as IALI depends upon their ability to understand the case and the consistency of their interpretations.

IV. CONCLUSION

What is presented in this paper is a starting point for integrated evaluation index of aquaculture lakes towards sustainable water resources management. An increasing need for integrative evaluation that measures the contribution of the lakes attributes to people well-being has recently been recognized.

It is necessary to design an index to evaluate the environmental quality of aquaculture lake environments in their present condition, potential for use and interest for conservation can be used to express these qualities in ways which are significant from both the scientific and the planning and management points of view. The major question addressed following this study is how to develop a procedure for integrated evaluation index of aquacultures lakes. Understanding this question, we established primary objectives of the study to response.

The conclusion was while there is no denying the fact that the aquaculture lakes are under great threat from the pressure of the increasing human activities, it must be realized that no evaluation methodology for aquaculture lakes can succeed by keeping the pristine condition. This evaluation methodology developed in this work can be used as an effective, low-cost evaluation methodology of aquaculture lakes for developing countries. Because it emphasizes the simplicity and understandability as it must communicate to decision makers and the experts. Moreover, stakeholders need to be helped to perceive their lakes so that sites can be accepted and valued by local people. For this site of lake development, accessibility and planning designation of the site is of decisive importance:

the local people want to know whether the lake condition is safe or whether it can be used.

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The Impact of Water Reservoirs on Biodiversity and Food Security and the Creation of Adaptation Mechanisms

Inom S. Normatov, Abulqosim Muminov, Parviz I. Normatov

Abstract**—**Problems of food security and the preservation of reserved zones in the region of Central Asia under the conditions of the climate change induced by the placement and construction of large reservoirs are considered. The criteria for the optimum placement and construction of reservoirs that entail the minimum impact on the environment are established. The need for the accounting of climatic parameters is shown by the calculation of the water quantity required for the irrigation of agricultural lands

Keywords**—**Reservoir, Central Asia, food, reserved zones, adaptation, agriculture.

I. Introduction

THE problem of food safety is important for all countries and at all times. Maintaining food safety is necessary not only from economic but also from social and political perspectives. A state that does not provide food independence cannot feel safe in the modern world. The modern situation has aggravated the problem of food safety, which is characterized by inconsistent processes in the global economy. On the one hand, food consumption is increasing in developing countries, and on the other hand, an economic and financial crisis has caused a slump in both food production and peoples' incomes in these countries [1].

Among the crises that the world has faced, climate change poses the most serious threat for all natural-economic systems, including water and land resources. Increased air temperatures and reduced precipitation combine to exacerbate the dryness of the climate. Most of Central Asia experiences arid environmental conditions, in which poor deposits, excessively low humidity, a high intensity of evaporation and excess solar radiation are characteristic. The sharp growth of the population, which has exceeded worldwide rates, has led to serious concern in the countries of Central Asia. Population growth has caused processes of economic intensification that have led to an increased pressure on water and land resources [2].

The food products manufactured in Tajikistan face many serious difficulties caused mainly by the growth of the population, the country's mountainous topography, and the limited farmlands available for grain crops and livestock because of steep slopes and unproductive microclimates. The average mean arable land per person of 0.14 ha is low compared with the global average of 0.26 ha/person. The degradation of the land, which has occurred as a result of the breaking of traditional norms of land tenure, such as the cutting down of woods and degradation of pastures, together with other processes, such as soil erosion, torrential rain events, flooding, soil salinization and desertification, have promoted an annual reduction of the volume of foodstuffs produced [3], [4].

One way to achieve minimum food safety in the vulnerable countries of the region is the development of new lands and the escalation of the production of agricultural products. In Tajikistan, for example, 800 Th. ha of suitable land is available for irrigation. An elementary analysis shows that approximately 0.2 ha/per of land is necessary for Tajikistan (in 2015, this number will result in between 650 and 850 Th. ha) to achieve an average regional indicator of the specific area of irrigation per capita. However, for this purpose, it is necessary to place in operation 10 Th. ha of newly irrigated land annually, and the possibility of such an expansion of the irrigated lands in Tajikistan is unlikely [5]. Nevertheless, another economically more favorable and ecologically useful solution to the given problem is an increase in the efficiency of the irrigated lands and water use. Increasing the efficiency of water use is a two-fold problem that entails both increased soil fertility and productivity and decreased water use. Thus, increasing the efficiency of water use is a complex problem in Tajikistan [6].

II. Ecological & Irrigation and Energetic Criteria for Reservoir Construction

The use of hydropower in agriculture is one of the key basic branches of the economy of the Republic of Tajikistan, which possesses major stocks of hydroelectric power. The total annual potential resources from water-power engineering projects amount to 527 Bln. kWt·h, of which only approximately 5% has been realized to date [7]. The existence of this large potential supply of power has led to plans for the construction of a number of hydroelectric power stations, accompanied by reservoirs, in the near future. These plans are reflected in the development strategy of the energy branch of the Republic of Tajikistan Government. Hence, when planning agricultural development in areas adjoining water reservoirs, it is necessary to consider that water reservoirs influence localized thermal and radiative balances that in turn alter the

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climatic characteristics over the reservoir and its adjoining lands. The meteorological conditions affected by the water reservoir will, in most cases, be transformed into a coastal zone, including an area several hundred meters around the reservoir, outside of which the intensity of the influence of the climatic conditions of the reservoir sharply decreases. However, in the dominant wind direction, the climatic influence of reservoirs can extend to 10 or more kilometers.

Through impoundment and increased residency times, dams alter water temperatures and chemistry, which in turn influence the rates of biological and chemical processes. Dams create barriers to the upstream-downstream movement of nutrients and organisms, thereby affecting physical and biological exchange processes. They also alter the timing and magnitude of the downstream fluxes of water, sediment, and ice, which modify biogeochemical cycles and the resulting structure and function of aquatic and riparian habitats. As dams occasionally collapse, they also present a risk to the built environment and downstream ecology [8].

The physical, biogeochemical, and biological processes occurring within a reservoir can affect the temperature and chemical composition of the water leaving the system to such an extent that its quality upon release no longer resembles that of the inflows. The degree to which water quality is affected on a daily, seasonal and/or annual basis depends on factors such as the surface to volume ratio and depth of the reservoir. Other factors include the geology and soil geochemistry of the surrounding catchment, the latitude of the reservoir, the rate and magnitude of sedimentation, the magnitude and timing of incoming flows and their residency time, and the level of biological productivity in the reservoir [8].

The water of a deep reservoir in temperate climates typically stratifies, with a large volume of cold, oxygen-poor water in the hypolimnion. Analysis of temperature profiles from 11 large dams in the Murray Darling Basin (Australia) indicated differences between the surface water and bottom water temperatures of up to 16.7 °C [9]. If the cold bottom water is released to maintain river flow, it can cause adverse impacts on the downstream ecosystem, including fish populations. Under a worst-case scenario (such as when the reservoir is full or nearly full, the stored water is strongly stratified and large volumes of water are released to the downstream river channel via bottom-level outlets), depressed temperatures can be detected 250-350 km downstream [10]. The operators of Burrendong Dam on the Macquarie River (eastern Australia) are attempting to address thermal suppression by hanging a geotextile curtain around the existing outlet tower to force the selective release of surface water [11,12].

Studies of changes in water temperature along the length of the river after discharge from reservoirs show that the influence of large reservoirs on water temperature is substantial: the daily and decadal scale change in water temperature before and after a reservoir is constructed, can reach 8-12 °C. The greatest difference in average monthly water temperatures in the tail water of reservoirs before and after the construction of a reservoir occurs in November-January, reaching 4.2-3.4 °C in the Vakhsh River. The warming influence of the waters discharged from large reservoirs lasts for 8 months and the cooling influence for four months (February-May). Thus, the length of the warming influence of large rivers is 1.74 times greater (209 km) than the cooling influence of the discharged waters (120 km) [13].

At present, to define the efficiency criteria for a hydropower station (HPS) with a reservoir, a method based on the analysis of key parameters of the HPS construction, such as the capacity and output of electricity by the HPS in relation to the area occupied for building the HPS, is widely applied. The relation between the capacity and electricity output is used as an index of the ecological-economic efficiency of the HPS per hectare of the land used for the construction of the HPS (Table I).

TABLE I
ECOLOGICAL-ECONOMIC EFFICIENCY OF THE CONSTRUCTION OF AN HPS WITH A RESERVOIR

Index efficiency of HPS	Capacity to the Area (MWt/ha)	Power output to the Area (TWt/ha)
Annual value for HPS with area of groundless 100 Th. ha	0. 123	0.406

Based on the data presented in Table I, we estimated the efficiency of the existing Nurek HPS and the Rogun HPS, which is planned for near-future construction, both of which include reservoirs (Table II).

TABLE II
ESTIMATION OF THE EFFICIENCY OF THE NUREK AND ROGUN HPSS WITH RESERVOIRS

Name	P, 10^2	W, 10^2	S	A	M	Index of efficiency			
						P/S	W/S	P/A	W/A
Bratsk	4400	22.6	547.0	357.3	70.0	0.008	0.041	0.012	0.06
Charvak	600	20.0	4.6	2.7	9.18	0.13	0.436	0.225	0.75
Toktogul	1200	41.0	31.9	-	29.3	0.038	0.128	-	-
Nurek	2700	112	21.5	0.2	1.50	0.126	0.522	13.50	56.00
Rogun	3600	133	17.0	6.800	16.0	0.212	0.782	0.529	1.96

P=capacity of HPS (MWt); W=power output (TWt·h); S=area for building of HPS (Th.ha); A=area of wooded vegetation (Th.ha); M=migration of population (Th. Pers).

For comparison, in Table III, the ecological-economic index values of the considered HPSs are compared with analogous indexes of other HPSs.

TABLE III
COMPARISON OF THE NUREK AND ROGUN HPS ECOLOGICAL-ECONOMIC INDEXES WITH THE OPTIMAL CRITERIA OF BUILDING OF HPS

Ecological-economic index efficiency of HPS	P/S (MWt/ha)	W/S (TWt/ha)
G	0.123	0.406
Bratsk HPS	0.008	0.041
Charvak HPS	0.130	0.436
Toktogul HPS	0.038	0.128
Nurek HPS	0.126	0.522
Rogun HPS	0.212	0.782

In Central Asia, with its climatic conditions, the selection of

an appropriate geographical location for a reservoir is a challenge. The estimation of the extent of the influence of reservoirs in arid zones on the surrounding environment is possible using the coefficient $K_{sur.env}$ [14]:

$$K_{sur.env} = \sum S_i / S_{oi} \cdot 100\% \quad (1)$$

where $K_{sur.env.}$ is the coefficient of reservoir influences on the environment; S_i is the area of the territory under the influence of the reservoir, km^2; and S_{oi} is the area of the basin, km^2.

Calculations of $K_{sur.env}$ demonstrated that the value for the influence on the surrounding environment of the Kairakkum reservoir is 0.11, that of the Nurek reservoir is 0.144, and that of the Muminabad reservoir is 0.00195 (Table IV).

TABLE IV
COEFFICIENT OF INFLUENCE OF RESERVOIRS ON THE SURROUNDING ENVIRONMENT

Reservoirs	Kairakkum	Nurek	Muminabad	Golovnoy
K	0.11	0.144	0.002	0.0011

Note that small, upland reservoirs have a greater influence on the microclimate than reservoirs in plains areas. For large reservoirs, an identical pattern is observed. The influence of the Nurek reservoir is 1.31 times greater than that of the Kairakkum reservoir.

Apparently, the degree of influence of reservoirs on the adjoining land decreases as their size and volume decrease, and at the same time, the influence of the adjoining land on the reservoir increases. This pattern should be considered when planning the construction of new reservoirs in Tajikistan and during the planning of coastal development by the tourism industry seeking to create zones of rest with a greater set of recreation services.

To estimate the role of a reservoir in local climate conditions, the expression $\Delta P/\sigma_{sp.dif.}$ can be employed, where ΔP is an influence indicator and $\sigma_{sp.dif.}$ is the mean square deviation of the difference in the influence of a given indicator between two stations located within a distance of 10-20 km from one another.

When $\Delta P/\sigma_{sp.dif.} \geq 1$, the influences of the reservoir on the local meteorological conditions are essential. We use these criteria to estimate the role of the reservoirs as factors in the formation of the local meteorological conditions, the agro-climatic parameters of the coastal zone and coasts, and the thermics of the rivers in downstream reaches [15].

Until the Nurek reservoir was filled with water that was the same temperature as the Vakhsh River, the water upstream of the Nurek HPS dam (kishlak Tutkaul) exhibited almost no difference in temperature from the water up to 17 km below the dam (kishlak Sariguzar). With the filling of the Nurek reservoir (1972), a drop in the water temperature in spring (February-May) and an increased temperature in summer, autumn, and winter (July-January) in comparison with natural conditions were observed. This finding can be partly explained by the fact that reservoir was not at its maximum level when water was discharged from its top horizon; only in 1980 did the reservoir reach its high surface level (HSL).

In 1980, the influence of the Nurek reservoir on the thermal conditions of the Vakhsh River water began, which is traced most precisely to 17 km of the river downstream from the Nurek HPS dams up to the Sariguzar hydrological station. The greatest difference in the average monthly temperature of the water before and after the construction of the reservoir for the Sariguzar hydrological station (4.2°C) is observed in November-December. During discharge periods, this difference decreases to 1.2°C. The influence of small-channel reservoirs on the change in water temperature along the length of the river can traced for a considerable distance (Table V). Thus, the change in the annual distribution of the average monthly values of the water temperatures below large reservoirs for a specified time interval is not due to a change in the annual mean air temperature but, rather, is influenced by the discharge of water from the reservoirs. However, according to the data of the "Nurek" meteorological station, the average monthly temperature decreased after construction of the Nurek HPS (Fig. 1).

TABLE V
AVERAGE MONTHLY TEMPERATURES OF VAKHSH RIVER WATER BEFORE AND AFTER BUILDING OF THE NUREK RESERVOIR

River-Post	Period	Month							
		I	II	III	IV	V	VI	VII	VIII
Vakhsh Tutkaul	1946 1967	2.6	4.3	7.6	11.0	12.8	14.3	15.0	14.9
Vakhsh Sariguzar	1967 1971	2.0	4.0	8.1	11.5	13.2	14.4	15.0	14.9
Vakhsh Sariguzar	1972 1980	5.4	3.9	5.5	10.0	13.0	14.9	15.9	16.0
Difference		-3.4	0.1	2.6	1.5	0.2	-0.5	-0.9	-1.1

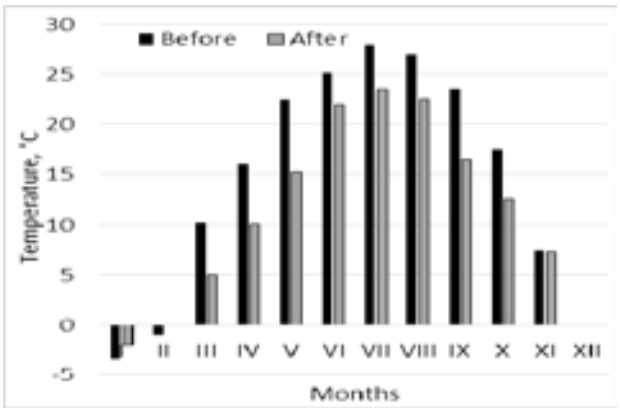

Fig. 1 Average monthly temperature before and after building of the Nurek reservoir

III. IMPACT OF RESERVOIRS ON IRRIGATION REGIME FOR AGRICULTURE

To determine the potential influences of climate change on agro-climatic resources, we analyzed the climatic parameters of three districts with developed agricultural sectors (Dangara, Fayzabad and Yavan) adjoining the Nurek reservoir. For this

purpose, data from the hydrometeorological stations (HMSs) located in these areas were used. Data on the temperature dynamics and relative humidity of air and the atmospheric precipitation for the years 1968-2000 were used. The evaporation and humidity coefficient were calculated (Table VI).

TABLE VI
AVERAGE MONTHLY TEMPERATURES OF VAKHSH RIVER WATER BEFORE AND AFTER BUILDING OF THE NUREK RESERVOIR

Hydropost	Index	Years	
		1968-1972	1995-2000
Dangara	*T* (°C)	15.3	16.4
	H (%)	57.0	56.9
	F (mm)	570.5	598.5
	I (mm)	1196.7	1438.0
Fayzabad	*T* (°C)	13.2	15.4
	H (%)	61.6	55.2
	F (mm)	709.0	675.4
	I (mm)	1013.0	1258.8
Yavan	*T* (°C)	17.2	16.9
	H (%)	47.2	50.4
	F (mm)	677.4	677.3
	I (mm)	1630.8	1567.5

T=temperature; H=humidity; F=precipitation; I=evaporation

The data presented in Table VI demonstrate that for 32 years (1968-2000), the average annual temperature increased 1.0-1.5 °C, which led to a 3-6% decrease in the relative humidity and a 10-26% increase in evaporation on an annual basis and of 12-30% in the period May-September. However, in the Yavan district, the dynamics of the changes in the listed parameters has had the opposite tendency: the temperature of the air and evaporation decreased by 0.5%, 7.2%, respectively, and the relative humidity and evaporation increased 7.2% and 10%, respectively.

The reduction of evaporation during the vegetative period in the Yavan district reached 12.2%. In view of these climatic changes, it is necessary to take corresponding corrective measures in planning agriculture-related water use. Planning for the development of irrigation regimes typically considers meteorological parameters for all periods analyzed. However, this approach can lead to essential errors. Studies based on previously irrigated fields and prospective irrigation regimes that ignore the effects of global climate warming often do not consider the growing needs for water. In contrast, data on the Yavan valley recommended irrigation regimes resulting in the overuse of water resources. For example, the most recent recommendations for irrigation regimes for the valley are based on the annual average means of the humidity coefficient (0.35), which suggest that this valley is a drought-prone area. However, the data presented in Table VI show that for the past 20 years, evaporation in the valley has decreased by almost 300 mm (17%), the quantity of precipitation has risen by 70 mm (11%) and the humidity coefficient has risen to 0.45. Hence, the present irrigating norms for cultivation of the middle-fibrous cotton of 1100 m^3/ha and 3000 m^3/ha in the Yavan valley and Lucerne, respectively, are overestimated. Calculations show that the unproductive losses of water in these two valleys exceed 60 mln.m^3.

IV. SEDIMENTATION OF RESERVOIRS

The analysis of previous results on the filtration characteristics of irrigation by clean water versus water with the suspended sediments shows that prior to the construction of the Nurek reservoir, each m^3 of Vakhsh River water contained up to 10 kg of sediments and that more than 100 t of sediments rich with minerals entered the agricultural fields annually. According to the Hydrometeorological Agency of the Republic of Tajikistan, the mid-annual discharges of suspended sediments in the Vakhsh River on the hydropost located on the kishlak Sariguzar—17 km below the Nurek HPS—since 1972 (the beginning of the filling of the Nurek reservoir) decreased from 1000 g/s to 82 g/s by 1980. The Nurek reservoir almost completely eliminated the suspended sediments from the Vakhsh River (Table VII).

TABLE VII
AVERAGE ANNUAL GRANULOMETRIC COMPOSITION OF THE VAKHSH RIVER SEDIMENT FLOW

Years	*D* (mm)					
	1-0.5	0.5-0.2	0.2-0.1	0.1-0.05	0.05-0.01	0.01-0.05
	Komsomolabad					
1972-1976	1.43	7.05	8.6	15.3	37.0	18,0
1977-1987	1.53	7.11	8.7	14.9	37.2	17,9
	Sariguzar					
1972-1976	0.63	1.77	3.9	8.7	47.3	22,1
1977-1987	0.72	1.94	3.9	9.1	48.2	21,5

The construction of the Nurek HPS dam was begun in 1961. Simultaneously with construction, a technical project investigating the suspended load was initiated. The prediction for reservoir sedimentation for a period of 11 years was included in the project.

For the period 1972-1989, the sediment flow of the Vakhsh River was measured in 1977 and 1980-1982 at Komsomolabad and in 1978 and 1985 at the Kishrog hydropost. In 1977 and 1985, the sediment flow measured at the Komsomolabad station changed in accordance with the change of the annual rainfall from 55.2 to 38.3 Mln. t at the station of Kishrog from 86 to 59 Mln. t.

Based on the estimation of the Institute of Mathematics of AS of Tajikistan, the additional value of the tributary sediment from Komsomolabad up to the Nurek reservoir is 4.0 Mln. t.

The input of sediment into the Nurek reservoir under average conditions for a dry year can be estimated at 60-65 Mln. t. The calculation demonstrates that by the sixth year of constant use, the useful volume of the reservoir would decrease by 200 Mln. m^3 and by the 11th year by 650 Mln. m^3. Table VIII presents the initial forecast for sedimentation of the Nurek reservoir.

During the planning for the reservoir, it was accepted that the process of sedimentation would conditionally begin in 1978, with an intensity of 40 Mln. m^3 per annum for the first five years, accelerating to 90 Mln. m^3 per annum in all

subsequent years.

TABLE VIII
PROGNOSES OF NUREK RESERVOIR SEDIMENTATION (REDUCTION OF THE FULL VOLUME)

Year	1978	1983	1988	1993	1998	2001
volume (km^3)	10.5	10.3	9.85	9.4	8.95	8.68

Early studies established that in connection with the increase in temperature, a longer vegetative period of agricultural crops can be expected. The intensity of increase of the air temperature and the increase in the stock of soil moisture in the spring will enable earlier spring sowing. Adhering to the mean annual starting dates of sowing without accounting for climate change will lead to a decrease in productivity of all agricultural crops. The displacement of sowing relative to an optimum for 5-10 days reduces productivity on the average by 10-20%. This decreased productivity is related to the fact that the most important period for the efficiency of crop growth occurs at air temperatures that are higher than the optimum. The influence of agro-climatic conditions on the rates of growth of agricultural crops can be reduced to an estimation of when each crop will pass through its phenological phases.

Understanding the forces exerted by large dams on local climate is key to establishing whether artificial reservoirs inadvertently modify precipitation patterns in impounded river basins. Using a 30-year record of reanalyzed data, the spatial gradients of atmospheric variables related to precipitation formation are identified around the reservoir shoreline for 92 large dams of North America. The large dams influence local climate most in Mediterranean and semi-arid climates, while for humid climates, the influence is least apparent. Clear spatial gradients of convective available potential energy, specific humidity and surface evaporation are also observed around the fringes between the reservoir shoreline and farther from these dams [16].

V. IMPACT OF RESERVOIR ON THE TIGROVAYA BALKA NATURE RESERVE

The Tigrovaya Balka Nature Reserve is a heavily forested area critical for the preservation of unique communities of flora and fauna. They species living in the reserve are not simply independent "units" that could be kept in zoos and botanical gardens but, rather, are members of a climax community that has developed over millennia, the infringement of which will lead to the irreversible degradation and disappearance of many species, as was the case with Turanian's tiger.

Until the settlement of the Vakhsh River and the building of the Nurek HPS, the vegetative ecosystems of the reserve were supported by annual spring-summer floods, and all the lakes of the reserve were filled with water. Since construction of the Nurek HPS and its reservoirs, the natural floods have stopped. This condition has led to a gradual reduction of the water level in the lakes of the reserve and complete drying of Lakes Blue and Kabane.

Now, to maintain the balance of water in the reserve, sewage from farmlands is input. Without the implementation of measures to prevent excess sewage from entering the reserve, even with small concentrations of salts, due to extremely high evaporation, the salinity of the soil and waters of the reserve will increase to fatal limits. There will be an intensification of processes of desertification and salinization that finally will change the heavily forested vegetation.

The present condition of the Tigrovaya Balka Nature Reserve requires the provision of uncontaminated water. In turn, the development of alternative means of supplying the reserve with water will considerably improve the condition of the flora and fauna of the reserve. Future studies should have the following objectives:

- To identify the impact of chemical pollution transition processes on environmental changes;
- To identify the impact of transition processes on changes in environmental standards and risk assessment criteria related to toxic elements;
- To review suitable remediation options;
- To develop methods of prioritizing urgent action areas (hot spots) within the boundaries of the "Tigrovaya Balka" reserve;
- To provide recommendations for risk management strategies to improve environmental conditions and water resources;
- To organize a preliminary purification system of water inflow to the reserve by building reservoirs.

Fluctuations or changes in one ecosystem component cause a number of collateral changes in other components. The change of the water dynamics and the chemical composition of waters cause physiological changes in the reserve plants due to the adaptation of the plants to the newly created conditions. This process is reflected in the food allowance and activity of the fauna and birds of the reserve. The potential mutation of species of plants, animals and other inhabitants of the reserve cannot be excluded. Considering the fact that the Tigrovaya Balka Nature Reserve is also a place of seasonal residence for migrating birds, the processes taking place in the reserve's flora and fauna can have far-reaching effects. In many areas, poachers have used the reserve as a place to hunt. The undesirable infections and illnesses caused by the adaptation of the inhabitants of the reserve to the disrupted natural conditions can be transmitted through food and thereby result in the mass distribution of an illness or infection.

In 2007, a set of tasks for inspection of the territory of the reserve, and certain measures were undertaken to improve the delivery of water to the reserve ecosystem. These measures include clearing an overgrown natural channel, building a channel for a supply of fresh water bypassing dams, and building a pumping station, among other measures. The systematic clearing of channels has proceeded since 2008. Despite an insignificant difference in the levels of the northern and southern parts of the reserve, the development of bogs in the north, where wastewaters were historically discharged, has almost ceased. The water in the cleaned channels and drains has been directed into a drying-up lake, which has been filled

with water, as in former years when the natural waterway was supported by regular floods.

At the direction of the Tajikistan government in 2007, the reserve has been increased by 21 thousand ha. The added territories improve the protection of ecosystems such as tugayes and adjoining deserts. Additionally, the increase in the reserve has facilitated the natural movement of animals; previously, they often left the limits of the protected territory.

In the autumn of 2008, the Tigrovaya Balka Nature Reserve had been in existence for 70 years. At this anniversary, the government of Tajikistan made the decision to increase the area of the reserve by 100 thousand ha. However, before becoming part of the reserve, these areas were used as a cattle pasture and for fuel gathering, which led to the degradation of grasslands and a total disappearance of the *Haloxylon* woods. *Haloxylon* restoration is ongoing in the reserve.

Now, the reserve area encompasses more than 47 Th. ha. Taking into account the transferred lands of the former collective farms and the intercollective-farm enterprises (1.3 Th. ha), the total area of the reserve comprises 50.9 thousand ha, including a wooded area of 24.1 Th. ha (47.4%), a non-wooded area of 26.8 Th. ha (52.7%) and a light forest, glades, mountains area of 8.0 Thousand ha (14.1%). Bogs and waters occupy 21.4% of the total reserve area. In the northern and southern parts of the reserve, 16 and 5 large and small lakes are present, respectively.

The water regime of the soils of tugay-inundated biogeocenosis sharply differs from the previous regime. First, there is increased humidity at all soil depths; second, the influence of seasonal atmospheric humidity is almost nonexistent, as the regime here is defined by additional humidity at the expense of the shallow (1-2 m) levels of groundwater. The water and salt regimes of the soils define the formation and seasonal development of vegetative cover. In deserted biogeocenosis, the seasonal development of vegetation has been accurately traced. Additional soil moisture in tugay-inundated biogeocenosis provides sufficient humidity for the soil profile throughout the year. The brief drying of the top horizon occurs only in the middle of summer, during the period of greatest evaporation and moisture consumption by transpiration (to 5%). The bottom horizons in contact with groundwater are provided with the greatest moisture throughout the year, and the limits of fluctuation of this parameter (30%) can serve as an indicator of the dynamics of the groundwater level. Thus, in the autumn, when evaporation and transpiration decrease, the level of groundwater rises, whereas it falls in the summer. The shallow groundwater on the inundated terrace, which is continuously fed and freshened by river water and by periodic floods in recent times, provides the vegetation of the reserve with moisture throughout the year. The ecological conditions also include a long summer drought, which causes extremely low relative humidity. These contrasting relationships of the soil and atmospheric humidity characterize the living conditions of the tugay vegetation. In the initial stage of development, tugay woods are connected with coastal open communities of grassy vegetation formed on young shallows and river-bottom terraces. Presently, the territory of the reserve includes 25 Th. ha.

VI. CONCLUSION

A review of the literature clearly shows the influence of reservoirs on the formation of the local microclimate, the landscape, habitat conditions and the physicochemical properties of water flows. However, the extent of this influence on various components of the environment differs and is primarily defined by the sizes and locations of the reservoirs. The main factor increasing the coefficient of influence of reservoirs is the surface area of the water body, the volume of water and the height of the dam. The essential change in the water temperature of the Vakhsh River after the construction of a reservoir is revealed. Meteorological data established a decrease in the temperature of the district after the construction of the Nurek reservoir. A reduction of the volume of the reservoir by more than 17% for the period 1978-2001 was observed. Thus, studies have established that mitigation of the impact of the changes in microclimate that are induced by reservoirs on the environment and the production of agricultural products can be achieved by developing effective mechanisms of adaptation and by carefully selecting the locations of future water reservoirs.

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Water Budget in High Drought-Borne Jaffna Area, Sri Lanka, in Dry Season

R. Kandiah, K. Miyamoto

Abstract— In Sri Lanka, the Jaffna area is a high drought affected area and depends mainly on groundwater aquifers for its water needs. Water for daily activities is extracted from wells. As households manually extract water from the wells it is not drawn from mid evening to early morning. The water inflow at night provides the maximum water level that decreases during the daytime due to extraction. The storage volume of water in wells is limited or at its lowest level during the dry season. This study analyzes the domestic water budget during the dry season in the Jaffna area. In order to evaluate the water inflow rate into wells, and storage volume and extraction volume from wells over time, water pressure is measured at the bottom of three wells, which are located in coastal area, denoted as well A, in nonspecific area, denoted as well B, and agricultural area, denoted as well C. The water quality at the wells A, B, and C, are mostly fresh, modest fresh, and saline respectively. From the monitoring, we can find that the daily inflow amount of water into the wells and daily water extraction depend on each other, that is, higher extraction yields higher inflow. And, in the dry season, the daily inflow volume and the daily extraction volume of each well are almost in balance.

Keywords—Domestic water, Water balance, Water budget, Ground water, Shallow well,

I.INTRODUCTION

SRI LANKA is located between latitudes 5° and 10° N and longitudes 79° and 82° E in the tropical climate region with an average temperature of 27° C. It receives about 2000 mm mean-annual rainfall with a range of less than 900 mm in dry areas to over 5000mm in wet areas from two monsoon seasons, hurricanes and interim rainfall. Sri Lanka has diverse geology and landforms including 103 river basins [1]. It is divided into three climatic zones as wet, intermediate and dry zones [2] as shown in Fig.1 (a).

Jaffna is located in the dry zone in the northern part of the country and has no river. It has only small ponds and seasonal water bodies on sedimentary rocks of Miocene age [3]. It receives 935mm-1800 mm rainfall mainly from the North-East monsoon [4]. This rainfall water is collected in the karstic aquifers, and shallow aquifers in coastal sands [5]. People mostly depend on this ground water from wells for their domestic needs and livelihoods.

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According to the statistical data in 2014 by District Secretariat, Jaffna [6], 141,113 families use ground water directly from 103,777 wells including shallow wells (80.64%) and tube wells (19.36%), 14,498 families have access to 1,865 pipelines which are connected to wells, and 31,070 families use other sources like bowser, ponds. The households directly using wells extract water from morning to early evening, mainly using manpower.

In dry season, the water level of wells decreases to the lowest levels leading to drought conditions. Though, historically, meteorological and hydrological droughts have occurred in these areas the water level in wells now decreases to low levels more rapidly than in the past. This stress condition challenges people and led to severe water scarcity. At this severe condition, it is significant to find out the basis of water scarcity in the drought area by studying the factors connects with scarcity like water use, consumption.

Jonathan and Munir [7] introduced the water scarcity indicators to explain the basis of scarcity, which are "(i) water use to water supply, (ii) water supply to water demand, and (iii) human water demand to human water access". Among these indicators, indicator (i) "water use to water supply" can be treated objectively and the water scarcity can be described as 'shortage of water supply compared to the water use' using the indicator. Therefore, we focus on the relationship between water use and water supply, and at the first step, we try to find the domestic water balance. To do it, we monitored temporal change of water depth in the several wells.

A. Concept of Water Balance

The water balance can be determined by the balance of all of water movements that is water inflows and outflows in a defined period. It is further explained as the changes in storage for a definite area which is the difference between inflow amount and outflow amount. In general, Precipitation (P) is the inflow; Evaporation (E) and Evapo-transpiration (ET) are the outflows; and the Groundwater Flow (GF) and Surface Runoff (SRO) have the characteristics both of inflow and part of outflow. The water balance, the changes in storage ΔS, can be written as follows [8].

$$\Delta S = P - E - ET \pm SRO \pm GF \qquad (1)$$

B.Application of the Concept of Water Balance to Well

For well, when we denote V_t for the volume of the water stored in a well, ΔS in (1) is considered as changing in the

volume (ΔV_t); P is to be rainfall amount on the open well surface (V_r); E and ET also correspond to Evaporation amount(V_e) and Evapo-transpiration amount (V_{et}) from open water surface of well; SRO does not exist due to lack of direct interaction between water on ground surface and water inside of the well; and GF can be divided into the flow (V_{if}) which comes from ground water aquifer to the well and extraction ($V_{ext.}$) from the well. Therefore, (1) can be

$$\Delta V_t = V_r - V_e - V_{et} + V_{if} - V_{ext} \qquad (2)$$

In addition, Evaporation (V_e), and Evapo-transpiration (V_{et}) could not evaluated directly and groundwater flow would be somehow affected by the existence of V_e and V_{et}. Therefore, both of V_e and V_{et} would be included into groundwater inflow. Then, (2) can be rewritten as

$$\Delta V_t = V_r - V_{ext} + V_{if} \qquad (3)$$

In dry season, especially in night, we could assume that it would be no rain, that is $V_r = 0$. Then, (3) can be modified as

$$\Delta V_t = V_{if} - V_{ext} \qquad (4)$$

More over during night, we could also assume no extraction, (4) can be developed as

$$\Delta V_t = V_{if} \qquad (5)$$

ΔV_t can be defined as the difference of the volumes at starting (t) and ending ($t+\Delta t$) of defined period (Δt) that is

$$\Delta V_t = V_{t(t+\Delta t)} - V_{t(t)} \qquad (6)$$

The volume of stored water (V_t) in the well with cross sectional area (A) is obtained as

$$V_t = A\,h \qquad (7)$$

In which, h is the water depth in the well. The water depth can be calculated using the monitored water pressure p at bottom of well by the following relation.

$$h = p/\rho g \qquad (8)$$

In which ρ is density of water and g is gravity. Substituting of equations (6), (7), and (8) into (5), (5) yields

$$V_{if} = \Delta V_t = A\left[p_{t(t+\Delta t)} - p_{t(t)}\right]/\rho g \qquad (9)$$

II. Site Selection and Monitoring

A. Study Area and Location of Wells

Maravanpulo in the Thenmaradchy Divisional Secretariat area [9] is one of the highest drought affected areas in Jaffna district, and is selected based on its drought history in recent years. More than seventy percentages (70%) of households have their own wells. We choose three domestic wells with different water uses for monitoring. The location of the wells is shown in Fig.1 (b).

Well A

It is located nearby coastal area of Jaffna lagoon. This well has fresh water and it is used for domestic needs, animal husbandry, and home gardening.

Well B

It is found in the boundary of residential area. The water of this well is modest fresh water.

Well C

It is located by ponds which are small surface water bodies. The water is saline water in dry season and modest fresh in rainy season.

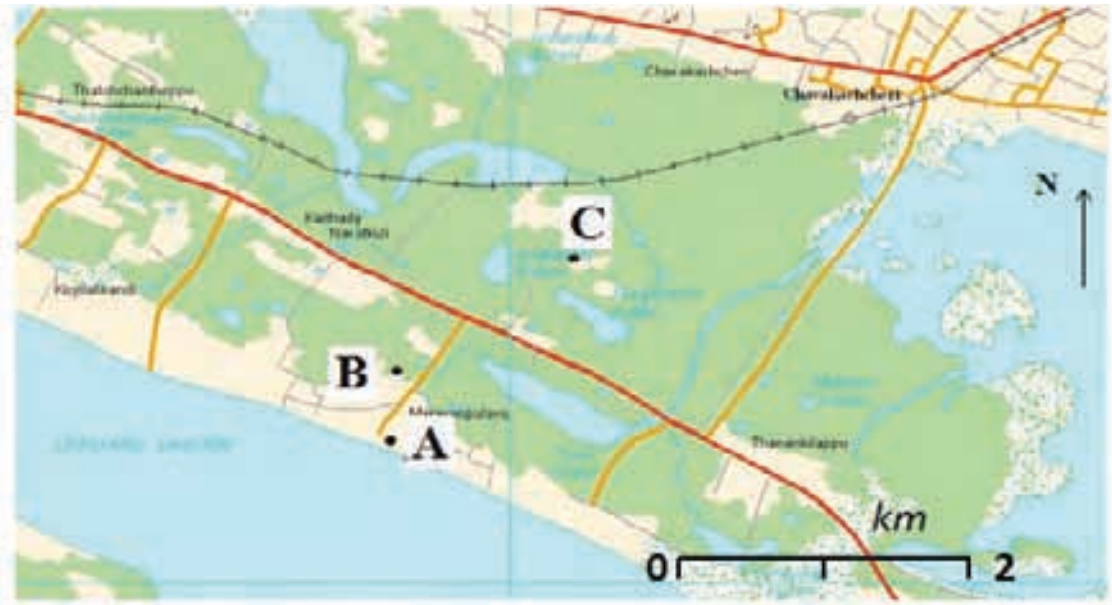
B.Shallow wells

The wells are commonly shallow and have free surface, and constructed with cemented sidewall and non-concreted basement to flow the water. The pressure gauge is kept at the bottom of the well as shown in Fig.2. The well does not have plants or trees on its water surface.

C.Monitoring

Three pressure gauges (HOBO® U20 Water Level Logger) which fixed on the bottom of the each well A, B, and C are used to monitor the water pressure and another one is kept in atmosphere at site B to measure atmospheric pressure to correct the bottom pressures. The pressures were monitored in 10 minutes interval from August 24, 2015 until December 06, 2015 during the dry and rainy seasons.

(a) Main Climatic Zones of Sri Lanka



(b) Topographic map of the study area and location of wells A, B, and C (adopted from Panabokke *et al*., 2002)

Fig. 1 The study site for monitoring wells A, B, C at Maravanpulo Jaffna, Sri Lanka

III. Results and Discussion

A. Water Balance to Study Area

The water depth (h) is calculated from the water pressure observed at bottom of wells A, B and C using (8). Fig.3 is the typical changes of water depths, h_A, h_B, and h_C, during three continuous days in dry season from 26th August to 28th August, 2015.

Fig. 3 shows that the water depth increases constantly at night and decreases quickly in several times during the daytime. It means that water recovers at night and it is used in daytime. And the daily maximum water depth is mostly found at from 5.00am to 10.00am. The water depth change of well C is smallest one compared to wells A and B in dry season.

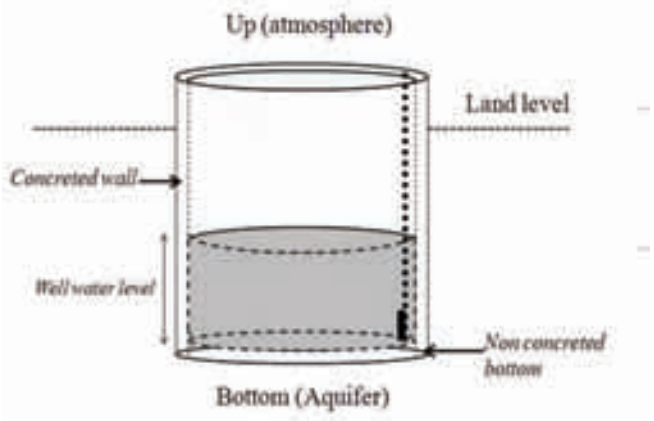


Fig.2 Structure of typical shallow well in the study area

Therefore we calculated the daily inflow volume ($V_{if\ (24)}$) using the inflow volume for the period from 1.10am to 5.00am (230min). The inflow volume for the period from 1.10am to 5.00am is obtained by using (9). The relationship between the inflow volume for the period from 1.10am to 5.00am, V_{if}, and daily inflow volume $V_{if(24)}$, is,

$$V_{if_{(24)}} = 24(\text{hours})/(230\text{min.}/60\text{min.})V_{if} \qquad (10)$$

The daily water balance can be expressed based on (3) as,

$$\Delta V_{t(24)} = V_{r(24)} - V_{ext(24)} \qquad (11)$$

In this equation, $V_{r(24)}$ is obtained by monitoring as daily precipitation, and $\Delta V_{if(24)}$ can be evaluated by (10). $\Delta V_{t(24)}$ in (11) means the daily change of the volume of the water in the well. Therefore, it can be evaluated by (9) using two pressure data, which are obtained at 24 hours difference. When we used the pressure at 0.00am every day, $\Delta V_{t(24)}$ can be evaluated by,

$$\Delta V_{t(24)} = V_{t(24)} - V_{t(0)} = A\,[p_{(24)} - p_{(0)}]/\rho g \qquad (12)$$

In which, $p_{(24)}$ and $p_{(0)}$ are the pressures at 0.00am in tomorrow and today morning, respectively.

$\Delta V_{t(24)}$, $V_{r(24)}$, and $V_{if(24)}$ in (11) are known by above discussion, then we can evaluate the daily extraction volume, $V_{ext(24)}$, from (11), as

$$V_{ext(24)} = V_{r(24)} - \Delta V_{t(24)} + V_{if(24)} \qquad (13)$$

In case that rain fall can be neglected, (13) is,

$$V_{ext(24)} = V_{if(24)} - \Delta V_{t(24)} \qquad (14)$$

B.Results and Discussion

Fig.4 shows the daily rainfall, $V_{r(24)}$, and cumulative rainfall in the monitoring period of 104 days [10].

We could recognize that the rainy season would start on 27 September 2015, and we could divide the rainy season into three periods, pre-intensive rain period, from 27th September to 26th October, intensive rain period, from 27th October to 15th November, and post-intensive rain period, from 16th November to end of the monitoring period.

Fig.5 shows the daily inflow rate, $V_{if(24)}/A$, daily extraction rate, $V_{ext(24)}/A$, and water depth observed at 09.00am, $h_{(9)}/A$ of each well A, B and C.

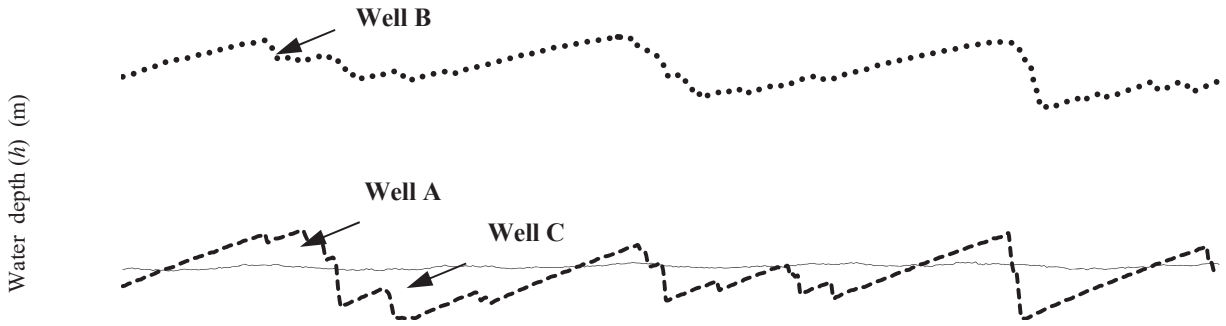


Fig. 3 Changing in water depths of wells A, B, and C in dry season from 26.08.2015 to 28.08.2015

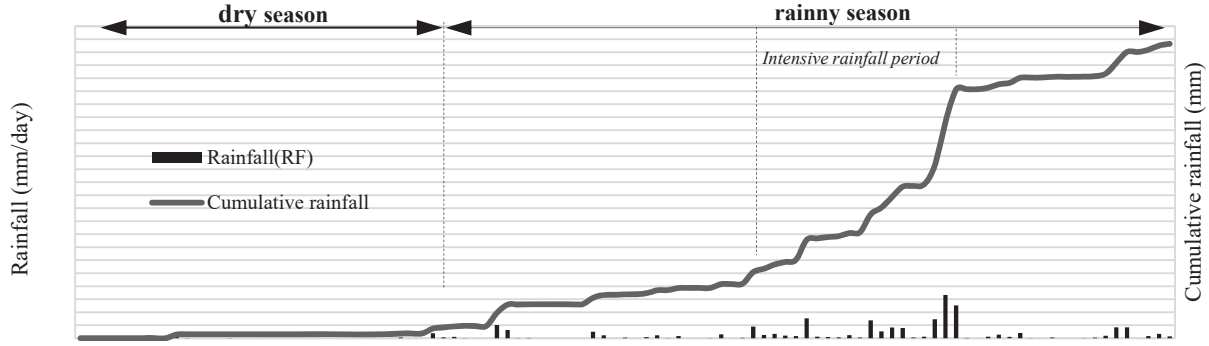


Fig.4 Rainfall of the study area from 2015.08.25 to 2015.12.05

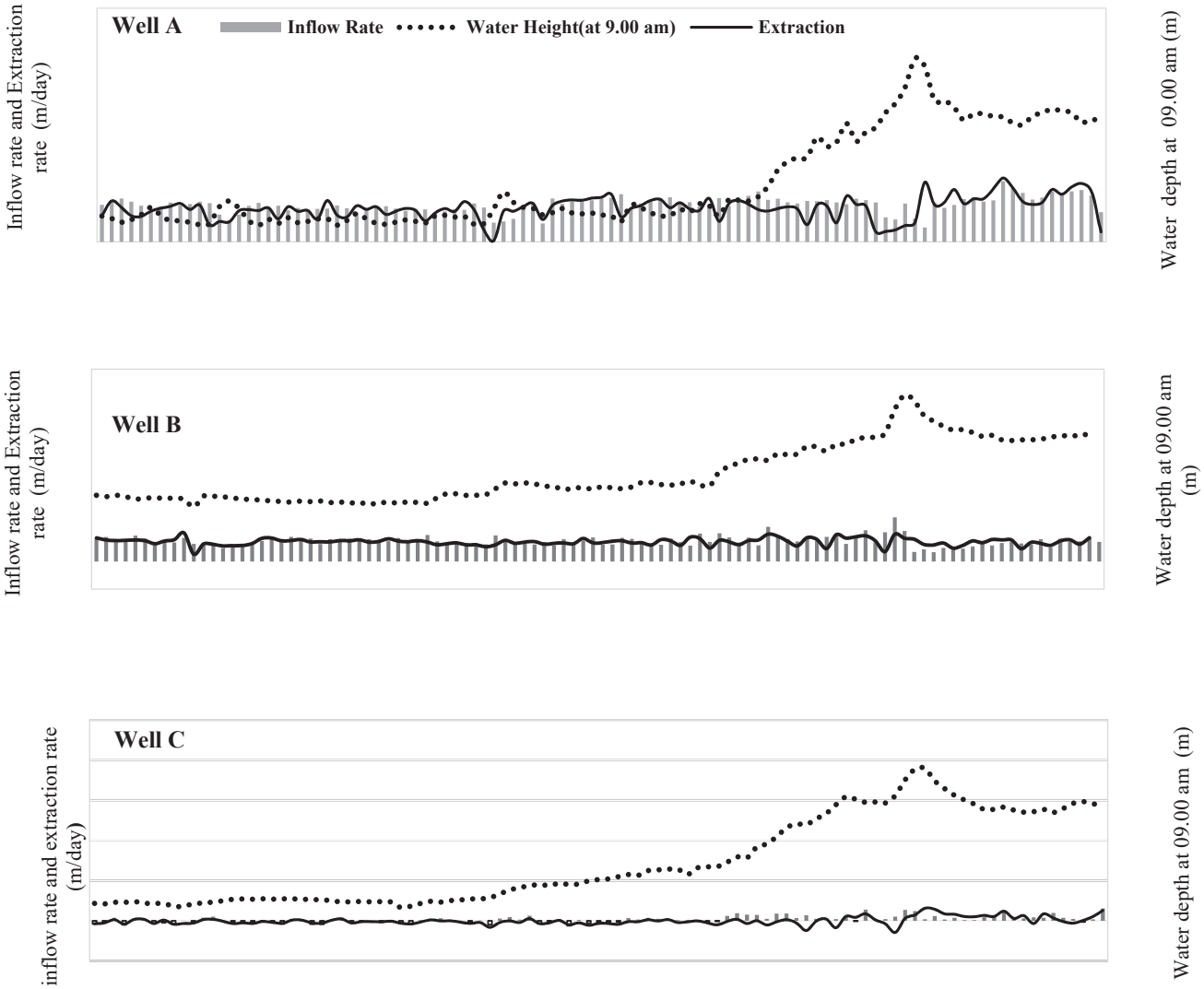


Fig.5 Comparison of wells A, B, and C during dry and rainy seasons from 2015.08.25 to 2015.12.05

TABLE I
THE INFLOW RATE AND EXTRACTION VOLUME OF WELLS A, B, AND C IN DRY AND RAINY SEASONS

	Unit	Dry season August 24 - September 26, 2015			Rainy season September 27- December 06, 2015		
		A	B	C	A	B	C
Average inflow rate ($V_{if(24)}/A$)	($x10^{-6}$) m/sec	6.0	4.3	-0. 1	6.7	4.3	0.4
Average inflow rate ($V_{if(24)}/A$)	m/day	0.518	0.374	-0.006	0.580	0.374	0.035
Average inflow volume ($V_{if(24)}$)	m^3/day	1.176	0.849	-0.015	1.318	0.851	0.071
Average extraction volume ($V_{ext(24)}$)	m^3/day	1.041	0.829	-0.031	1.209	0.811	0.014

TABLE II
INFLOW RATE AND EXTRACTION VOLUME OF WELLS A, B, AND C IN *PRE-, MID-, AND POST*-INTENSIVE RAINY PERIODS

	Unit	pre-Intensive rainy period Sep 27- Oct 26, 2015			mid-Intensive rainy period Oct 27- Nov 15, 2015			post-Intensive rainy period Nov 16 -Dec 05, 2015		
		A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Inflow rate ($V_{if(24)}/A$)	($x10^{-6}$) m/sec	6.2	4.2	0.1	6.9	5.2	0.5	7.3	3.7	0.5
Inflow rate ($V_{if(24)}/A$)	m/day	0.537	0.363	0.006	0.594	0.450	0.046	0.631	0.318	0.047
Average inflow volume ($V_{if(24)}$)	m^3/day	1.220	0.776	0.009	1.350	0.963	0.065	1.432	0.680	0.066
Average extraction volume ($V_{ext(24)}$)	m^3/day	1.149	0.741	-0.025	0.986	0.818	-0.021	1.521	0.743	0.086

Table I denotes the average values of inflow rate, inflow volume and extraction volume in dry and rainy seasons. And Table II also denotes them in every period of *pre*-, *mid*-, and *post*-intensive rainy period.

Table I shows that the average daily inflow volume and extraction volume at each well are almost same in both of dry and rainy seasons. The inflow and extraction volumes of well A are the highest among three wells in both seasons. This means that well A is the most actively used in the three wells.

From Fig.4, Fig.5, and Tables I and II, we can explain the water balance status of each well as follows.

Well A

The household of five persons using well A draws an average of 1,041 liters of water, which is correspond to almost 200 liters par one parson a day, in dry season and 1,209 liters, correspond to 240 liters per person a day, in rainy season for daily needs from the well according to the Table I.

In dry season, from Fig. 3, the household extracts almost all of water from well every day.

According to Table II and Fig.5, the daily inflow volume is gradually increasing from pre-intensive rainfall period to post-intensive rainfall period. However, the daily extraction volume in mid-intensive rainfall period is decreasing, and it causes the increasing of water depth in the well in this period. The highest depth is recorded at end of the mid-intensive rainfall period as 2.75m. In the post-intensive rainfall period, the average daily inflow rate takes highest value. Further, the average extraction rate takes also highest value and is higher than the average daily inflow rate in this period. This yields the reduction of water depth. The extra extraction of water in this period may be used for the watering to home gardening. The gardening perhaps starts in the middle of October. And they may face water shortage for gardening at this period due to relatively small rainfall compared with intensive rain period.

Well B

Table I and Fig. 5 show that it is not found any considerable difference in daily inflow and extraction rates/volumes in both of dry and rainy seasons. However, from Table II, the highest inflow and extraction rate/volume are found in the intensive rainy period.

From Table I, we find household B with six members extracts 829 liters of water from the well, which is correspond to 140 liters a person a day, in dry season, and 811 liters, correspond to 135 liters a person a day, in rainy season for domestic needs.

Well C

Fig.5 shows almost no extraction and no inflow at well C, especially in dry season. It means that the water in the well and the water in the aquifer outside of well are not exchanged because of the static equilibrium state between the well and aquifer.

The reason why they don't extract water in the dry season is high saline. They actually use common well, ditch and pond nearby to them.

In rainy season, the positive value of inflow rate is observed according to Tables I and II. Fig.5 also shows that the water depth increases to its maximum value due to the positive inflow rate. The Household C starts to extract the water in well C because its salinity may become small in post intensive period.

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Quantitative Analysis of Nutrient Inflow from River and Groundwater to Imazu Bay in Fukuoka, Japan

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Abstract**—**Imazu Bay plays an important role for endangered species such as horseshoe crabs and black-faced spoonbills that stay in the bay for spawning or the passing of winter. However, this bay is semi-enclosed with slow water exchange, which could lead to eutrophication under the condition of excess nutrient inflow to the bay. Therefore, quantification of nutrient inflow is of great importance. Generally, analysis of nutrient inflow to the bays takes into consideration nutrient inflow from only the river, but that from groundwater should not be ignored for more accurate results. The main objective of this study is to estimate the amounts of nutrient inflow from river and groundwater to Imazu Bay by analyzing water budget in Zuibaiji River Basin and loads of T-N, T-P, NO_3-N and NH_4-N. The water budget computation in the basin is performed using groundwater recharge model and quasi three-dimensional two-phase groundwater flow model, and the multiplication of the measured amount of nutrient inflow with the computed discharge gives the total amount of nutrient inflow to the bay. In addition, in order to evaluate nutrient inflow to the bay, the result is compared with nutrient inflow from geologically similar river basins. The result shows that the discharge is 3.50×107 m3/year from the river and 1.04×107 m3/year from groundwater. The submarine groundwater discharge accounts for approximately 23 % of the total discharge, which is large compared to the other river basins. It is also revealed that the total nutrient inflow is not particularly large. The sum of NO3-N and NH4-N loadings from groundwater is less than 10 % of that from the river because of denitrification in groundwater. The Shin Seibu Sewage Treatment Plant located below the observation points discharges treated water of 15,400 m3/day and plans to increase it. However, the loads of T-N and T-P from the treatment plant are 3.9 mg/L and 0.19 mg/L, so that it does not contribute a lot to eutrophication.

Keywords**—**Eutrophication, groundwater recharge model, nutrient inflow, quasi three-dimensional two-phase groundwater flow model, Submarine groundwater discharge.

I. Introduction

IN semi-enclosed bays with slow water exchange, high nutrient loading causes eutrophication. For example, pollution loads flowing into Tokyo Bay increased during the period of high economic growth, which resulted in outbreaks of red tide and poor oxygen water mass [1]. Water quality in Tokyo Bay has been improved by the regulation of COD, phosphorus and nitrogen based on the Water Pollution Prevention Law, but tideland or seaweed bed cannot be restored after eutrophication occurs.

A semi-enclosed bay, located in Fukuoka City, is a suitable place for wild birds to live. Approximately 180 kinds of birds, such as sandpipers or ducks, come to this bay and spawning horseshoe crabs have been reported in the beach of the bay. Thus, it is obvious that Imazu Bay biologically plays an important role. On the other hand, the bay does not have active water exchange and it is very possible that eutrophication occurs. Accordingly, it is needed to prevent eutrophication, which is mainly caused by high nutrient loadings, and therefore, the quantification of the nutrient loadings into the bay is of great importance. Generally, analysis of the nutrient inflow into a bay takes into consideration the nutrient loadings from the river only; however, recent research has shown that submarine groundwater discharge is also a significant pathway of nutrient loading to the sea [2].

In this paper, the main objective is estimate nutrient loadings from river and groundwater into Imazu Bay. First, the water budget in Zuibaiji River Basin, whose water is flowing into Imazu Bay is analyzed using various models. Next, the measured nutrient loadings are multiplied with the computed discharge from Zuibaiji River Basin into the bay to estimate nutrient inflow. Finally, in order to evaluate nutrient loading into Imazu Bay, the result is compared with the nutrient loadings from geologically similar river basin to bays.

II. Method and Materials

A. Study Area and Observations

Zuibaiji River is a second grade river which flows through the western part of Fukuoka and Itoshima City, Japan. It is 13.2 km long and its basin covers an area of 52.9 km^2. Zuibaiji Dam is located upstream of the river and helps to supply drinking water to Fukuoka and Itoshima City. The basin is composed of granodiorite in the upstream basin and transported sediments, such as mud, sand or gravel, in the downstream basin.

In the river basin, rainfall has been monitored at Ikeda and Zuibaiji Dam. The river flow rate and water quality was observed at Ikada in November, 2014. (Table I), and, groundwater quality was observed at four points (A, B, C and D) in October, 2011. (Table II)

TABLE I
River Water Quality at IKEDA

Parameters	
Water temperature (°C)	23.8
pH	7.1
EC(μS/cm)	193
ORP(mV)	275
T-N(mg/L)	1.6
T-P(mg/L)	0.086
NO_3^-(mg/L)	5.8
NO_4^+(mg/L)	«0.03

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Fig. 1 Observation points

TABLE II
GROUNDWATER QUALITY AT THE 4 POINTS

Parameters	A	B	C	D
Water temperature (°C)	19.7	22.5	20.6	22.0
pH	7.20	7.41	6.89	6.64
EC(μS/cm)	2110	1870	6040	8040
DO(mg/L)	1.4	1.0	1.2	1.3
ORP(mV)	-179	-351	-209	-13
T-N(mg/L)				
T-P(mg/L)				
NO_3^-(mg/L)	ND	ND	ND	ND
NO_4^+(mg/L)	0.03	0.07	0.08	0.50

B. Estimation Methods

The two models, the groundwater recharge model and quasi three-dimensional two-phase groundwater model, are applied to estimate river discharge and submarine groundwater discharge, respectively. The study area is divided into 13,005 grids, with a grid length of 50 m in the x and y directions, and land use distribution data were assigned to all grids. The simulation period is three years from 2009 to 2012.

The groundwater recharge model is applied to calculate groundwater recharge, evapotranspiration and direct surface runoff from the input data, such as rainfall, temperature, topographical data and so on. The groundwater recharge calculated in the GRM is used as the input data for the quasi three-dimensional two-phase groundwater model which estimates groundwater discharge into river. The amount of river discharge is the summation of direct surface runoff and groundwater discharge into river, and the submarine groundwater discharge is the difference between groundwater recharge and groundwater discharge into the river.

After the simulation for the river discharge and the submarine groundwater discharge, the observed nutrient loads (T-N, T-P, NO_3-N and NH_4-N) in the river and groundwater are multiplied with the estimated discharge, resulting in the total nutrient loads from the river and groundwater to the bay.

III. DESCRIPTION OF MODELS AND PARAMETERIZATION

A. Groundwater Recharge Mode

The conceptual groundwater recharge model is illustrated in Fig. 1. It functions as a vertical tank storage with an outlet at height R_0 and an outlet coefficient a_L. The R_0 corresponds to the field capacity of the soil and a_L controls the groundwater recharge rate $q_w(t)$ from the tank. The recharge induces a rise of the groundwater table. Further, the rainfall interception is denoted by $r_{int}(t)$ and rainfall that reaches the ground surface r(t) is calculated by $r(t) = r_{total}(t) - r_{int}(t)$, where $r_{total}(t)$ is the total rainfall intensity. For areas without trees, $r(t) = r_{total}(t)$.

The rainfall that reaches the ground surface is then separated into two components: the surface runoff, whose rate is given as $F(r)\cdot r(t)$ and the infiltration, with the rate $[1 - F(r)]\cdot r(t)$, as shown in Fig. 1. Here, F(r) denotes the surface runoff coefficient as a function of rainfall intensity.

$$F(r)=\frac{r(t)}{r(t)+(r)_{1/2}}\cdot F_{\infty} \tag{1}$$

where $(r)_{1/2}$ is the value of r(t), when Fi(r) is equal to $F_{i\infty}/2$. If typical $F_{i\infty}$ values are adopted, such as exemplified in Table I, then only $(r)_{1/2}$ is an undetermined parameter in the equation.

Evapotranspiration reduces water stored in the tank by $EVT_1(t)$. If the water in the tank is exhausted, evapotranspiration can still occur by water uptake denoted by $EVT_2(t)$ from the groundwater through the unsaturated zone and the root zone, as explained by Bouwer (1978) [3]. This may occur if the vertical distance between the ground surface and the unconfined groundwater table is less than the extinction depth Hg*, which needs to be evaluated separately. A similar approach was introduced by Anderson & Woessner (1992) [4], who considered the water uptake rate from the groundwater as a linear function of depth of the water table less than Hg*. The actual evapotranspiration can thus be estimated as the sum of $r_{int}(t)$, $EVT_1(t)$ and $EVT_2(t)$. It is obvious that the actual evapotranspiration by the present procedure varies over the region depending on the tank properties and the groundwater level.

The following equations describe the change in water level stored in the tank as illustrated in Fig. 2. Equation (2) expresses the change in the tank water level, $h_w(t)$, and (3) gives the recharge rate to the unconfined groundwater:

$$\frac{dh_w}{dt}=\{1-F(r)\}\cdot r(t)-q_w(t)-EVT_1(t) \tag{2}$$

$$q_w(t)=a_L\cdot\{h_w(t)-R_0\}\times Y[h_w(t)-R_0] \tag{3}$$

where $Y\{h_w(t) - R_0\}$ is a step function equal to 1 for $hw(t)>R_0$ and 0 for $h_w(t)<R_0$. The outlet coefficient a_L has the unit h-1, and $q_w(t)$ is the recharge rate to groundwater. The $q_w(t)$ divided by effective porosity n_e can be approximated as the groundwater table rising rate.

The parameters n_e, a_L, R_0, F_{∞}, $(r)_{1/2}$, were assigned values depending on land use (Table III) to represent its effect to direct

runoff, infiltration, and groundwater recharge from a previous study by [5].

Fig. 2 Groundwater recharge model

TABLE III
PARAMETERS FOR LAND USE

Land use	n_e	a_L	R_0	$r_{(1/2)}$	F_∞
Paddy field	0.175	0.39	7	9	0.2
Agriculture field	0.175	0.19	5	5.7	0.2
Forest area	0.225	0.13	17	6.6	0.3
Building area	0.225	0.3	19	4.5	0.3
Golf field	0.08	0.5	8	12	0.1
Lake or river	0.08	0	0	0	1
Unused bare area	0.08	0.43	7	2.5	0.3

B. Quasi Three-Dimensional Two-Phase Groundwater Flow Model

The quasi three-dimensional salt- and freshwater two-phase groundwater flow model was applied to the present simulation, since one of the main interests was to calculate outflow from groundwater into the river. Not only freshwater, but also saltwater is taken into consideration in the model. The model employs basic groundwater flow equations for unconfined aquifer and confined aquifer. Figs. 3 and 4 show the quasi three-dimensional two-phase groundwater flow model for unconfined aquifer and confined aquifer.

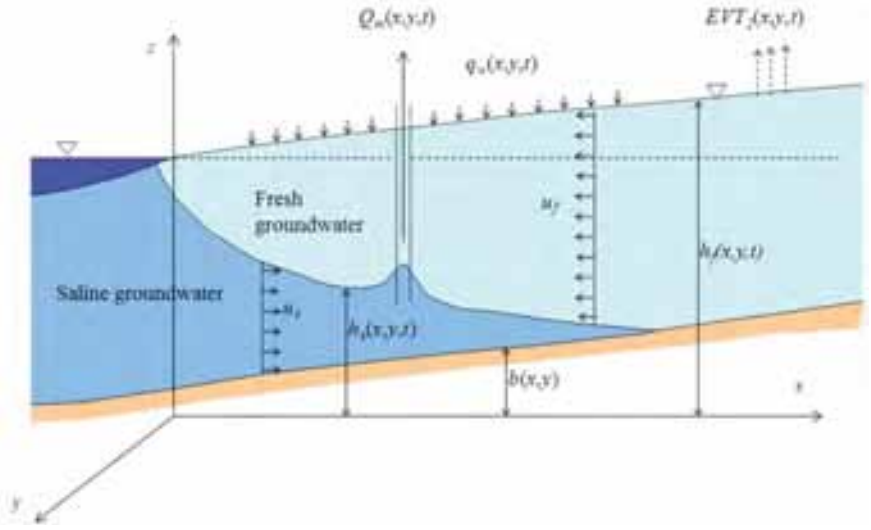

Fig. 3 Quasi three-dimensional two-phase groundwater flow model for unconfined aquifer

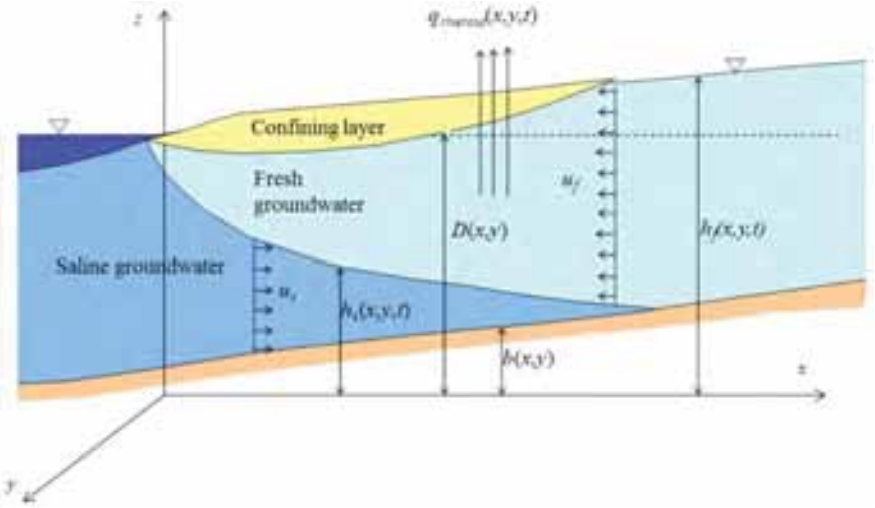

Fig. 4 Quasi three dimensional two-phase groundwater flow model for confined aquifer

The basic groundwater flow equations in the unconfined aquifer are;

- Freshwater phase:

$$n_e\frac{\partial(h_f-h_s)}{\partial t}=-\frac{\partial\{(h_f-h_s)\cdot u_f\}}{\partial x}-\frac{\partial\{(h_f-h_s)\cdot v_f\}}{\partial y}-\sum_m\begin{matrix}Q_m(x,y,t)\delta(x-x_m)\delta(y-y_m)\\+q_w(x,y,t)-EVT_2(x,y,t)\end{matrix} \quad (4)$$

- Saltwater phase

$$n_e\frac{\partial h_s}{\partial t}=-\frac{\partial[\{h_s-b(x,y)\}\cdot u_s]}{\partial x}-\frac{\partial[\{h_s-b(x,y)\}\cdot v_s]}{\partial y} \quad (5)$$

The basic groundwater flow equations in the confined aquifer are;

- Freshwater phase

$$S\cdot\frac{\partial h_s}{\partial t}-n_e\frac{\partial h_s}{\partial t}=-\frac{\partial[\{D(x,y)-h_s\}\cdot u_f]}{\partial x}-\frac{\partial[\{D(x,y)-h_s\}\cdot v_f]}{\partial y}-\sum_m\begin{matrix}Q_m(x,y,t)\delta(x-x_m)\delta(y-y_m)\\-q_{riverout}(x,y,t)\delta(x-x_{out})\delta(y-y_{out})\end{matrix} \quad (6)$$

- Saltwater phase

$$n_e\frac{\partial h_s}{\partial t}=-\frac{\partial[\{h_s-b(x,y)\}\cdot u_s]}{\partial x}-\frac{\partial[\{h_s-b(x,y)\}\cdot v_s]}{\partial y} \quad (7)$$

where $h_f(x,y,t)$, $h_s(x,y,t)$, $b(x,y)$ and $D(x,y)$ are fresh groundwater elevation, two-phase interface elevation, impermeable base elevation taken from the reference level and the elevation of the base of the confining layer taken from the reference level, respectively. The term $Q_m(x,y,t)$ is the water extraction rate by pumping at location (x_m,y_m) at time t. The delta functions $\delta(x-x_m)$ and $\delta(y-y_m)$ represent the location of the pumping well. The term $q_w(x,y,t)$ represents the groundwater recharge. The term $q_{riverout}$ is the groundwater discharge into the river at location (x_{out},y_{out}).

Darcy's law gives the relationship as shown in (8).

$$u_f=-k\frac{\partial\phi_f}{\partial x},v_f=-k\frac{\partial\phi_f}{\partial y},\phi_f=h_f$$
$$u_s=-k\frac{\partial\phi_s}{\partial x},v_s=-k\frac{\partial\phi_s}{\partial y},\phi_s=\frac{\rho_f}{\rho_s}\cdot h_f+\frac{\Delta\rho}{\rho_s}\cdot h_s \quad (8)$$

where the terms u_f, u_s, and v_s, v_f represent the velocity components in x- and y- direction. The subscripts s and f denote saltwater and freshwater, respectively. The terms ϕ_f and ϕ_s are the piezometric heads, and the density difference is written as $\Delta\rho=\rho_s-\rho_f$ at the saltwater intrusion area. The permeability k varies spatially but is assumed uniform in the vertical direction.

C. Water Balance Analysis

Based on the result of the simulations, several hydrological components are investigated. As seen in Fig. 5, river discharge and submarine groundwater discharge can be estimated as;

$$Q_r=q_r+q_{riverout} \quad (9)$$

$$q_{riverout}=q_w-q_{riverout} \quad (10)$$

IV. Results and Discussion

A. Simulation Results

The annual river discharge and groundwater discharge are $3.50\times10^7 m^3$/year and $1.04\times10^7 m^3$/year, accounting for approximately 77 % and 23 %. Taniguchi (2001) [6] reported that the percentage of submarine groundwater discharge is generally approximately a few percent to 10 percent. Thus, the submarine groundwater discharge into Imazu Bay is larger than average, the cause of which is much spring water in the area.

Fig. 5 Water balance in river basin

B. Validity of the Simulation

In order to verify the validity of the result, the simulated river discharge is compared with that which is observed at Ikeda. The comparison of the simulated specific discharge with the observed specific discharge is shown in Fig. 6. The simulated specific discharge is much smaller than the observed one. Generally, the h-q curve for the calculation of the observed discharge is defined where there are no sediments on the bottom. But, it is considered that the bottom level rise due to sedimentation caused the error, since the runoff rate which equals the observed river discharge divided by rainfall is more than one. Although there is a difference between the simulated value and the observed value, both values show the same tendency. Therefore, it is assumed that the simulated river discharge and groundwater discharge are valid and could be used for the estimation of nutrient loads.

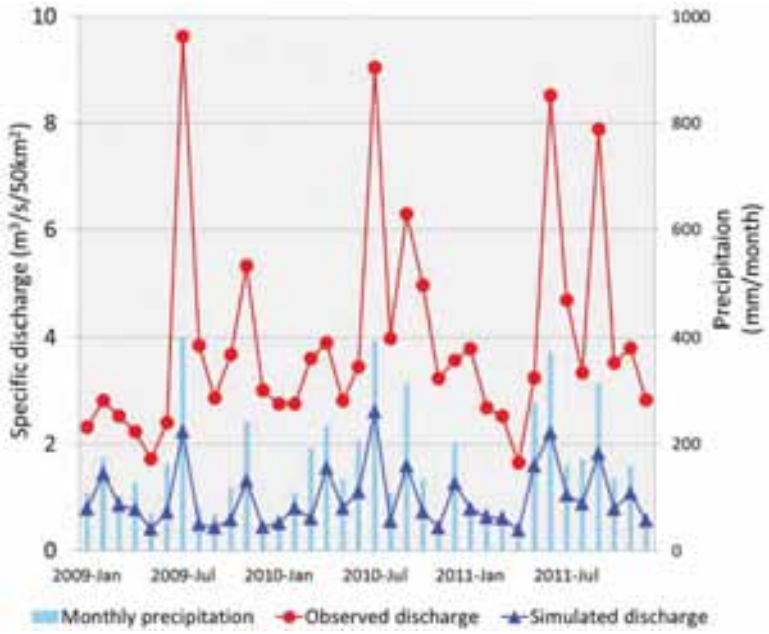

Fig. 6 Observed vs simulated river discharge

C. Estimated Nutrient Loads

Table IV shows the estimated nutrient loads flowing into Imazu Bay from river and groundwater.

1. Nutrient Loads from the River

There is a wastewater treatment plant named Shin Seibu Water Treatment Plant around Imazu Bay. The inflow into the wastewater plant was expected to increase due to urbanization resulting from the new campus of Kyushu University, and as such, the Shin Seibu Water Treatment Plant was established in

2014. The outflow from the treatment plant needs to be taken into consideration since it is located below the observation points. The treatment plant reported that the amount of treated water discharge is 15,400 m^3/day and loads of T-N and T-P from the plant are 3.9 mg/L and 0.19 mg/L, which indicates that the annual loads of T-N and T-P are 21.9 and 1.1 t/year [7]. These values are not too large to contribute to eutrophication in the bay.

The estimated nutrient loads are compared with those from other river basin with nearly same the areas as the study area. In Fukuoka City, there are the Mikasa River, Naka River, Okei River, besides the Zuibaiji River. Fukuoka City has already estimated the nutrient loads into Hakata Bay from the rivers [8]. The loads of T-N and T-P from Zuibaiji River are not large, compared with those from other rivers. (Fig. 7) Even if the loads from the treatment plant are added to those from Zuibaiji River, they are not considerable.

TABLE IV
NUTRIENT LOADS FROM RIVER AND GROUNDWATER

	T~N	NO_3~N	NH_4~N	T~P
River (t/year)	53	44	0.78	2.9
Groundwater (t/year)		0	0.28~4.73	

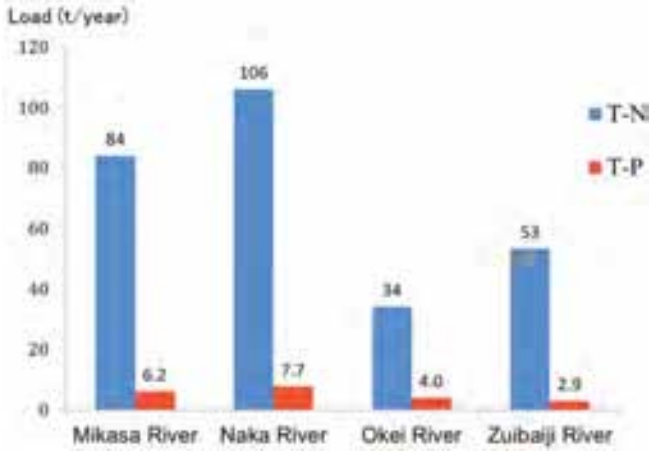

Fig. 7 Comparison of T-N and T-P loads from river

2. Nutrient Loads from Groundwater

Due to lack of data, the loads of T-N and T-P are not estimated. The load of NO_3-N is 0 t/year since the observed concentration of NO_3^- is lower than the detection limit as seen in Table II. The load of NH_4-N ranges from 0.28 to 4.73 t/year because of the different concentration of NH_4^+ depending on the observation points. Table III shows that the sum of NO_3-N and NH_4-N loadings from groundwater is less than 10 % of that from the river.

V. CONCLUSION

In this study, the nutrient loads into Imazu Bay from river and groundwater are respectively estimated using a groundwater recharge model coupled with a quasi three-dimensional two-phase groundwater model.

The following results were obtained:

- The percentage of submarine groundwater discharge to the total discharge into Imazu Bay is 23 %, which is larger than average according to Taniguchi.
- The summation of the NO_3-N and NH_4-N loads from groundwater is less than 1/10 of that from the river, which is mainly caused by that of the NO_3-N load is 0t/year due to the denitrification of the groundwater.
- Although a wastewater treatment plant is located around the bay, the loads of T-N and T-P from the treatment plant are 3.9 mg/L and 0.19 mg/L which do not contribute a lot to eutrophication in the bay. However, we need to pay attention to discharge from the plant since it plans to increase discharge.

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The Taiwan Environmental Impact Assessment Act Contributes to the Water Resources Saving

Feng-Ming Fan, Xiu-Hui Wen

***Abstract*—**Shortage of water resources is a crucial problem to be solved in Taiwan. However, lack of effective and mandatory regulation on water recovery and recycling leads to no effective water resource controls currently. Although existing legislation sets standards regarding water recovery, implementation and enforcement of legislation are facing challenges. In order to break through the dilemma, this study aims to find enforcement tools, improve inspection skills, develop an inspection system, to achieve sustainable development of precious water resources. The Taiwan Environmental Impact Assessment Act (EIA Act) was announced on 1994. The aim of EIA Act is to protect the environment by preventing and mitigating the adverse impact of development activity on the environment. During the EIA process, we can set standards that require enterprises to reach a certain percentage of water recycling based on different case characteristics, to promote sewage source reduction and water saving benefits. Next, we have to inspect how the enterprises handle their waste water and perform water recovery based on environmental assessment commitments, for the purpose of reviewing and measuring the implementation efficiency of water recycling and reuse, an eco-friendly measure. We invited leading experts in related fields to provide lecture on water recycling, strengthen law enforcement officials' inspection knowledge, and write inspection reference manual to be used as basis of enforcement. Then we finalized the manual by reaching mutual agreement between the experts and relevant agencies. We then inspected 65 high-tech companies whose daily water consumption is over 1,000 tons individually, located at 3 science parks, set up by Ministry of Science and Technology. Great achievement on water recycling was achieved at an amount of 400 million tons per year, equivalent to 2.5 months water usage for general public in Taiwan. The amount is equal to 710 billion bottles of 600 ml cola, 170 thousand international standard swimming pools of 2,500 tons, irrigation water applied to 40 thousand hectares of rice fields, or 1.7 Taipei Feitsui Reservoir of reservoir storage. This study demonstrated promoting effects of environmental impact assessment commitments on water recycling, and therefore water resource sustainable development. It also confirms the value of EIA Act for environmental protection. Economic development should go hand in hand with environmental protection, and it's a mainstream. It clearly shows the EIA regulation can minimize harmful effects caused by development activity to the environment, as well as pursuit water resources sustainable development.

***Keywords*—**The environmental impact assessment act, water recycling environmental assessment commitment, water resource sustainable development, water recycling and reuse.

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Grading of Emulsified Agarwood Oil Using Gel Electrophoresis Technique

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***Abstract*—**In this study, encapsulation of agarwood oil with non-ionic surfactant, Tween 80 was prepared at critical micelle concentration of 0.0167 % v/v to produce the most stable nano-emulsion in aqueous. The encapsulation has minimized the bioactive compounds degradation in various pH conditions thus prolong their shelf life and maintained its initial oil grade. The oil grading of the prepared samples were conducted using the gel electrophoresis instead of using common analytical industrial grading such as gas chromatography- mass spectrometry (GC- MS). The grading method was chosen due to their unique zeta potential value after the encapsulation process. This paper demonstrates the feasibility of applying the electrophoresis principles to separate the encapsulated agarwood oil or grading of the emulsified agarwood oil. The results indicated that the grading process are potential to be further investigate based on their droplet size and zeta potential value at various pH condition when the droplet were migrate through polyacrylamide gel.

***Keywords*—**Electrophoretic mobility, essential oil, nanoemulsion, polyacrylamide gel electrophoresis, Tween 80, zeta potential.

I. Introduction

AGARWOOD is resin impregnated heartwood from *Aquilaria malaccensis,* which belongs to the family of genus *Aquilaria (Thymelaeaceae).* It is also known as gaharu, eaglewood, aloeswood, oud, jinkoh, and chenxiang and can be found in Southeast Asian countries such as Malaysia. The aromatic resin is formed as the tree sap gradually become harder and change it physical form from dark brown to black colour due to response to injury and fungal infection. Agarwood is recognized as one of the most valuable natural product trade internationally due to its endless uses, ranging from large sections of trunk to finished products such as incense and perfumes.

The quality of the agarwood oil which industrially extracted through hydrodistillation is depending on the degree of injury and its resin formation. High quality agarwood oil (grade A) may cost between USD 93- 465 per tola [1]. Many previous studies on grading agarwood oil quality have been carried out. Conventionally, grading of agarwood was based on its physical properties such as colour, aroma, density which mainly depend on individual perception and experience. Recently, several analytical methods such as gas chromatography- mass spectrometry (GC- MS), electronic nose, and nuclear magnetic resonance (NMR) were recognized to determine the chemical properties of agarwood oil [2]-[5]. However, some of the industrialist unable to afford the burden of the analytical cost due to their limited product for sampling purpose. Thus, gel electrophoresis is introduced as an alternative method to assess and grade the quality of the agarwood oil based on its electrical properties.

Gel electrophoresis is a powerful, yet, simple and cheaper tool used for separate nucleic acid and protein on the basis of their size and charges in an applied electric field [5]. Small and high charge molecule will travel faster through the gel than a large and low charge molecule. Sieving mechanism is used to describe the electrophoretic mobility of the molecules throughout the gel matrix [6]. The types of gel most commonly used are agarose and polyacrylamide gels. Agarose gel is used for separating large size molecules and has a relatively low resolving power; whereas, polyacrylamide gels have greater resolving power for small size molecule. Also, the average pore size is typically 200-500 nm for agarose, but 5-100 nm for polyacrylamide gel [7]. Therefore, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) was chosen for the emulsified agarwood oil droplets separation in this study.

Up to the author's knowledge, agarwood oil quality grading system based on its electrical properties has not been being reported elsewhere in the literature. The objective of this study was to separate or to grading the emulsified agarwood oil droplets with SDS PAGE based on their size and zeta potential value. Since pH is one of the most important factor that affects zeta potential value, thus relationship between pH and zeta potential was also studied. The size of emulsified oil droplets lying within nanometre range and response to specific zeta potential value and electrophoretic mobility when they are varies in different pH conditions, so that separation using SDS PAGE can be evaluated.

II. Experimental

A. Materials

Agarwood oil was purchased commercially from YSG Excellence Sdn. Bhd., Malacca, Malaysia; Tween 80

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(Polyoxyethylene (20) sorbitanmonooleate), acrylamide, bisacrylamide, sodium dodecyl sulfate (SDS), tris base, ammonium persulfate (APS), Tetramethylethylenediamine (TEMED), bromophenol blue, coomassie brilliant blue, acetic acid, and methanol were purchased from Sigma Aldrich (St. Louis, MO, USA).

B. Sample Preparation

The droplets of agarwood oil were dispersed in a continuous phase of surfactant solution according to [8], [9]. However, some modifications were done on the formulation. The emulsion formulations were prepared by using various surfactant concentration of 0.01, 0.0125, 0.0167, 0.025, 0.05, 0.1% (v/v) with a constant volume of agarwood oil. The emulsion was prepared by probe sonicator (Fisher Scientific Model 705 Sonic Dismembrator, Waltham, MA, USA) inside an ice bath for 4 minutes and 70 % amplitude ultrasonication.

The emulsion that produced at critical micelle concentration (CMC) was then subjected to pH variation in order to obtain large significant differences in zeta potential values so that separation based on charges can be performed through SDS PAGE.

C. Droplet Size and Zeta Potential

The stability of emulsion and pH- adjusted emulsion produced were evaluated in term of size and zeta potential [10]. Droplet size distribution and zeta potential were measured using built in dynamic light scattering technique and electrophoretic light scattering technique, respectively by zeta/ nano particle analyser (Nanoplus, Particulate Systems, USA) at ambient temperature. The zeta potential was not measurable directly but it was calculated using Smoluchowski equation which relate with the experimentally determined electrophoretic mobility.

D. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE)

The experimental setup of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) was performed using a protocol [11], but with a little modification, as shown in Fig. 1. Electrophoresis gel can be divided into stacking gel and separating gel. Resolving gel was prepared by mixing 0.1 mL of 10 % APS (ammonium persulfate) and 0.01 mL of N, N, N', N'-tetramethylethylenediamine (TEMED) for 10 mL of resolving gel solution and pour up to ¾ of short plate height. About 0.2 ml of isopropanol was added on top of the gel solution to make the surface even and to remove the bubbles from the top layer. The isopropanol was then removed after 30 minutes with Whatman filter paper and the polymerized gel surface was rinsed with distilled water. Whereas, stacking gel solution was prepared by mixing 0.15 mL of 10 % APS and 0.02 mL of TEMED for 10 mL of stacking gel solution prior pouring to fill the remaining volume of the glass plates. The comb was immediately inserted to avoid the entrapment of bubbles inside the gel. Then, the comb was removed from the glass plates and the glass plates was washed to remove the gel adhered on the outer surface of the glass plates after 30 minutes. The glass plate was then placed properly in the inner chamber and the chamber was tightened properly by clamping frame in order to avoid the leaking of running buffer from the inner chamber. Next, the chamber was filled with 1x running buffer. Finally, dyed samples were loaded into wells and electrophoresis was carried out at 50 mA, 150 V, for 70 minutes. The gel was then stained with staining solution for half an hour and destaining for an hour prior image scanning. The relative mobility of the bands appeared were then calculated based on:

$$\text{Relative mobility} = \frac{Migration\ distance\ of\ band}{Migration\ distance\ of\ dye} \quad (1)$$

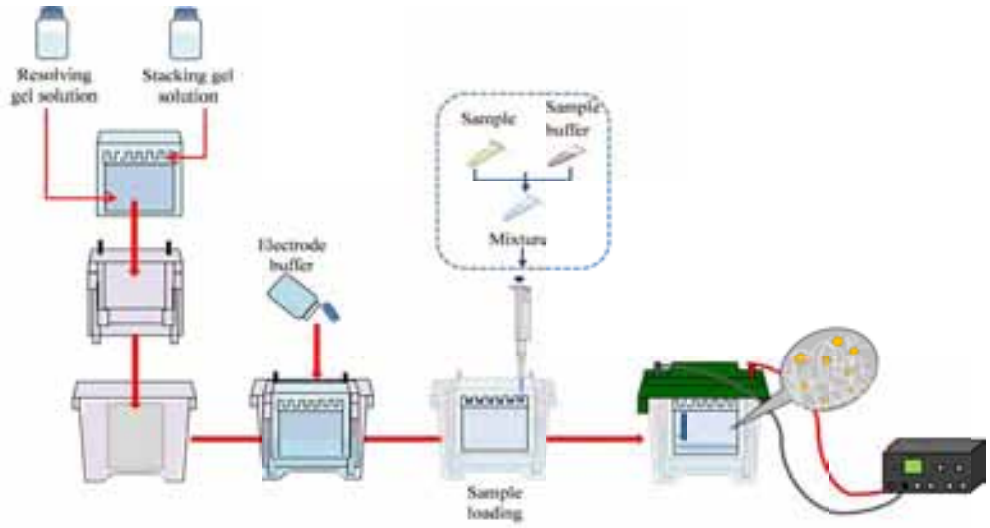

Fig. 1 Experimental setup for SDS PAGE

III. Results and Discussion

A. Effect of Surfactant Concentration on Size Distribution and Stability of Emulsion

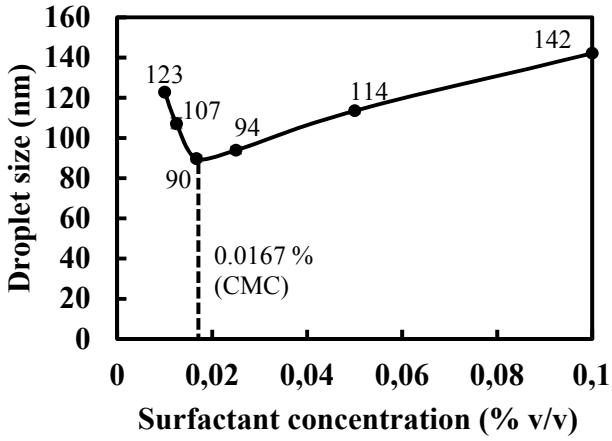

Fig. 2 Surfactant concentration effect on emulsified droplets size

The size of emulsified oil droplets is controlled by the interaction between the droplet breakup and droplet coalescence [12], [13]. Droplet break up is controlled by the type and amount of energy applied to the droplets whereas droplet coalescence is controlled by the ability of surfactant adsorb at the droplet interface, which depend on surfactant surface activity and concentration. In the present study, surfactant Tween 80 was chosen and its concentration effect on droplet size was shown in Fig. 3. Droplet surface coverage by surfactant can used to describe the relationship between droplet size and surfactant concentration. Initially increasing in surfactant concentration resulted in a large decrease in droplets size as the surfactant adsorbed at the oil/ water interface and instantaneously oriented themselves between the two phases. Next, the surfactant facilitated reduction of interfacial tension, and formed a protective film around the droplet to prevent droplets from coalescence [14], [15]. Large droplet size was observed at low surfactant concentration which owing to insufficient coverage of surfactant at the interface of newly formed droplets.

When there was an excess addition of surfactant, at 0.0167% v/v, smallest droplets size about 88 nm was obtained. This was owing to the sufficient concentration of surfactant that rapidly adsorbed at the interface of newly formed droplets, facilitates a maximum reduction in the interfacial tension [16] and provided strongest repulsive forces to prevent droplets from coalescence. Further increase in surfactant concentration, above 0.0167% v/v, led to increasing of droplets coalescence. This might due to changes of sphere micelle to rod shape, lamellar or mesophase owing to excessive surfactant in the system. In this system, Tween 80 concentration of 0.0167% was close to the limit of the reduction of droplet size, thus, 0.0167% of Tween 80 was recognized as critical micelle concentration (CMC) along this study.

Fig. 3 showed the effect of surfactant concentration on zeta potential value of emulsion produced. Zeta potential is one of the factors determining the physical stability of emulsions. The higher the zeta potential value, the higher is the electrostatic repulsion between the droplets and the higher is the physical stability. In the present study, zeta potential values ranged from -20 to -40 mV for emulsion produced with Tween 80, a non-ionic surfactant were obtained, but in principle, droplets stabilized by non-ionic surfactants should have no charge. Negative charge values were obtained may be explained by the presence of composition of agarwood oil, pH, electrolyte or other ionic impurities remaining from their production or generated during storage [17].

The CMC 0.0167% v/v gave highest zeta potential of -39.6 mV among the others surfactant concentration. This might be caused by the strongest repulsive force provided by sufficient coverage of surfactant barrier around the oil droplets, providing the most stable emulsion. However, there was no significant change for zeta potential value of emulsified oil with less or excess addition of surfactant which might be due to the non-ionic characteristic of surfactant used.

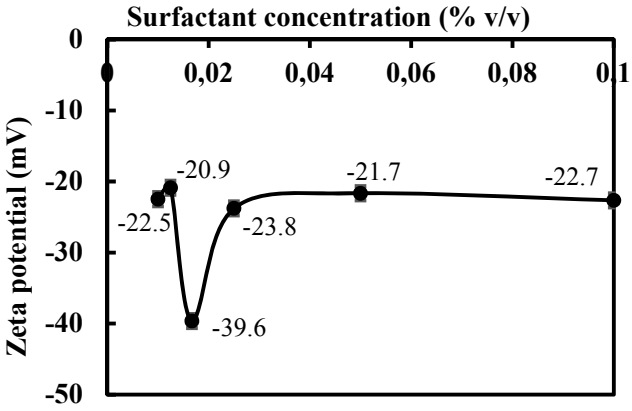

Fig. 3 Zeta potential values of emulsified agarwood oil as a function of surfactant concentration

B. Effect of pH on Size Distribution and Stability of Emulsion Produced at CMC

The oil droplets which comprise of bioactive compounds tend to undergo physical and chemical changes when they are subjected to an extreme pH condition. However, when the most stable emulsion which produced at CMC with pH 6.5 was subjected to various pH conditions starting from pH 3 to pH 10, the droplet size of emulsified oil has no significant changes as shown in Fig. 4. This might be due to the sufficient protection provided by the surfactant barrier toward pH condition as the droplets were fully encapsulated by surfactant monomers.

The zeta potential of the particles is used as a measure of particle charge and electrostatic repulsion, and is one of the fundamental parameters that affects stability. In aqueous media, the pH is one of the most important factors that affects zeta potential value. The relationship between the effects of pH on the zeta potential of emulsion produced at CMC was shown in Fig. 5. It can be seen that small changes in pH has caused significant changes in the zeta potential values of the emulsified oil in which the zeta potential values decreased at low pH and increased at high pH. This may be owing to the adsorption of H^+ and OH^- ions, which are the zeta potential- determining ions

at the oil/ water interface. In addition, the effect of pH on the zeta potential of the emulsified oil may be explained by considering that the surfaces of the oil droplets might contain pH- dependent ionizable functional groups which can undergo dissociation and protonation [18]. Highest zeta potential at simultaneously low electrolyte concentration led to formation of the smallest particle size. Consequently, the emulsified oil produced initially at CMC value (approximately pH 6.5) has high zeta potential value of -39.6 mV in correlate with smallest droplet size. However, the zeta potential value of the emulsified oil tended to decrease with decreasing pH value.

At acidic pH condition (pH 3- 4), the emulsified oil showed destabilisation due to its low zeta potential values that less than 30 mV which is the marker value of stabilisation. This might be explained as the negative charge density was gradually reduced by the adsorption of H^+ ions at the oil/ water interface, which resulting in reduction of zeta potential value as well as the electrostatic repulsion force between droplets. As pH increased to pH 6-7, a significant increase in zeta potential value was observed indicating that the formation of stable emulsion. The highest zeta potential value of -75.6 mV was achieved at pH 8, which might owing to strongest electrostatic repulsion force acting between droplets. Also, OH^- ions concentration have greatly increased with increasing pH and their adsorption at the fully surfactant covered oil/ water interface significantly increased the negative charge density of oil droplets and thus, increased the zeta potential value. As pH value further increase up to pH 10, an increase in OH- ions concentration in the system reduced the emulsion zeta potential to -60.5 mV by compressing the electrical double layer [19].

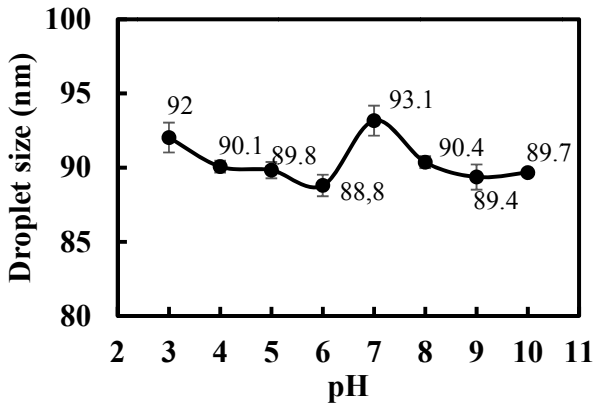

Fig. 4 Measurement of droplet size as a function of pH for emulsion produced at CMC

A. SDS-PAGE Electrophoresis

Electrophoresis refers to the movement of a molecule through a matrix of gel under an electric field. The separation of molecules within a gel was determined by the relative size of the pores formed within the gel. While, the pore size of the gel can be controlled by choosing appropriate concentration of acrylamide and cross- linking agent. Higher percentage gels with smaller pores are used to separate smaller molecules and vice versa. In the present study, single-percentage gel of 15 % was used to separate emulsified droplets with size below 100 nm. Fig. 6 represented the SDS PAGE of emulsion produced at CMC with pH varies from 3-10 at 15% of gel concentration.

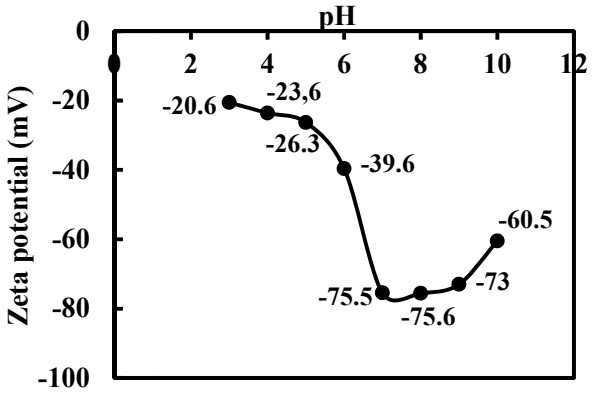

Fig. 5 Measurement of zeta potential as a function of pH for emulsion produced at CMC

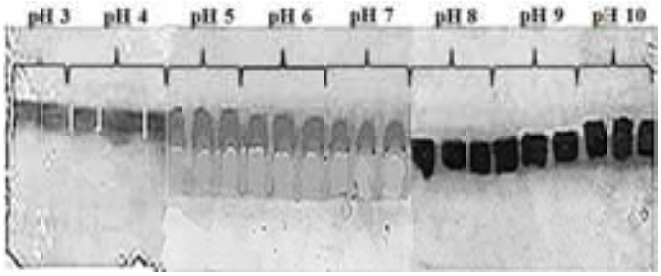

Fig. 6 Visible bands of emulsion produced at CMC 0.0167 % v/v at various pH conditions

Since bioactive compounds of agarwood oil with close molecular weight, for example, γ-eudesmol, α-Elemol, β-Caryophyllene, alloaromadendrene, α-Gurjunene with molecular weight 222.4 g/mol, 222.4 g/mol, 222.4 g/mol, 204.4 g/mol and 204.4 g/mol, respectively were encapsulated within a droplet, thus a broad and single band was observed for each sample lane. As mentioned earlier, there was not much different in the size distribution of the emulsified oil droplets produced at CMC with pH varies from pH 3-10, thus separation was mainly based on the significant difference values of zeta potential at various pH condition. Also, by referring to the band mobility pattern, the emulsified droplets tended to follow the zeta potential trend during pH variation. Thus, a hypothesis was made in which the zeta potential was the domain factor in separating emulsified droplets instead of molecular weight using SDS PAGE.

IV. Conclusion

The paper studied the feasibility of applying SDS PAGE in separation of the emulsified agarwood oil. Characteristics of emulsions produced were evaluated in term of size and zeta potential. Emulsion produced at CMC provided the most stable condition with smallest z-average diameter ~90 nm, and highest zeta potential value of -39.6 mV. Emulsion produced below CMC or above CMC value contributed to large oil droplet and low zeta potential. Variation of pH has a significant

effect on zeta potential of emulsion, owing to H^+ and OH^- ions adsorption at the oil/water interface. Visible bands were observed for emulsified droplets produced at various pH condition within size range of 85 to 95 nm and zeta potential range of -20 to -80 mV due to suitable pore size of 15 % of SDS PAGE gel. Zeta potential value was the dominant factor for emulsified agarwood oil separation as compared to droplet size.

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Utilization of *Juncus acutus as* Allternative Feed Resource in Ruminants

N. Cetinkaya

***Abstract*—** The aim of this paper is to bring about the utilization of *Juncus acutus* as an allternative roughage resource in ruminant nutrition. In Turkey, JA is prevailing plant of the natural grassland in Kizilirmak Delta, Samsun. Crude nutrient values such as crude protein (CP), ether extract (EE), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin(ADL) including antioxidant activity, total phenolic and flavonoid compounds, total organic matter digestibility (OMD) and metabolisable energy (ME) values of *Juncus acutus* stem, seed, and also its mixture with maize silage were estimated. and published. Furthermore, the effects of JA over rumen cellulolitic bacteria were studied. The obtained results from different studies conducted on JA by our team show that *Juncus acutus* may be a new roughage source in ruminant nutrition.

***Keywords*—** Antioxidant activity, cellulolitic bacteria, *Juncus acutus*, organic matter digestibility

I. Introduction

Juncus acutus (JA) is worldwide common plant species of wetlands and it is readily available as green forage for grazing animals in natural grassland of wetlands throughout the year. It has been observed that JA plants are consumed by water buffaloes during grazing period starting from April to November in Kizilirmak Delta. Potential production capacity of JA is arround 8.650 tons only in Kizilirmak Delta. If the other 23 wetlands of Turkey are considered about their JA content, the total available capacity may roughly reaches to 85.537 tons/year [1].

The *in vitro* gas production method is one of powerful and practical method which has been widely used to estimate organic matter digestibility and metabolisable energy values of feedstuffs [2]- [7].

Because the many developing countries including Turkey have been in severe scarcity of roughage supplies due to the adverse effects of global warming, alternative forage sources are to be created and led into the use of feeding ruminant animals. The objective of present paper is to introduce the utilization of *Juncus acutus* as a new roughage resource in ruminant nutrition.

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II. Material and Methods

JA samples were collected from Kizilirmak Delta and Hamsiloz Bay. Collected JA samples were analyzed for DM, CP, EE and OM [8]. NDF, ADF and ADL were determined by [9]. OMD and ME values of JA samples were determined by using in-vito gas production method [10]. The effects of JA on rumen bacteria were estimated by real-time PCR method [11]. JA samples were also analyzed for antioxidant activity [12], [13] and for total phenolic and flavonoid contents[12], [14].

III. Results and Discussion

The OM, EE, CP, NDF, ADF and ADL values of JA were reported between 93.57- 93.89 %, 1.46-1.48 %, 9.77-10.03 %, 73.76-73.70 %, 46.08-46.58 % and 12.01-12.27 % repectively. Moreover OMD and ME values were between 42.64-42.48 % and 6.78-6.82 MJ/kg DM consecutively [1].

The CP, EE and crude fiber contents of stem and seed of JA plant were found 51.00 and 93.10; 47.90 and 26.40; 17.20 and 27.80; 295.70 and 184.50 g/kg DM respectively. Total antioxidant activities of the stems and seeds were found to be similar each other while the total phenolic and total flavonoid concentrations of the seeds were higher than those of the stems. However, crude nutritive values, OMD and ME values of JA seeds were estimated higher than the stems Both stems and seeds of JA may be considered as alternative feed sources for ruminants. The use of these stems and seeds JA as an energy and protein source in ruminant nutrition would also provide phenolic compounds to show antioxidant value[15].

OMD %, ME and b values of three different ratios of JA and maize silage 100:0 (A), 50:50 (B) and 0:100 (C) were 42.06, 51.06 and 60.21%; 6.72, 8.16 and 9.63 MJ/kg DM; 20.85, 35.24 and 48.11 mL respectively. There were significant variations between the chemical composition, gas production, OMD % and ME values of A, B and C ($P<0.05$). Abundance of ruminal bacteria were as following *Fibrobacter succinogenes>Ruminococcus flavefaciens>Ruminococcus albus* values at all incubation times. In conclusion, mixing of JA with maize silage in 50:50 ratio increased the amount of rumen cellulolytic bacteria and 22% of both OMD and ME of JA. Supplementation of maize silage to JA in ruminant diet may improve the utilization of JA through providing of nitrogen and fermentable carbohydrates to rumen bacteria [7].

It may be concluded that JA may be a new alternative feed resouce for ruminant animals. However, further studies are required to see its effects on productive and reproductive parameters such as live weight gain, pregnancy, calving rate,

lactation and milk yield by conducting in vivo trials in ruminanat animals.

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Evaluation of Hazelnut Hulls as an Alternative Forage Resource for Ruminant Animals

N. Cetinkaya, Y. S. Kuleyin

***Abstract*—**The aim of this study was to estimate the digestibility of the fruit internal skin of different varieties of hazelnuts to propose hazelnut fruit skin as an alternative feed source as roughage in ruminant nutrition. In 2015, the fruit internal skins of three different varieties of round hazelnuts (RH), pointed hazelnuts (PH) and almond hazelnuts (AH) were obtained from hazelnut processing factory then their crude nutrients analysis were carried out. Organic matter digestibility (OMD) and metabolisable energy (ME) values of hazelnut fruit skins were estimated from gas measured by *in vitro* method. Their antioxidant activities were determined by spectrophotometric method. Crude nutrient values of the fruit skins of three different varieties were organic matter (OM): 87.83, 87.81 and 87.78%), crude protein (CP): .97, 5.93 and 5.89 %, neutral detergent fiber (NDF): 30.30, 30.29 and 30.29 %, acid detergent fiber (ADF): 48.68, 48.67 and 48.66% and acid detergent lignin (ADL): 25.43, 25.43 and 25.39% respectively. OMD from 24 h incubation time of RH, PH and AH were 22.04, 22.46 and 22.74%; ME_{GP} values were 3.69, 3.75 and 3.79 MJ/kg DM; and antioxidant activity values were 94.60, 94.54 and 94.52 IC 50 mg/mL respectively. The fruit internal skin of different varieties of hazelnuts may be considered as an alternative roughage for ruminant nutrition regarding to their crude and digestible nutritive values. Moreover, hazelnut fruit skin has a rich antioxidant content so it may be used as a feed additive for both ruminant and non-ruminant animals.

***Keywords*—**Antioxidant activity, hazelnut fruit skin, metabolizable energy, organic matter digestibility.

I. Introduction

THE world hazelnut production shows fluctuations depending on climatic conditions. Turkey is a leading country in hazelnut production; an average inshell production is around 650.000 t/year which covers approximately 75-80% of total world production. The remaining 20% of hazelnut production is shared by Italy, USA, Azerbaijan, Georgia and Spain[1]. Turkey is producing 16 different hazelnut varieties in Giresun, Ordu, Trabzon, Rize, Artvin, Sinop, Samsun, Kastomonu, Bartın, Kocaeli, Duzce, Sakarya and Zonguldak provinces which are located in Black Sea Region of Turkey[1].

Hazelnut produced in Turkey are generally classified in three main groups according to fruits shape and features, they are named as RH, PH and AH. Hazelnut hull or hazelnut fruit internal skin is a by-product or waste obtained during hazelnut processing in factories [1].Hazelnut fruit internal skin is obtained as waste in the amount of 4-5% of the total processed hazelnuts according to data received from the hazelnut processing factory. The amount of this waste is around 26.000 – 32.500 t/year. Hazelnut has also been consumed by people without removing internal skin of hazelnut which indicates that internal skin of fruit is edible [2].

The crude nutritive value of a ruminant feedstuffs is determined by chemical analysis [3]. *In-vitro* gas production technique is useful to evaluate the nutritive value of feedstuffs in which produced gas is regarded as an indicator of carbohydrates degradation[4]. Sallam suggested that gas volume is a good parameter from which to predict digestibility and microbial protein synthesis of the substrate by rumen microorganisms in the *in-vitro* system [5]. OMD and ME values of feedstuffs have mostly been determined by using *in-vitro* gas production method [4], [6] and [7].

Nowadays, natural antioxidant sources as health promoting nutrients are gaining great importance in human nutrition [8]. There are several extraction procedures and determination methods for evaluation of the total antioxidant activity of plants[9],[10].The 2,2 diphenyl-1-picrylhydrazyl radical (DPPH) method has widely been used due to its simplicity and its simple reaction system which involves only direct reaction between radical and antioxidant [11].

Since synthetic antioxidants may be toxic and carcinogenic which they have also been well demonstrated with many studies, limitations or prohibitions on their use have been put in the application [12]- [14]. These consequences are directed animal nutrition scientists to search safe and natural resources.

The objective of the present study was to estimate the digestibility and antioxidant activity of the fruit internal skin of different varieties of hazelnut to propose hazelnut fruit skin as an alternative feed source as roughage in ruminant nutrition.

II. Material and Methods

A. Animal Material

The rumen fluid was collected from slaughtered cattle in Florya Meat Joint-Stock Company, Samsun, Turkey. Collected rumen fluids were immediately transferred from Florya slaughterhouse to the laboratory approximately in 5 minutes.

B. Feed Material

In 2015, the fruit internal skins of three different varieties of RH, PH and AH were obtained four times from hazelnut processing factories.

C. Experimental Procedure

Chemical analysis, *in-vitro* gas production experiment and

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total antioxidant activity analysis were carried out with quartet four samples in the Ruminant Feed Evaluation Laboratory of Department of Animal Nutrition and Nutritional Diseases and in Laboratory of Department of Biochemistry, Faculty of Veterinary Medicine, OMU, Samsun, Turkey.

D.Chemical Analysis

Collected fruit internal skins samples were milled through a 1 mm sieve for total antioxidant activity, chemical analysis and *in-vitro* gas production method. Dry mater (DM), ash, ether extract (EE) contents and nitrogen (N) contents were determined according to AOAC procedure[3]. Crude protein (CP) was calculated as N x 6.25. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by using ANKOM fiber analyzer[15].

E. In-vitro Gas Production Method

The ANKOM ^{RF} gas production system which consists of incubator, 12 glass jars named modules, each one having of 250 mL capacity was connected to computer. Gas accumulating in the headspace of module was automatically released when the pressure inside the units reached to 1.5 kPa above ambient pressure. The produced gas pressure was recorded at 10 minutes intervals by using ANKOM ^{RF} gas production system program.

Approximately 1 g of grounded samples were weight and put into 12 modules. The prepared artificial salivia [4] was mixed with rumen fluid 4:1. A mixture of 100 mL of this solution was added to preheated sample containing modules under anaerobic conditions by continuously flushing CO_2. Then modules transferred to incubator at temperature about 39 ^{0}C and pH about 6.5 to 6.8 and in-vitro gas production system was started. After 96 hours system was stopped.

The average cumulative pressure recorded at 0, 3, 6,12, 24, 48, 72 and 96 hours were converted to mL of gas at standard temperature and pressure. Cumulative gas production data at 24 h was fitted to the model (1) of Ørskov and McDonald[16].

$$\text{Gas (Y)} = b\,(1-e^{-ct}) \quad (1)$$

where b: the gas production from the insoluble fraction(mL), c: the gas production rate constant for the insoluble fraction (mL/h), t: incubation time (h).

$T_{1/2:}$ the time taken to produce the half of the gas volume was calculated[17 16], using by the equations (2) and (3).

$$T_{1/2}=Ln2/c \quad (2)$$

$$T_{1/2}=0.693/c \quad (3)$$

OMD %, $ME_{GP,}$ and ME_{OMD} (MJ/ kg DM) values of samples were estimated from gas measured by *in-vitro* method at 24 h by using below equations [5].

$$ME_{GP}\,(MJ/kg\ DM) = 2.2+0.136\ GP+0.057CP+0.0029\ EE \quad (4)$$

$$OMD\ (\%) = 57.2+0.365\ GP+0.304\ CP-1.98\ ADL \quad (5)$$

GP (mL/200 mg DM)

$$ME_{OMD}\ (MJ/kg\ DM) = 0.16\ OMD \quad (6)$$

$ME_{GP:}$ ME calculated from gas production
$ME_{OMD:}$ ME calculated from OMD

F. Determination of Total Antioxidant Activity

Total antioxidant activity and free radical scavenging activity of fruit internal skin of different varieties of hazelnut samples were determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [17], [18]. The absorbances were measured at 520 nm. Quercetin (0–50 mg/L) and ascorbic acid (0–40 mg/L) were used as positive controls.

The radical scavenging activity was calculated by using following equation (7).

$$\text{Inhibition \%} = [(\text{blank absorbance} - \text{sample absorbance})/\text{blank absorbance}] \times 100 \quad (7)$$

The mean concentrations of samples were calculated from three readings causing 50% inhibition values (IC50).

G. Statistical Analysis

The data obtained from the chemical analysis, antioxidant and *in-vitro* gas production experiments was analyzed by the procedure of the software package SAS[18]. Differences between mean values of fruit internal skin of different varieties of hazelnut samples were performed by t-test.

III. Results And Discussion

Chemical composition of fruit internal skin of three different varieties RH, PH and AH of hazelnut is shown in Table I. DM % in air dried of RH, PH and AH were calculated as 91.17, 91.11 and 91.07 % respectively. A statistically significant difference were not observed between chemical composition and ME estimated from ADF values of hazelnut varieties RH, PH and AH at 24 h of incubations with the exception of CF or ether extract. Mean ME_{ADF} values of RH, PH and AH were not significantly different and they were higher than the reported values for wheat straw, maize straw and black wheat straw[21], however, they were close to marc, chick pea straw[22] and *Juncus acutus* [23].

TABLE I
CHEMICAL COMPOSITION OF FRUIT INTERNAL SKIN OF THREE DIFFERENT VARIETIES OF HAZELNUT

Crude Nutrients (%)	RH (n=16)	PH (n=16)	AH (n=16)
	Mean±SE	Mean±SE	Mean±SE
DM	91.17±0.01	91.11±0.03	91.07±0.04
CA	3.34±0.02	3.30±0.04	3.29±0.01
OM	87.83±0.02	87.81±0.03	87.78±0.03
CP	5.97±0.04	5.93±0.03	5.89±0.02
CF	21.16±0.08a	20.32±0.06b	17.15±0.03c
NDF	30.30±0.05	30.29±0.03	30.29±0.05
ADF	48.68±0.05	48.67±0.03	48.66±0.02
ADL	25.43±0.08	25.43±0.07	25.39±0.08
ME_{ADF}, MJ/kg KM	8.27 ±0.03	8.27 ±0.04	8.27 ± 0.02

[a, b, c] Means in the same row with different letters in their supercripts differ (P<0.05). DM=Dry Matter, CA=Crude Ash, OM=Organic Matter,CP=Crude Protein, CF=Crude Fat, NDF:= Neutral Detergent Fiber, ADF=Acid Detergent Fiber; ADL= Acid Detergent Lignin; ME_{ADF}= ME Calculated from ADF.

Estimated OMD %, ME_{OMD} (MJ/KG DM), ME_{GP} (MJ/KG DM) values based on at 24 hour *in-vitro* gas production volume ($P_{PSİ}$/1 G DM, GP_{ML}/200MG DM) of RH, PH and AH are shown in Table II. Changes of gas production volume with *in-vitro* incubation times for RH, PH and AH is shown in Fig. 1. The mean ME_{GP} values of internal skin of three different fruits of RH, PH and AH were found significantly different($P<0.05$). This differences may be originated from different gas production of RH hulls as seen in Table II. ME_{GP} values of of three different hazelnut varities were found similar to wheat straw[24], *M. indica, L. arborea ve S mexicana* tree leaves[6].The estimated OMD % and ME_{OMD} as well as c, b and $T_{1/2}$ values of internal skin of three different fruits of RH, PH and AH at 24 h incubations were significantly different($P<0.05$).The reason may be originated from low gas production at 24 h incubation of RH besides high ADL values of hazelnut fruit hulls.The mean OMD % values were changed between 22.04-22.74 % which are similar to reported values of *M. indica, L. arborea* and *S mexicana* tree leaves[7].

TABLE II
ESTIMATED OMD %, ME_{OMD} (MJ/KG DM), ME_{GP} (MJ/KG DM) VALUES BASED ON AT 24 HOUR *IN-VITRO* GAS PRODUCTION VOLUME ($P_{PSİ}$/1 G DM, GP_{ML}/200MG DM) OF RH, PH AND AH

In-vitro Gas Production Parameters	RH(n=16) (Mean±SE)	PH(n=16) (Mean±SE)	AH(n=16) (Mean±SE)
P_{psi}	3.23±0.15c	3.42±0.17b	3.54±0.14a
GP_{mL}	8.0±0.30c	8.47±0.32b	8.77±0.23a
OMD	22.04±0.04c	22.46±0.08b	22.74±0.05a
ME_{OMD}	3.53±0.04c	3.60±0.03b	3.64±0.02a
ME_{GP}	3.69±0.02c	3.75±0.02b	3.79±0.04a
b	8.82±0.35c	9.31±0.41b	9.69±0.36a
c	0.28±0.032c	0.35±0.021a	0.30±0.028b
$T_{1/2}$	2.52±0.23a	1.98±0.32c	2.31±0.18b

[a,b,c]Means within a row with different superscripts differ ($P< 0.05$). ME_{OMD}=Metobolisable energy estimated from OMD, ME_{GP}= Metobolisable energy estimated from *in-vitro* gas production, b=Potantial gas production, c= The gas production rate constant for the insoluble fraction (mL/h), $T_{1/2}$= The time taken to produce the half of the total gas pool (h).

The cumulative volume of gas production increased with increasing incubation time as seen in Fig.1.

Total antioxidant activity values of RH, PH and AH were 94.60, 94.54 and 94.52 IC 50 mg/mL respectively. There were no significant differences between studied varieties. The mean total antioxidant values were higher than the reported values of different varieties of soybean [25] and rice straw[26] but similar to *Juncus acutus*[27].

In conclusion, the obtained nutritive values of fruit internal skin of different varieties of hazelnut showed similar profiles when compared with common crop residues like wheat or barley straw, therefore, it can be proposed as an alternative roughage source in ruminant feeding. Furthermore, it may also be considered as food additive because of its high antioxidant content in animal even in human nutrition.

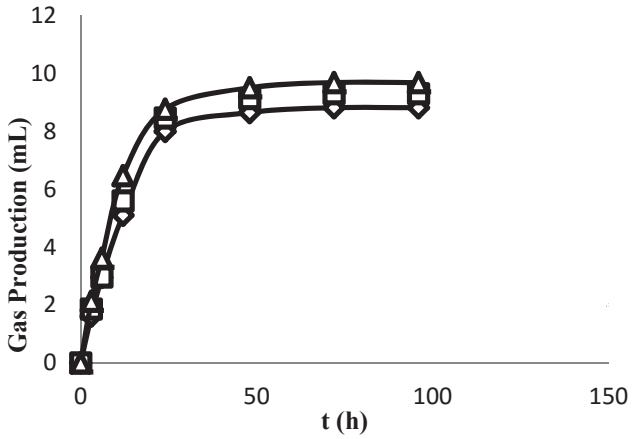

Fig. 1 Changes of gas production volume(mL) with *in-vitro* incubation times(h) for round(◊), pointed(□)and almound(Δ) hazelnuts.

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PSE (Pale, Soft, Exudative) Turkey Meat in a Brazilian Commercial Processing Plant

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***Abstract*—** Over the past decade, the Brazilian production of turkey meat increased by more than 50%, indicating that the turkey meat is considered a great potential for the Brazilian economy contributing to the growth of agribusiness at the marketing international scenario. However, significant color changes may occur during its processing leading to the pale, soft and exudative (PSE) appearance on the surface of breast meat due to the low water holding capacity (WHC). Changes in PSE meat functional properties occur due to the myofibrils proteins denaturation caused by a rapid postmortem glycolysis resulting in a rapid pH decline while the carcass temperature is still warm. The aim of this study was to analyze the physical, chemical and histological characteristics of PSE turkey meat obtained from a Brazilian commercial processing plant. The turkey breasts samples were collected (n=64) at the processing line and classified as PSE at L* ≥ 53 value. The pH was also analyzed after L* measurement. In sequence, PSE meat samples were evaluated for WHC, cooking loss (CL), shear force (SF), myofibril fragmentation index (MFI), protein denaturation (PD) and histological evaluation. The abnormal color samples presented lower pH values, 16% lower fiber diameter, 11% lower SF and 2% lower WHC than those classified as normal. The CL, PD and MFI were, respectively, 9%, 18% and 4% higher in PSE samples. The Pearson correlation between the L* values and CL, PD and MFI was positive, while that SF and pH values presented negative correlation. Under light microscopy a shrinking of PSE muscle cell diameter was approximately 16% shorter in relation to normal samples and an extracellular enlargement of endomysium and perimysium sheaths as the consequence of higher water contents lost as observed previously by lower WHC values. Thus, the results showed that PSE turkey breast meat presented significant changes in their physical, chemical and histological characteristics that may impair its functional properties.

***Keywords*—** Functional properties, Histological evaluation, Meat quality, PSE.

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Pale, Firm and Non-Exudative (PFN): an Emerging Major Broiler Breast Meat Group

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***Abstract*—** The quality of broiler breast meat is changing as a result of continuing emphasis on genetically bird's selection for efficiently higher meat production. The consumer is experiencing a cooked product that is drier and less juicy when consumed. Breast meat has been classified as PSE (pale, soft, exudative), DFD (dark, firm, dry) and normal color meat. However, recently variations of this color have been observed and they are not in line with the specificity of the meat functional properties. Thus the objective of this work was to report the finding of a new pale meat color group characterized as Pale, Firm and Non-exudative (PFN) based on its pH, color, meat functional properties and micro structural evaluation. Breast meat fillets samples (n=1045) from commercial line were classified into PSE (pH ≤5.8, L* ≥ 53.0), PFN (pH > 5.8 and L* ≥ 53.0) and Normal (pH >5.8 and L* < 53.0), based on pH and L* values. In sequence, a total of 30 samples of each group were analyzed for the water holding capacity (WHC) and shear force (SF). The incidence was 9.1% for PSE meat, 85.7% for PFN and 5.2% for Normal meat. The PSE meat presented lower values of WHC (P ≤ 0.05) followed in sequence by PFN and Normal samples and also the SF values of fresh PFN was higher than PSE meat (P ≤ 0.05) and similar to Normal samples. Under optical microscopy, the cell diameter was 10% higher for PFN in relation to PSE meat and similar to Normal meat. These preliminary results indicate an emerging group of breast meat and it should be considered that the Pale, Firm and Non-exudative should be considered as an ideal broiler breast meat quality.

***Keywords*—**Broiler PSE meat, Light microscopy, Texture, Water holding capacity.

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Occurrence of Broiler Chicken Breast White Striping Meat in Brazilian Commercial Plant

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Abstract— White Striping (WS) is becoming a concern for the poultry industry, as it affects the look of breast broiler chicken meat leading it to rejection by the consumers. It is characterized by the appearance of varying degrees of white striations on the *Pectoralis major* muscle surface following the direction of the muscle fiber. The etiology of this myopathy is still unknown, however it is suggested to be associated with increased weight gain rate and age of the bird, attributing the phenomenon to the genetically bird's selection for efficiently higher meat production. The aim of this study was to evaluate the occurrence of *Pectoralis major* WS in a commercial plant in southern Brazil and its chemical characterization. The breast meat samples (n=660) from birds of 47 days of age, were classified as: Normal NG (no apparent white striations), Moderate MG (when the fillets present thin lines <1 mm) and Severe SG (white striations present >1 mm thick covering a large part of the fillet surface). Thirty samples (n = 10 for each level of severity) were analyzed for pH, color (L*, a*, b*), proximate chemical composition (moisture, protein, ash and lipids contents) and hydroxyproline in order to determine the collagen content. The results revealed the occurrence for NG group was 16.97%, 51.67% for MG group and 31.36% for SG group. Although the total protein content did not differ significantly, the collagen index was 42% higher in favor to SG in relation to NG. Also the lipid fraction was 27% higher for SG group. The NG presented the lowest values of the parameters L* and a* ($P \leq 0.05$), as there was no white striations on its surface and highest b* value in SG, because of the maximum lipid contents. These results indicate there was a contribution of the SG muscle cells to oversynthesize connective tissue components on the muscle fascia. In conclusion, this study revealed a high incidence of White Striping on broiler commercial line in Brazil thus there is a need to identify the causes of this abnormality in order to diminish or to eliminate it.

Keywords— Collagen content, Commercial line, *Pectoralis major* muscle, Proximate composition.

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Meat Qualities and Death on Arrival (DOA) of Broiler Chickens Transported in a Brazilian Tropical Conditions

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Abstract— The objective of this work was to evaluate the influence of microclimatic profile of broiler transport trucks under commercial conditions over the breast meat quality and DOA (Death On Arrival) in a tropical Brazilian regions as the North East where routinely the season is divided into dry and wet seasons. The temperature remains fairly constant and obviously the relative humidity changes accordingly. Three loads of 4,100 forty seven days old broiler were monitored from farm to slaughterhouse in a distance of 4.3 km, morning period of October 2015 rainy days. The profile of the environmental variables inside the container truck throughout the journey was obtained by the installation of thermo anemometers in 6 different locations by monitoring the heat index (HI), air velocity (AV), temperature (T), and relative humidity (RH). Meat qualities were evaluated by determining the occurrence of PSE (pale, soft, exudative) meat and DFD (dark, firm dry) meat. The percentage of birds DOA per loaded truck was determined by counting the dead broiler during the hanging step at the slaughtering plant. The analysis of variance was performed using statistical software (Statistica 8 for windows, Statsoft 2007, Tulsa, OK, USA). The Tukey significance test ($P<0.05$) was applied to compare means from microenvironmental data, PSE, DFD and DOA. Fillet samples were collected at 24h post mortem for pH e color (L*, a* e b*) determination through the CIELAB system. Results showed the occurrence of 2.98% of PSE and 0.66% de DFD and only 0.016% of DOA and overall the most uncomfortable container location was at the truck frontal inferior presenting 6.25% of PSE. DFD of 2.0% were obtained from birds located at central and inferior rear locations. These values were unexpected in comparison to other results obtained in our laboratories in previous experiments carried out within the country south state. The results reported herein were lower in every aspect. Reasonable explanation would be the shorter distance, wet conditions throughout around 15-20 min journeys and lower T and RH values as observed in samples taken from the rear location as higher DFD values were obtained. These facts mean the animals were not under heat stressful condition but in fact under cold stress conditions as the result of DFD suggested in association to the lower number of DOA.

Keywords— Cold stress, DFD, Microclimatic profile, PSE.

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Study of Aerosol Deposition and Shielding Effects on Fluorescent Imaging Quantitative Evaluation in Protective Equipment Validation

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Abstract—The leakage of protective clothing is an important issue in the occupational health field. There is no quantitative method for measuring the leakage of personal protective equipment. This work aims to measure the quantitative leakage of the personal protective equipment by using the fluorochrome aerosol tracer. The fluorescent aerosols were employed as airborne particulates in a controlled chamber with ultraviolet (UV) light-detectable stickers. After an exposure-and-leakage test, the protective equipment was removed and photographed with UV-scanning to evaluate areas, color depth ratio, and aerosol deposition and shielding effects of the areas where fluorescent aerosols had adhered to the body through the protective equipment. Thus, this work built a calculation software for quantitative leakage ratio of protective clothing based on fluorescent illumination depth/aerosol concentration ratio, illumination/Fa ratio, aerosol deposition and shielding effects, and the leakage area ratio on the segmentation. The results indicated that the two-repetition total leakage rate of the X, Y, and Z type protective clothing for subject T were about 3.05, 4.21, and 3.52 (mg/m2). For five-repetition, the leakage rate of T were about 4.12, 4.52, and 5.11 (mg/m2).

Keywords—fluorochrome, deposition, shielding effects, digital image processing, leakage ratio, personal protective equipment

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Volatile Profile of Monofloral Honeys Produced by Stingless Bees from the Brazilian Semiarid Region

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***Abstract*—**In Brazil, there is a diverse fauna of social bees, known by meliponinae or native stingless bees. These bees are important for providing a differentiated product, especially regarding unique sweetness, flavor, and aroma. However, information about the volatile fraction in honey produced by stingless native bees is still lacking. The aim of this work was to characterize the volatile compound profile of monofloral honey produced by jandaíra bees (Melipona subnitida Ducke) which used chanana (Turnera ulmifolia L.), malícia (Mimosa quadrivalvis) and algaroba (Prosopis juliflora (Sw.) DC) as their floral sources; and by uruçu bees (Melipona scutellaris Latrelle), which used chanana (Turnera ulmifolia L.), malícia (Mimosa quadrivalvis) and angico (Anadenanthera colubrina) as their floral sources. The volatiles were extracted using HS-SPME-GC-MS technique. The condition for the extraction was: equilibration time of 15 minutes, extraction time of 45 min and extraction temperature of 45°C. Through the results obtained, it was observed that the floral source had a strong influence on the aroma profile of the honey under evaluation, since the chemical profiles were marked primarily by the classes of terpenes, norisoprenoids, and benzene derivatives. Furthermore, the results obtained suggest the existence of differentiator compounds and potential markers for the botanical sources evaluated, such as linalool, D-sylvestrene, rose oxide and benzenethanol. These reports represent a valuable contribution to certifying the authenticity of those honey and provides for the first time, information intended for the construction of chemical knowledge of the aroma and flavor that characterize these honey produced in Brazil.

***Keywords*—**Aroma, honey, semiarid, stingless, volatiles.

I. Introduction

HONEY is a complex mixture of carbohydrates produced by nature, being considered a very important energetic food due to its nutritional properties as well as its aroma and flavor [1]. Traditionally, the botanical origin of honey can be determined through a series of techniques known as melissopalinology. However, this type of analysis is expensive, requires a large amount of time and depends a great deal on the qualification and judgment of the analyzer. Therefore, there is a tendency to replace pollinic analysis with analytic markers through the discrimination of volatile compounds and of other physicochemical parameters of honey [2]. The volatile compounds of bee honey can be derived from a variety of sources: from the plant or nectar; from the transformation of plant compounds by bee metabolism; from the heating or handling during processing and storage of honey, or even from microbial or environmental contamination.

Brazil possesses the greatest vegetal genetic diversity in the world, which, combined with its vast territory and climatic variability, enables the production of honey throughout the year. The Brazilian semiarid region stands out for having a type of vegetation which is adapted to the typical climatic conditions of the rainy season and the dry season, thus providing a continuous flux of nectar and pollen during the whole year and favoring the production of different types of honey with singular properties [3].

This region is home to endemic species of stingless bees, also known as meliponas or Meliponini. Among these, two stand out: *Melipona subnitida* Ducke (jandaíra) and *M. scutellaris* Latrelle (uruçu) [4]. These bees produce honey which has been consumed since before the arrival of European colonizers in the American continent. Furthermore, some medicinal properties have also been accredited to this type of honey [3]. Nevertheless, there is almost no information regarding the profile of volatile compounds present in honey produced by stingless bees.

Therefore, the aim of this work was to characterize the profile of volatile compounds found in monofloral honeys produced by stingless bees (jandaíra and uruçu) from specific botanical sources which are typically found in the Brazilian semiarid region. This type of information will shed more light on the chemistry of these natural resources, which are still relatively unknown, and will also help with the characterization of the floral and geographical identities of the honeys which are produced exclusively in this region.

II. Material and Methods

A. Sample Acquisition

The samples of honey obtained from stingless bees M. *subnitida* Ducke (jandaíra) were produced by the floral sources of chanana (***Turnera ulmifolia* L.**), malícia (*Mimosa quadrivalvis* L.) e algaroba (*Prosopis juliflora* (Sw.) DC). Meanwhile, the samples of honey produced by stingless bees uruçu (Melipona *scutellaris* Latrelle) used the floral sources of chanana (***Turnera ulmifolia* L.**), malícia (*Mimosa quadrivalvis* L.) and angico (*Anadenanthera colubrina*). The honey was obtained directly from meliponary located in the semiarid region of Northeastern Brazil (Seridó region, state of Rio Grande do Norte and Agreste region of state of Paraíba). The honey was collected directly from hives by suction with the aid of disposable syringes. After collection, the honey was stored for a period not exceeding 30 days in sterilized glass jars at 4°C until analysis.

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B. Melissopalynological Analysis

For this analyses, 10 g of each honey sample were diluted in 20 mL of distilled water and then centrifuged at 4000 g for 20 min. The sediment was dried at 40 °C and then mounted with Entellan Rapid (Merck, 1.07961.0500). The honeydew elements and pollen grains (n ¼ 500) were counted and identified in 20 distinct optical areas using an optical microscope (Nikon Optiphot II microscopio; 400 and 1000) [5]. The pollen grains were compared to reference images of the University of São Paulo, São Paulo, Brazil. All samples contained more than 65% pollen grains of the same botanical origin.

C. Extraction and Analysis of Volatiles

The volatiles compounds of honey from stingless bees were extracted by HS-SPME. The Fiber used was a PDMS / DVB, 65 µm (Supelco, Bellofonte, PA, USA). The extraction conditions of volatiles were: 10 g of honey in 10 ml of Milli-Q in 60 ml vials sealed with polytetrafluroethylene silicone septum (Supelco, Bellenfont, PA, USA); equilibrium time of 15 minutes, extraction time of 45 minutes and temperature of extraction of 45°C.

For separation and identification of compounds extracted from honeys, Varian Saturn 3800 gas chromatograph coupled to Varian Saturn 2000R mass detector and VF-5MS (60m x 0.25mm x 0.25µm) was used. The temperature programming of the gas chromatograph oven started at 40°C for 2 min, followed by a 2°C / min ramp up to 60°C, 3°C / min up to 90°C and 4°C / min up to 240°C, maintaining this temperature for 10 minutes. The injector temperature was maintained at 250°C and detector temperature at 250°C. Helium was used as carrier gas at constant flow rate of 1.0 mL / min gas. Mass spectrometer was operated by electron impact at source temperature of 200°C and ionization energy of 70V and scan range from m/z 29 to m/z 400 at 3.33 scan/s. The identification of volatile compounds was based on the comparison of their mass spectra with spectra of compounds previously analyzed, with NIST / EPA / NIH Mass Spectral Database (Version 2008), or spectra published in journals. To confirm the identity of each component, the linear retention index (LRI) was calculated using the retention times of a homologous series of *n*-alkanes C8 - C25 and also by comparing the LRI of authentic compounds analyzed under similar conditions [1], [6]-[8].

D. Data Analysis

The volatile data were analyzed by Principal Component Analysis (PCA) using the Multi-Variate Statistical Package (MVSP v.3.13).

III. Results and Discussion

The use of gas chromatography associated with mass spectrometry (GC/ME) allowed the detection of 114 compounds in the headspace of honey from stingless bees. Terpenes were the main class of volatile compounds found in honey – a total of 48 were detected. The terpenes were accompanied by the following compounds: 14 esters, 11 norisoprenoids, 11 benzene compounds, 7 furans, 5 ketones, 4 hydrocarbons, 4 alcohols, 4 aromatic aldehydes, 3 aldehydes, 2 acids and 1 sulfur compound. There were terpenes in all of the honeys evaluated, while the acids and the sulfur compound were only found in the honey obtained from algaroba (Fig. 1).

The number of compounds was higher, for both bees, in chanana compared to the other floral species; meanwhile, angico honey presented the lowest number of volatiles in the honeys produced by stingless bees.

In the sample of honey produced by uruçu bees from malícia, a total of 33 compounds were identified. The volatile chemical profile of this samples is characterized primarily by the presence of terpenes (90.9 % of the total chromatogram area), followed by norisoprenoids (2.8 %), alcohols (2.2 %) and other minority compounds. Meanwhile, in the sample of honey produced by jandaíra bees, also from malícia sources, 35 compounds were identified. Once more, the predominant class of compounds were terpenes (85.5 % of the total area). The two different honeys produced using malícia (by jandaíra bees and by uruçu bees) had a total of 21 compounds in common.

As for the analysis of the honeys produced using chanana as the floral source, 41 compounds were detected in the honey produced by jandaíra bees, while the honey generated by uruçu presented 55 compounds in total. Those two honeys had 36 compounds in common. It can be observed that terpenes were once again predominant, occupying 54.5 % and 70.3 % of the total chromatogram area, in the uruçu and jandaíra honeys, respectively. The chemical profile of these samples was also characterized by the class of norisoprenoids, which represented 17.8 % of the total area in uruçu-made honey, and 7.5 % in jandaíra-made honey.

In the analysis of the honey sample produced by jandaíra bees from algaroba sources, 32 compounds were detected. Terpenes accounted for 42% of the total chromatogram, while norisoprenoids and alcohols occupied 16 % and 11 % respectively.

In the honey generated by uruçu bees from angico plants, 15 compounds were identified. They belonged to the following classes: benzene derivatives (41.4 %), terpenes (22.3 %), furans (18.9 %) and aromatic aldehydes (17.4 %).

The chemical classes present in the aroma of the honey samples analyzed were submitted to principal component analysis (PCA) (Fig. 2). The principal components (PC) I e II accumulated 71% of the variation which took place between the samples. In this figure, the chemical classes are represented by vectors whose size is associated with the importance of that class to the segmentation of samples. The direction of the vectors indicates in which samples that chemical class displays a greater influence [9]. The samples are represented by black circles.

Fig. 2 reveals the separation of honeys with different botanical origins. The honey produced by uruçu bees using angico, located in the negative axis of PC I, distanced itself from the other samples and was characterized by a higher concentration of furans and aromatic aldehydes. The sample of honey produced by jandaíra bees using algaroba as a floral

source also distanced itself from the other honeys, being mostly influenced by the class of sulfur compounds and being located in the negative axis of PC I and II.

The honeys produced by the different bee species using chanana plants were located in the positive axis of PC I and II, drawing closer due to the influence of terpenes, norisoprenoids, esters, alcohols and hydrocarbons. However, the fact that the uruçu-made sample occupies the farthermost region to the right of axis 1 in Fig. 2 indicates that this sample displayed a higher concentration of the aforementioned compounds, when compared to the jandaíra-made honey.

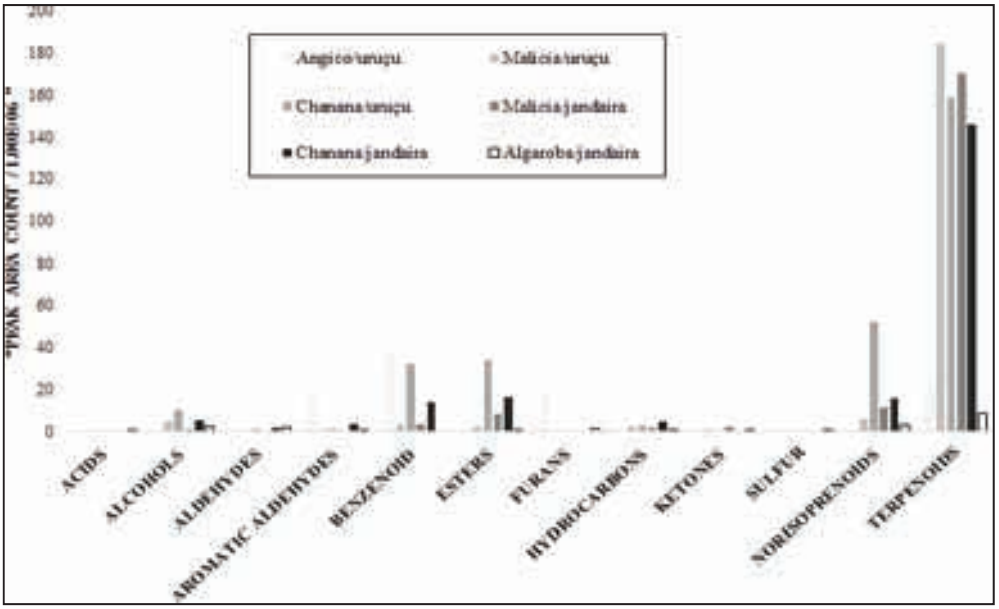

Fig. 1 Distribution of chemical classes of volatiles of monofloral honeys produced by different stingless bees (jandaíra and uruçu) in the semiarid region of Northeastern Brazil from different floral sources

The honeys obtained from malícia plants displayed very similar profiles to one another, which were distinct from the profiles of honeys produced using other botanical sources. The malícia honeys were characterized by the high influence exerted by the ketone class.

The volatile compounds found in honey belong to various classes, including hydrocarbons, aldehydes, alcohols, ketones, acids, esters, benzene and its derivatives, furan and pyran, norisoprenoids, terpenes and its derivatives, and cyclic compounds [10]. However, only compounds derived from plants and its metabolites (terpenes, norisoprenoids, benzenes and its derivatives) should be used to identify the floral origin of honey [2].

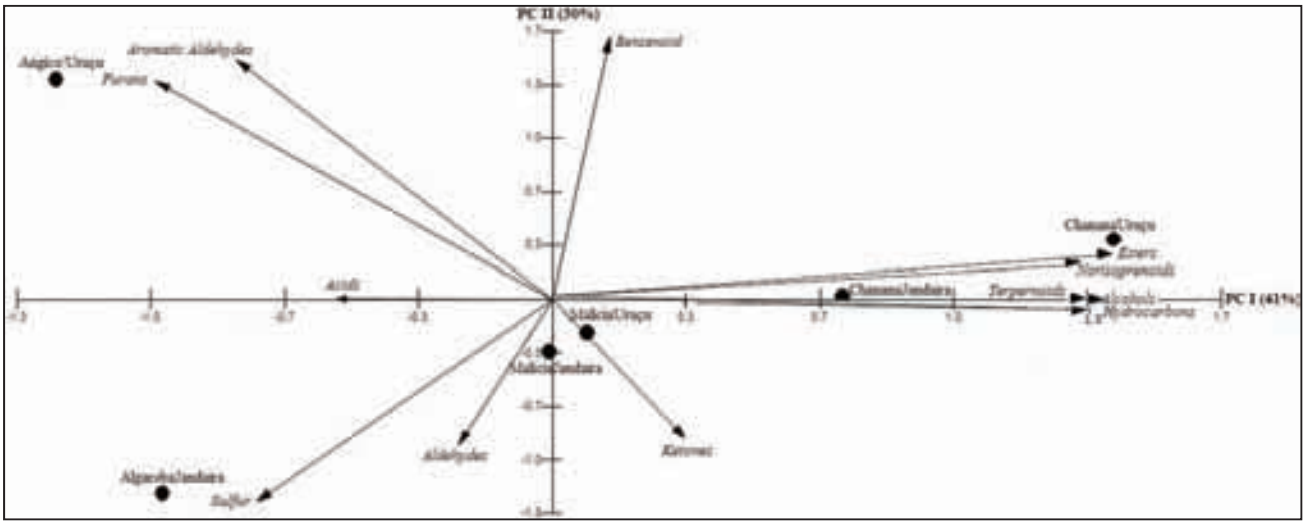

Fig. 2 Principal Component Analysis (PCA) of the chemical classes of volatiles of monofloral honeys produced by different stingless bees (jandaíra and uruçu) in the semiarid region of Northeastern Brazil from different floral sources

The honeys generated from malícia plants were marked by a number of different compounds; for uruçu-made honey, the most abundant compounds were linalool (53.1 %), cis-Linalool oxide (23.7 %) and hotrienol (4.3 %). Meanwhile, in the jandaíra-made honey, the most plentiful compounds were linalool (47.6 %), cis-Linalool oxide (16.4 %), nerol oxide (8.2 %) and hotrienol (5.2 %). These compounds have been frequently reported in studies as being the main constituents of the volatile profile of honey, regardless of the geographical origin [11]-[13]. Cis-linalool oxide (furanoid) detected in honeys from cashew and quince trees native to Northeastern Brazil [14]. Linalool and its derivatives, such as hotrienol, cis-linalool and trans-linalool oxides (piranoid), and lilac aldehydes were all mentioned [15] as volatile compounds which can mark the floral origin of citric honeys collected in Greece. In a similar study, [16] characterized 10 Spanish citric honeys based on their volatile profile, and suggested compounds such as linalool, linalool oxide, µ-terpineol, lilac aldehyde and lilac alcohol isomers, methyl anthranilate be considered floral markers for citric honeys. High levels of linalool oxides, linalool, hotrienol, epoxylinalool, and 2,6-dimethyl-3,7-octadiene-2,6-diol, whose concentrations exceed their detection threshold, are responsible for sweet and floral notes in honey [17].

The monoterpene D-Sylvestrene was the main compound in the two different honeys produced from chanana. Even though this floral origin monoterpene is not commonly found in honeys, its presence may not be a differentiator for honeys made from chanana because this terpene was also found in the honeys produced using the botanical source malícia.

Rose oxide (22.9 % of the total chromatogram) was the most abundant compound found in algaroba honey. This monoterpene is found in flowers, fruit and in essential oils from *Eucalyptus citriodora*, *Dracocephalum heterophyllum*, Damask rose, geranium, *Laggera* spp., and tropical fruit [18]-[20]. It contributes to the fruit and floral notes found in fruit such as lychee and grapes, and has also been synthesized from citronellol for industrial purposes, due to its characteristic rose aroma [21]. Cis-rose oxide has been proposed as a marker for honey from *Tilia cordata* [22]. This is the first time that rose oxide has been reported in Brazilian honey. Even though in literature no data were found regarding the volatile composition of algaroba honey, rose oxide can be considered a differentiator for this particular honey, since it is not commonly found in other honeys (being absent in the other samples analyzed) and it also comes from a botanical source and appears in high concentrations.

The main compound in the honey made from angico was benzenethanol, which reached 40% of the total chromatogram area. While studying tropical honeys produced by *Apis mellifera* bees using angico plants native to the Atlantic Forest, [23] detected benzenethanol as the second most abundant compound, contributing to floral and spicy notes. Benzenethanol has been considered an important odorant for the aroma of tília honey (*Tilia* sp.) and haze honey (*Corylus* sp.), both from Europe [22]; it also contributes to the aroma of honeys made from cashew trees found in the Caating biome [14] and to the aroma of honey made using morrão de cadeia (Atlantic Forest biome) and assa-peixe (Cerrado biome) [24].

The compounds 5-hydroxymethylfurfural, 2,5-dimethyl furan, furfural, 2-furanmetanol and furaneol were only present in the angico and/or algaroba honeys. These compounds are considered a negative indicator of the honey's quality, possibly having been generated by the excessive heating of the sample. However, some authors state that HMF can naturally occur in honey, especially in regions with a hot climate [25].

TABLE I
POTENTIAL VOLATILES MARKS OF MONOFLORAL HONEYS PRODUCED BY DIFFERENT STINGLESS BEES (JANDAÍRA AND URUÇU) IN THE SEMIARID REGION OF NORTHEASTERN BRAZIL FROM DIFFERENT FLORAL SOURCES

Compounds[2]	LRI	Peak Area Count[1]					
		Uruçu			Jandaíra		
		Angico	Malícia	Chanana	Malícia	Chanana	Algaroba
BENZENOID COMPOUNDS							
Benzeneethanol	1118	37.3	nd[3]	nd	nd	nd	nd
FURANS							
2.5-dimethyl furan	<800	nd	nd	nd	nd	nd	0.07
Furfural	836	8.3	nd	nd	nd	nd	nd
2-Furanmethanol	860	0.4	nd	nd	nd	nd	nd
Furaneol	1052	2.3	nd	nd	nd	nd	0.1
5-Hydroxymethylfurfural	1234	6.7	nd	nd	nd	nd	nd
TERPENOIDS							
D-Sylvestrene	1024	nd	6.5	58.9	7.1	46.8	nd
Cis-Linalool oxide	1076	10.6	47.9	5	32.5	1.7	0.5
Linalool	1091	nd	107	nd	94.3	nd	nd
Hotrienol	1092	1.4	8.8	3.7	10.4	12.1	0.3
Rose oxide	1116	nd	nd	nd	nd	nd	4.6
Nerol oxide	1143		7.3	1.6	16.3	2.5	nd

[1] Valour of peak area count / 10^6 average data from triplicate injections; [2] Compounds identified by MS and LRI; [3] nd = not detected

IV. Conclusion

This study demonstrates that the botanical sources had a strong influence on the volatile profile of monofloral honeys produced by two stingless bees (jandaíra and uruçu) from the semiarid region of Northeastern Brazil. The chemical profiles were characterized primarily by the classes of terpenes, norisoprenoids and benzene derivatives. Furthermore, the results obtained indicate the existence of 'differentiator' compounds and potential markers for the botanical sources evaluated, such as linalool for malícia honeys; D-sylvestrene for chanana honeys; rose oxide for algaroba honeys; and benzenethanol for angico honeys. These reports represent a valuable contribution to certifying the authenticity of those honeys.

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Further Evidence for the Existence of Broiler Chicken PFN (Pale, Firm and Non-Exudative Meat) and PSE (Pale, Soft and Exudative) in Brazilian Commercial Flocks

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***Abstract*—**The quality of broiler breast meat is changing as a result of the continuing emphasis on genetic selection for a more efficient meat production. Breast meat has been classified as PSE (pale, soft, exudative), DFD (dark, firm, dry) and normal color meat, and recently a third group has emerged: the so-called PFN (pale, firm, non-exudative) meat. This classification was based on pH, color and functional properties. The aim of this work was to confirm the existence of PFN and PSE meat by biochemical characterization and functional properties. Twenty four hours of refrigerated fillet, *Pectoralis major*, m. samples (n= 838) were taken from Cobb flocks 42-48 days old, obtained in Northeastern Brazil tropical region, the Northeastern, considered to have only dry and wet seasons. Color (L*), pH, water holding capacity (WHC), values were evaluated and compared with PSE group samples. These samples were classified as Normal (46<L*<53; pH>5.8), PSE meat (L*≥53; pH<5.8) and PFN (L*≥53; pH>5.8). The occurrence of control meat, PSE and PFN was 69.09%, 11.10% and 19.81%, respectively. Samples from PFN presented 4.0-5.0% higher WHC in relation to PSE meat and similar to control group. These results are explained by the fact that PSE meat syndrome occurs because of higher protein denaturation as the consequence of a simultaneous lower pH values under warm carcass sooner after slaughtering impairing the myofibril proteins functional properties. Conversely, PFN samples follow normal glycolysis rate maintaining the normal proteins activities. In conclusion, the results reported herein confirm the existence of this emerging broiler meat group with similar properties as control group and it should be considered as normal breast meat group.

***Keywords*—**Broiler breast meat, functional properties, PFN, PSE.

I. Introduction

THE increase in the production of broiler meat, boosted by growing consumer demand, has been reflected in a series of changes in the poultry industry over the last decades. These changes demanded a great deal of effort and intensive genetic selection by producers and breeders, in order to attain an increased growth rate, associated with a better breast muscle yield and better nutritive efficiency. As a result, the birds which are sold nowadays take half the time to grow, and display double the body weight, when compared to birds from previous decades [1], [2].

However, the selection process implemented, which looks to attain the desired performance in the shortest possible amount of time, has made the animal more susceptible to stress, which in turn leads to a decrease in the quality of its meat [3].

There are countless pre-slaughter factors which are determinant to the quality of meat; among these are: the stress caused by environment factors (temperature, air humidity, thermal radiation and air movement), animal handling conditions, fasting regime and hydric restriction, inadequate handling during loading and unloading of the birds, water showers in farms, transporting conditions, amount of rest and season of the year [4]-[6]. Moreover, slaughter conditions and post-slaughter processing must also be taken into account [7]. Those factors cause physicochemical alterations in the interior of the muscle, which lead to changes in color, pH and functional properties. In chickens, these changes have been linked to PSE (pale, soft, exudative) and DFD (dark, firm, dry) syndromes, and represent a problem for the meat processing industry, since they diminish the technological value of broiler meat [8].

In relation to pork meat, in addition to the Normal, PSE and DFD categories, variants of these anomalies have been observed, which have been named PFN (pale, firm, non-exudative) and RSE (reddish-pink, soft, exudative), according to reports by Kauffman et al [9]. PFN pork meat displays the firmness and water retention capacity of normal pork meat, but a pale color similar to PSE. Meanwhile, RSE pork meat has normal color, but displays a soft texture and an exudative aspect equivalent to PSE meat [10]. In studies carried out in the South of Brazil, a region known for its milder, colder climate, a new anomaly group was detected, related to the color of broiler meat obtained at the processing line. This group was described as having pale color, firm texture and non-exudative aspect, which suggests a new category for broiler meat quality – PFN [11].

Therefore, considering that the incidence of PFN syndrome in broiler meat and the changes caused by this defect are still

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not consolidated, this work aims to investigate the incidence of this anomaly and of PSE in broiler meat, as well as the physicochemical alterations provoked by those anomalies, by using animals from a commercial plant in Northeastern Brazil.

II. Materials and Methods

A. Sample Preparation

This study used broilers of Cobb lineage, of either sex, between 42 and 48 days old. The animals came from a commercial plant located in the town of Guarabira, in the Agreste mesoregion of the state of Paraiba, Northeastern Brazil. The plant slaughters on average 90,000 birds per day. The experiment was carried out during the rainy season, from May to August 2015. The town of Guarabira lies 97 meters above sea level. The climatic conditions in this region are characterized by a maximum temperature and minimum temperature of 32.1 and 19.5 ° C, respectively. The relative humidity (RH) value varied between 80.9 % to 84.7 % during the experiment; the average monthly rainfall for this region is 132.58 millimeters [12].

The animals were slaughtered according to the standard industry practice, consisting of electrical stunning, bleeding, scalding, defeathering, evisceration, pre-chilling through water immersion in two continuous refrigerators. The pre-chiller's temperature was lower than 16 °C, with average duration of 12 minutes, while the chiller's temperature was lower than 4 °C with average duration of 55 minutes, followed by deboning.

After that, the broiler breast fillets (n=838) were collected and stored in polyethylene plastic bags and refrigerated at -3 °C ≤ T ≤ 2 °C for 24 hours for subsequent color (L*) and pH measurements and classification. The breast fillets were classified into three groups (Table I) according to Soares et al [13] and Kaminishikawahara [11].

TABLE I
Classification of Broiler Breast Fillets (*Pectoralis Major,* m.) According to Color Anomalies

Description	L*	pH_{24h}	Reference
Normal	≥ 44 e ≤ 53	≥ 5.80	Soares et al. [13]
PSE (pale, soft, exudative)	> 53	< 5.80	Soares et al. [13]
PFN (pale, firme, no exudative)	> 53	≥ 5.80	Kaminishikawahara [11]

pH_{24h}: pH values measured 24 h post-mortem; L*: colour

B. Characterization of Broiler Breast Fillets

The broiler breast fillets were classified as Normal, PSE and PFN, according to the following parameters: color (L*), pH, water holding capacity (WHC), cooking loss, shear force (SF) and mineral profile: Ca^{2+}, Mg^{2+}, K^{+} e Na^{+}. For the color anomalies PSE and PFN, 20 repetitions were carried out, while the group classified as Normal went through 40 repetitions. Each repetition corresponds to one breast fillet, totaling 80 samples.

C. Color Measurements

A colorimeter [14] was used to evaluate the lightness (L*) at the dorsal surface of the intact skinless breast muscles. The L* values were measured at 3 sites on the same sample: the proximal extremity of the muscle, the distal extremity, and the medial side halfway between the proximal and the distal extremity [15].

D. pH Measurements

The pH was measured by inserting electrodes into the ventral cranial part of the fillet using a contact pH meter system [16], according to Boulianne and King [17]. Analyses were performed in triplicate 24 h postmortem.

E. Water-Holding Capacity

WHC was determined, based on the technique described by Hamm [18]. Twenty-four-hour post-mortem samples were collected from the cranial side of the breast fillets and cut into 2.0-g (±0.10) cubes. They were placed between two filter papers and then left under a 10 kg weight for 5 min. The samples were weighed and WHC was determined by the exudated water weight via the following formula:

{100 – [(Wi–Wfp)/Pi x100]}, where Wi and Wf are the initial and final sample weights.

F. Cooking Loss

CL was expressed as the percentage of water loose during the cooking procedure. It was measured according to Honikel [19]. The samples were weighed (75 ± 5 g) before and after 15 min of cooking, which was the time required for the internal temperature to reach 75°C.

G. Texture Measurement

Tenderness was evaluated by measuring shear force, as described by Honikel [19]. It was measured in raw and cooked breast fillets. The samples were packed in plastic bags and cooked in a water bath until the internal temperature was 75°C. After refrigeration at 2 ± 1°C for 12 h, the samples were cut into 1 cm^3 pieces, and analysed on a TATX-2i texturometer [20]. The results were expressed in Newtons (N).

H. Mineral Profile

The quantification of mineral elements (Ca^2+, Mg^2+, K+ e Na+) was performed based on the official AOAC methods [21] using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) [22]. The system was totally controlled by ICP software using 99.996% liquid argon as plasma gas (Air Liquid, SP, Brazil). Operating conditions of the ICP-OES equipment were: reflected power, 1.0kW; spray flow, 0.9 $Lmin^{-1}$; auxiliary argon flow, 1.5 $Lmin^{-1}$; main argon flow, 15 L min^{-1}; nebulizer pressure, 200kPa.

I. Statistical Analysis

Data were submitted to analysis of variance and the means were compared by the F test for a completely randomized design in a 3×1 factorial arrangement (three treatments Normal, PSE and PFN), having three replicates. Differences in means among treatments were evaluated for significance using the Assistat, version beta 7.7. [23], applying the Turkey test. Graphics were generated by using of Software GraphPad Prism version 6.0 [24].

III. Results and Discussion

A.Incidence of Broiler Breasts (Pectoralis Major, m.) with Abnormal Coloration

The incidence of broiler breast fillets (n = 838) with abnormal coloring was superior to 30%; PFN had the highest incidence (n = 166), that is, 20% of total breast fillets, followed by PSE, which was present in 11.1% of fillets (n = 93). Normal fillets accounted for 69% of total breasts (n = 579). In the USA, Woelfel et al. [25] demonstrated that 47% of the broiler fillets evaluated displayed a pale color and lower WHC values. Meanwhile, in the South of Brazil, Soares et al. [13] and Droval et al. [26] reported PSE percentages of 12% and 22% respectively. Furthermore, still in Southern Brazil, Simões et al. [27] observed that, the longer the distance and journey time for chickens, the higher the occurrence of PSE. In addition to this, it was also confirmed that water showers in chicken farms helped reduce PSE occurrence. The presence of PSE syndrome may be due to the effects of high temperatures and relative humidity [5]. These conditions are typical of the summer in Southern Brazil [28], and are also predominant in the Agreste mesoregion of the state of Paraiba, Northeastern Brazil [29]. Therefore, the lower incidence of PSE observed in Northeastern Brazil may be associated with the time of the year in which the breast fillets were collected; in this case, during the rainy season, which is characterized by its milder temperatures (20 °C). Various researchers [6], [30] observed that seasonal differences increased the occurrence of pale meats, suggesting that thermal stress during the summer led to a higher rate of PSE syndrome in birds. The same was also observed in pigs [31]. As for the PFN anomaly, Kuminishikawahara et al. [32] reported that broiler breast fillets from Southern Brazil had a PFN incidence of 85.7%, and PSE incidence of 9.1%. The first anomaly, which displayed a high level of incidence, was also reported in Northeastern Brazil, even though there are currently no reports on these pale, firm and non-exudative (PFN) broiler breasts.

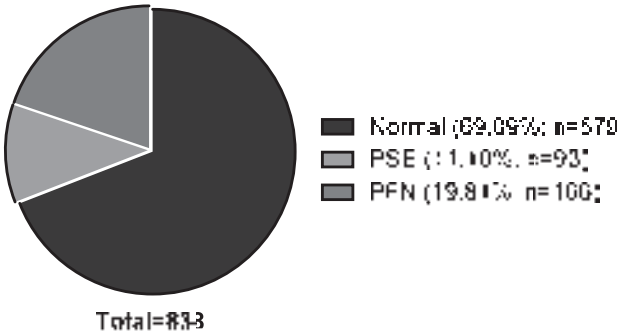

Fig. 1 Incidence of broiler breasts (*Pectoralis major,* m.) with abnormal coloration (PSE, PFN) in Northeastern Brazil

B.Characterization of Broiler Breast (Pectoralis Major, m.) Fillets Displaying Abnormal Coloration (PSE, PFN)

The physical parameters and the functional properties of Normal, PSE and PFN breast fillets are presented in Table II. The pH and L* values were considered typical for normal and PSE fillets [25], [26], [33]-[35] as well as for PFN fillets [32].

Normal, PSE and PFN fillets all presented different values of pH from one another. The PFN group had a lower pH than the Normal group, but it was still higher than the pH displayed by the PSE group. Moreover, there was no difference in color between PFN and PSE values, and both displayed a higher L^*_{24h} value than did the Normal fillets.

Normal fillets and PFN fillets had similar WHC values, which in turn were 4.2% higher than the one displayed by PSE breasts. This result is in accordance with what was reported by Kuminishikawahara et al. [32].

As for CL levels, the highest values were observed in the PSE group, indicating that, during heating, those fillets were more susceptible to heat, and thus ended up losing more weight than the other groups. The CL value in PSE breasts was 18.4% higher than in Normal breasts; however it, was not as high as the 31.0% reported by Droval et al. [26]. PFN fillets displayed CL values which were similar to the ones shown by PSE and Normal breasts.

The shear force values of raw PFN fillets did not differ from those of raw Normal fillets, but were 21% higher than the SF of raw PSE fillets. This shows that PSE breasts had less firmness, being consistent with the results noted by Kuminishikawahara et al. [32]. Wilhem et al. [35] observed that the intense protease activity in PSE meats, caused by the excessive release of sarcoplasmic Ca^{2+} ions, ended up affecting the muscular fiber structure and impairing the functionality of meat proteins, and, as a consequence, led to a loss of texture. However, the SF of raw Normal fillets and raw PSE fillets did not differ from one another, and were similar to what was reported by Droval et al. [26].

On the other hand, when measuring shear force in cooked fillets, it was observed that PSE fillets displayed 16.6% higher SF than Normal fillets. The firmest texture was observed in the PSE cooked fillets, which resulted in a lower WHC and higher CL. According to Anadón [36], meat texture is directly proportional to the intramuscular water level; therefore, the higher the level of water held inside the muscle, the softer the meat. SF in PFN breasts were no different from the normal group.

TABLE II
Comparison of Biochemical and Physicochemical Parameters Between Normal, PFN and PSE Broiler Breast Meat (*Pectoralis major m.*) Samples from the Northeastern Region of Brazil

Parameters	Normal (n=40)	PFN (n=20)	PSE (n=20)	p
pH_{24h}	6.13±0,22^{a}	5.93±0.05^{b}	5.70±0.04^{c}	<0.0001
L^*_{24h}	47.90±2.10^{b}	55.07±0.91^{a}	55.04±0.78^{a}	<0.0001
WHC (%)	67.02±2.57^{a}	66.90±1.98^{a}	64.21±1.86^{b}	0.0006
CL (%)	13.66±1.63^{b}	15.19±3.13^{ab}	16.18±2.05^{a}	0.0016
SF_{raw} (N)	39.03±5.78^{ab}	42.43±6.70^{a}	35.21±6.57^{b}	0.0093
SF_{cooked} (N)	23.45±4.30^{b}	26.38±4.58^{ab}	27.34±4.58^{a}	0.0014

$^{a–b–c}$ Means within each line with different superscripts are significantly different ($p<0.05$).

pH_{24h}: pH values measured 24 h post-mortem; L*: colour; WHC: water-holding capacity; CL: cooking loss; SF = shear force, N = Newton. SF_{raw}: Shear Force raw meat, N = Newton; SF_{cooked}: Shear Force cooked meat, N = Newton.

The mineral profile of Normal, PSE and PFN fillets is presented in Table III. The calcium levels differed ($p < 0.001$) from one group to the other; PFN fillets had the lowest concentration, followed by Normal fillets. PSE meat displayed a 14.7% calcium level, which was 42.2% higher than in Normal and PFN meats. These results are in accordance with literature, which links higher calcium levels to the lower pH presented by this meat. Fujji et al. [37] and Mickelson and Louis [38] described a defect in calcium regulation in pigs submitted to stressor agents, in which they observed a mutation in the ryanodine (RyR1) receptor – which belongs to the calcium channel in the sarcoplasmic reticulum – being responsible for a greater availability of these ions. This excessive amount of Ca^{2+} ions accelerated metabolism and thus increased the pace at which the pH declined. In chickens, Oda et al. [39] reported lower mean relative quantification (RQ) values for the β-RyR gene expression in PSE fillets, with this being the first sign of PSE syndrome occurring as a result of the excessive release of calcium ions.

No differences were observed in the magnesium, sodium and potassium levels of Normal, PSE and PFN fillets. According to Keeton, Ellerbeck and Núñez de González [40], the minerals magnesium, sodium, and potassium, together with calcium, play a fundamental role in muscle contraction. Magnesium acts directly in the process of muscle relaxation; high concentrations of this mineral in the sarcoplasm tend to inhibit the interaction between actin and myosin. Calcium plays a part in the opposite process; when there is a high calcium concentration in the sarcoplasm, the ions stimulate ATPase activity and also act on the interaction between actin and myosin, promoting muscle contraction. Sodium and potassium are responsible for maintaining membrane potential through the sodium-potassium pump, and are also linked to the transmission of nervous stimuli and muscle contraction [41].

TABLE III
MINERAL PROFILE OF BROILER BREAST (*PECTORALIS MAJOR,* M.) FILLETS WITH ABNORMAL COLORATION (PSE, PFN), FROM NORTHEASTERN BRAZIL

Minerals	Normal (n=12)	PFN (n=6)	PSE (n=6)	p
Ca^{2+} (mg/kg)	462.57±26.79[b]	373,02±43.33[c]	530,64±28.20[a]	0.001
K^{+} (mg/kg)	4987.15±171.63	4761.85±82.84	5000.01±159.50	0.0649
Mg^{2+} (mg/kg)	936.20±2.988	928.65±17.53	948.95±67.35	0.7668
Na^{+} (mg/kg)	1208.49±244.84	1305.01±82.98	1218.86±65.03	0.6932

[a–b-c] Means within each line with different superscripts are significantly different (p<0,05).

The L* value obviously shows that the PFN breast fillets have affinity with the pale color, as was observed with the PSE fillet samples. The other comparative results have shown that the values of CL, SF_{raw} , SF_{cooked}, Ca^{2+}, and especially WHC which were displayed by PFN fillets all had a greater affinity with the samples from the Normal group. It is possible, therefore, to associate the groups termed PFN and Normal as having similar characteristics, typical of normal meats, which justifies the popular assertion that broiler breast is a white meat.

IV. CONCLUSION

The existence of an emerging group of abnormal color in broiler meat, described as pale, firm and non-exudative (PFN) was confirmed. However, the functional properties were similar to those of the Normal group and, therefore, it would be perfectly acceptable if both belonged to a single Normal group. In addition to that, the results reported indicate that the PSE syndrome in broilers is related to a flaw in calcium regulation, which led to a decrease in meat pH, and thus compromised functional properties.

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Sandy Soil Properties under Different Plant Cover Types in Drylands, Sudan

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Abstract—This study investigated the effects of Acacia Senegal, Calotropis procera, Leptadenia pyrotechnica, Ziziphus spina Christi, Balanites aegyptiaca, Indigofera oblongigolia, Arachis hypogea and Sesimum indicum grown in the western region of White Nile State on soil properties of the 0-10, 10-30, 30-60 and 60-90 cm depths. Soil properties were: pH(paste), electrical conductivity of the saturation extract (ECe), total N (TN), organic carbon (OC), soluble K, available P, aggregate stability and water holding capacity. Triplicate Soil samples were collected after the end of the rainy season using 5 cm diameter auger. Results indicated that pH, ECe and TN were not significantly different among plant cover types. In the top 10-30 cm depth, OC under all types was significantly higher than the control (4.1 to 7.7 fold). The highest (0.085%) OC was found under the Z. spina Christi and A. Senegal whereas the lowest (0.045%) was reported under the A. hypogea. In the 10-30 cm depth, with the exception of A. hypogea, Z. spina christi and S. indicum, P content was almost similar but significantly higher than the control by 72 to 129%. In the 10-30 cm depth, K content under the S. indicum (0.46 meq/L) was exceptionally high followed by Z. spina christi (0.102 meq/L) as compared to the control (0.029 meq/L). Water holding capacity and aggregate stability of the top 0-10 cm depth were not significantly different among plant cover types. Based on the fact that accumulation of organic matter in the soil profile of any ecosystem is an important indicator of soil quality, results of this study may conclude that (1) cultivation of A.senegal, B.aegyptiaca and Z. spina Christi improved soil quality whereas (2) cultivation of A. hypogea or soil that is solely invaded with C. procera and L.pyrotechnica may induce soil degradation.

Keywords—canopy, crops, shrubs, soil properties, trees

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Bioproduction of Phytohormones by Liquid Fermentation Using a Mexican Strain of Botryodiplodia theobromae

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Abstract—Plant hormones are a group of molecules that control different processes ranging from the growth and development of the plant until their response to biotic and abiotic stresses. In this study, the capacity of production of various phytohormones was evaluated from a strain of Botryodiplodia theobromae by liquid fermentation system using the modified Mierch medium added with a hydrolyzate compound of mead all in a reactor without agitation at 28 °C for 15 days. Quantification of the metabolites was performed using high performance liquid chromatography techniques. The results showed that a microbial broth with at least five different types of plant hormones was obtained: gibberellic acid, zeatin, kinetin, indoleacetic acid and jasmonic acid, the last one was higher than the others metabolites produced. The production of such hormones using a single type of microorganism could be in the future a great alternative to reduce production costs and similarly reduce the use of synthetic chemicals.

Keywords—biosystem, plant hormones, Botryodiplodia theobromae, fermentation

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Packaging Processes for the Implantable Medical Microelectronics

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Abstract—Electrostimulation medical devices for neural diseases require electroactive and biocompatible materials to transmit signals from electrodes to targeting tissues. Protection of surrounding tissues have become a great challenge for long-term implants. In this study, we designed a back-end processes with compatible, efficient, and reliable advantages over the current state-of-the-art. We explored a hermetic packaging process with high quality of adhesion and uniformity as the biocompatible devices for long-term implantation. This approach is able to provide both excellent biocompatibility and protection to the biomedical electronic devices by performing conformal coating of biocompatible materials. We successfully developed a packaging process that is capable of exposing the stimulating electrode and cover all other faces of chip with high quality of protection to prevent leakage between devices and body fluid.

Keywords—biocompatible package, medical microelectronics, surface coating, long-term implantation

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Blood Volume Pulse Extraction for Non-Contact Photoplethysmography Measurement from Facial Images

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***Abstract*—** According to WHO estimation, 38 out of 56 million (68%) global deaths in 2012, were due to noncommunicable diseases (NCDs). To avert NCD, one of the solutions is early detection of diseases. In order to do that, we developed "U-Healthcare Mirror", which is able to measure vital sign such as heart rate (HR) and respiration rate without any physical contact and consciousness.

To measure HR in the mirror, we utilized digital camera. The camera records red, green, and blue (RGB) discoloration from user's facial image sequences. We extracted blood volume pulse (BVP) from the RGB discoloration because the discoloration of the facial skin is accordance with BVP. We used blind source separation (BSS) to extract BVP from the RGB discoloration and adaptive filters for removing noises. We utilized singular value decomposition (SVD) method to implement the BSS and the adaptive filters. HR was estimated from the obtained BVP.

We did experiment for HR measurement by using our method and previous method that used independent component analysis (ICA) method. We compared both of them with HR measurement from commercial oximeter. The experiment was conducted under various distance between 30~110 cm and light intensity between 5~2000 lux. For each condition, we did measurement 7 times.

The estimated HR showed 2.25 bpm of mean error and 0.73 of pearson correlation coefficient. The accuracy has improved compared to previous work. The optimal distance between the mirror and user for HR measurement was 50 cm with medium light intensity, around 550 lux.

***Keywords*—** Key words: Blood volume pulse, Heart rate, Photoplethysmography, Independent component analysis.

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Investigation into Black Oxide Coating of 410 Grade Surgical Stainless Steel Using Alkaline Bath Treatment

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***Abstract*—**High reflectance of surgical instruments under bright light hinders the visual clarity during laparoscopic surgical procedures leading to loss of precision and device control and creates strain and undesired difficulties to surgeons. Majority of the surgical instruments are made of surgical grade steel. Instruments with a non reflective surface can enhance the visual clarity during precision surgeries. A conversion coating of black oxide has been successfully developed 410 grade surgical stainless steel .The characteristics of the developed coating suggests the application of this technique for developing 410 grade surgical instruments with minimal reflectance.

***Keywords*—**Conversion coatings, 410 stainless steel, black oxide, reflectance.

I. Introduction

STAINLESS steels are widely used for the manufacture of surgical instruments [1]. Reflection under bright lights from the surgical instruments made of surgical grade stainless steel creates undesired difficulties to the surgeons like loss of precision, lack of device control etc in laparoscopic surgeries [2]. The precision and device control in laparoscopic and key-hole surgeries are vital . Matte black oxide coating of surgical steels have been suggested to reduce the problems created due to the reflection of light from the surgical instruments made of surgical grade stainless steel. Black oxide is a conversion coating formed on the metal surfaces as a result of chemical reaction of the metal atoms with an oxidizing agent like air, alkaline aqueous salt solution, and molten salts. The conversion coating is a film of chemical compound formed by the reaction of the substrate in air and the properties do not vary from the main substrate [3].

Black oxide coating provides reduced light glare, dimensional stability, anti-galling property, improved lubricity, superior finish, corrosion protection etc [4]. The various black oxide processes are hot alkaline black oxide process, cold black oxide process, and molten salt black oxide process [7]. These methods have been tried for coating 300 series grade surgical stainless steel. Currently surgical equipments are manufactured from superior grade 410 stainless steels. The martensitic stainless steel is strong, hard, has good wear resistance but less resistance to corrosion [5]. In the present study all three methods suggested for conversion coating of 300 series stainless steel has been investigated on 410 grade steel by altering the various parameters like the composition of the mixture ,time and temperature. It was found that a black oxide (Ferric oxide, Fe3O4) conversion coating with good characteristics can be developed on grade 410 stainless steel using the molten salt process with a composition of 400g/L of sodium hydroxide, 320g/L sodium nitrate, and 200g/L of sodium dichromate in water at a temperature of 1390C and coating duration of 40 minutes. The reflectance, thickness and surface roughness parameters of the coating suggest its application for black oxide coating of surgical instruments made of 410 grade stainless steel so as to obtain minimal reflecting surgical surfaces.

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II. Experimental

A.Materials and Sample Preparation

Type SAE grade 410, 316L & 310, stainless steel mechanically cleaned ,degreased in alkaline solution followed by a pickling process in 20% diluted Hydrochloric acid were used as the specimens[6]. Prepared samples are shown in Fig. 1.

Fig. 1 Prepared samples ready for coating process

B. Deposition Procedure

Coating of black oxide was attempted by three methods as detailed below. For each method the coating solution was transferred to a heating container of 50 litres capacity with two heating coils with heating capacity up to 3000C .The solution was allowed to reach the required coating temperature for each method. Then the specimens were partially dipped in the high temperature solution so as to get the required coating. The specimens were taken out at intervals of 5, 15, 25, 35, 45 and 55 minutes monitored by a calibrated stopwatch.

Method 1: By treating in hot solution of sodium hydroxide, sodium nitrate and sodium nitrite in water with a composition of 700g/L of sodium hydroxide, 100g/L of sodium nitrate and 100g/L of sodium nitrite in water. A 10 litre of solution was used to completely immerse the heating coils in the heater vessel used. After weighing in the chemicals and mixing them in water, the

mixture was transferred to the heating container. The mixture was maintained at a temperature of 1400C during the coating process. [7]

Method 2: Coating by treating in solution having composition of 110g/L of potassium hydroxide, 200g/L of potassium nitrate in 10 litres of water. The mixture was maintained at a temperature of 1350C during the coating process. [7]

Method 3: Using composition of 400g/L of sodium hydroxide, 320g/L sodium nitrate, and 165g/L of sodium dichromate in 10 litres of water. The mixture was heated to a temperature of 1450C during the coating process. Fig. 2 shows the actual coating process in progress.[7]

Fig. 2 Setup showing the coating process

As the preliminary samples of 410 grade on physical verification showed uniform coating only by the method 3 and that too at longer intervals, this method was chosen for further investigation to get optimal time and temperature and composition for uniform black oxide coating of 410 grade stainless steel. This was done by subjecting 410 grade samples to coating by method 3 at temperatures varying between 1150C-1500C. The process was also further explored with varying percentage of the sodium dichromate (Na3Cr3O7) (50-350g/L) while maintaining the same composition for sodium hydroxide (NaOH) and sodium nitrate (NaNO3).

C. Surface Characterization

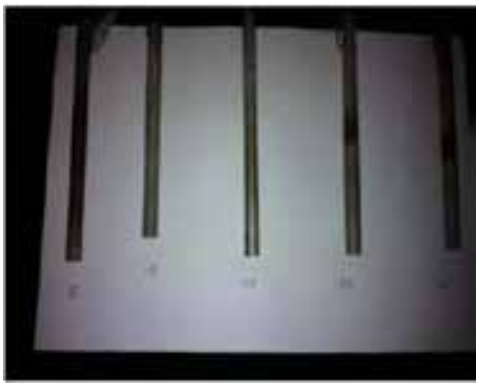
Measurement of reflectance : Reflectivity is a vital parameter in this work. In optics and photometry, reflectivity is the fraction of incident radiation reflected by a surface. In general it must be treated as directional property that is the function of the reflected direction, incident direction, and the incident wavelength. However it is also commonly averaged over the reflected hemisphere to give the hemispherical spectral reflectivity. When reflection occurs from thin layers of material, internal reflection effects can cause the reflectance to vary with surface thickness. Reflectance is the fraction of electromagnetic power reflected from a specific sample, while reflectivity is a property of the material itself, which could be measured on a perfect machine if the material filled half of the space [8]. The reflectance of the samples were measured with UV-VIS-NIR spectrophotometer Varian, Cary 5000 with Spectral range: 175 – 3300 nm Wave length accuracy: ± 0.1 nm (UV –Vis), ± 0.4 nm (NIR) Wave length reproducibility: 0.025 nm Limiting resolution: 0.05 nm (UV –Vis), 0.2 nm (NIR)

Measurement of thickness and roughness : Average thickness of the films and average roughness was measured using a Dektak 6M stylus profilometer (Veeco, USA) [9]-[11]. The stylus profiler takes measurements electromechanically by moving the sample beneath a diamond-tipped stylus. The film undergoing measurement is deposited with a region masked; this creates a step on the sample surface, and the thickness of the sample can be measured accurately by measuring the vertical motion of the stylus over the step. The profilometer measures the average thickness and roughness of the sample.

III. Results and Discussion

The results of the coating produced by the three methods on SAE grade steel samples of 310, 316 L and 410 are tabulated in Table I. On preliminary observation it was found that a good uniform dull matte black coating was formed on the grade 410 steel specimens coated by method 3 at a coating time of 45 minutes. No coating was observed on the 316 L samples. As the base interest was to coat surgical steel of 410 grade, method 3 was taken up for further investigation with changes in coating parameters. Fig. 3 shows the samples after the coating procedure. Samples in Fig. 3(c) clearly show black oxide conversion coating.

(a)



(b)

(c)

Fig. 3 Photographs of 410 grade samples after the primary coating (a) Method 1 (b) Method 2; and (c) Method 3

The coating characteristics obtained by varying the temperature of the bath under method 3 without altering the composition of the solution and time of coating are tabulated in Table II. A thin layer of conversion coating was observed on the specimen for all temperatures from 1200C to 1450C. The coating formed at a temperature of 1300C returned the lowest reflectance along with the maximum thickness and minimum roughness. Fig. 4 shows the variation of thickness, roughness and percentage reflectance of the samples for the various temperatures. Fig. 5 shows the variation in sample colour in different samples from black to brown.

TABLE I
TABULATED RESULTS OF BLACK OXIDE COATING ON VARIOUS GRADE OF STEEL BY THE THREE METHODS

Sl no	Chemical composition Chemical	quantity	Steel Grade	Coating Time	coating	Colour	Visual examination Results
			Method 1 (max temp 140^{0}C)				
				5	No	-	
	Sodium Hydroxide	0.700 kg		15	No	-	
			SAE 410	35	No	-	No coating was observed
1.	Sodium Nitrate	0.100 kg		35	No	-	
				45	No	-	
	Sodium Nitrite	0.100 kg	SAE 310	45	Uniform	Black	Fine uniform coating
	water	1 litre	SAE316L	45	No	-	No coating observed
			Method 2 (max temp 135^{0}C)				
				5	No	-	
	Potassium Hydroxide	1.100 kg		15	No	-	
			SAE 410	35	No	-	No coating observed
2.				35	No	-	
	Potassium Nitrate	0.200 kg		45	No	-	
			SAE 310	45	Uniform	Black	Fine uniform coating
	water	1 litre	SAE316L	45	No	-	No coating observed
			Method 3 (max temp 140^{0}C)				
				5	Traces	Black	
	Sodium Hydroxide	0.400 kg		15	Traces	Black	Black coating was observed which became more uniform with increase in coating time
	Sodium Nitrate	0.320 kg	SAE 410	35	Non uniform	Black	
3.				35	Uniform	Black	
	Sodium Dichromate	0.165 kg		45	Uniform	Black	
			SAE 310	45		Black	Uniform coating
	water	1 litre	SAE316L	45			No coating observed

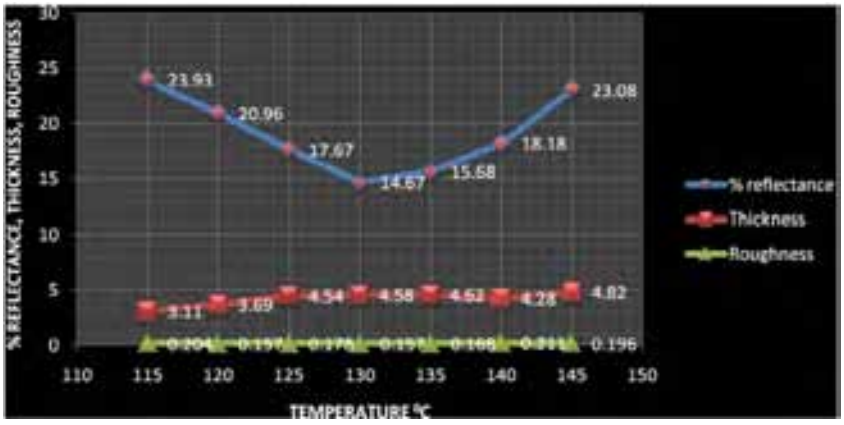


Fig. 4 Variance of Reflectance, thickness, and roughness at different temperatures

TABLE II
TABULATED RESULTS OF BLACK OXIDE COATING ON 410 GRADE SAMPLES FOR THE VARIOUS TEMPERATURES

Exp no	Temperature	Specimen	Time of exposure	Coating	Colour	% R	t	Ra
1	115^0C	1	45	None	--	23.93	3.11	0.204
3	120^0C	2	45	Traces	Black	20.96	3.69	0.157
3	125^0C	3	45	Non uniform	Black	17.67	4.54	0.178
4	*130^0C*	*4*	*45*	*Uniform*	*Black*	*14.67*	*4.58*	*0.157*
5	135^0C	5	45	Uniform	Brown black	15.68	4.62	0.168
6	140^0C	6	45	Uniform	Brown	18.18	4.28	0.211
7	145^0C	7	45	Uniform	Brown	23.08	4.82	0.196

*t- thickness of coating obtained μm; %R- average percentage of reflectance obtained in the visible region (380-750 nm); Ra- average roughness values in μm

Fig. 5 Colour variation of the coated samples

As austenitic steels have a higher reflectance than the ferritic and martensitic steels and their integrated solar reflectance of 68% is considered too much for their use in endoscopy two of the natural oxides on stainless steel, Fe2O3 and Cr2O3 have been studied as separate films, and their optical constants determined by combined transmission and reflectance measurements. It has been found that Fe3O4 and Cr2O3 gives better selectivity than Fe2O3. Hence it is concluded that the ferritic and martensitic steel base is to be preferred for developing surgical instrument because of its lower reflectance [12]. Based on this the surface conversion of SAE 410 grade steel was further investigated with dichromate leading to a surface layer of Fe2O4 and Cr2O3 which will have lower reflectivity.

TABLE III
TABULATED RESULTS OF BLACK OXIDE COATING ON 410 GRADE SAMPLE BY VARYING SODIUM DICHROMATE COMPOSITION

Exp No	Composition in kg/L ($Na_3Cr_3O_7$)	Sample	Time of coating	Boiling point	Coating	Colour	%R	t	Ra
1	0.050	8	30	125^0C	Traces	-	23.08	1.02	0.128
		9	40			Black	18.95	1.93	0.168
3	0.100	10	30	133^0C	Non Uniform	Black	17.33	1.96	0.179
		11	40			Black	18.51	1.91	0.210
3	0.150	12	30	137^0C	Uniform	Black	17.06	2.21	0.236
		13	40			Black	15.68	3.16	0.162
4	0.200	14	30	139^0C	Uniform	Black	15.73	4.69	0.140
		15	40			Black	13.45	4.83	0.152
5	0.250	16	30	144^0C	Uniform	Black	15.29	4.47	0.195
		17	40			Brown	17.67	4.54	0.178
6	0.300	18	30	150^0C	Uniform	Brown	19.73	4.13	0.152
		19	40			Brown	21.16	3.39	0.139
7	0.350	20	30	153^0C	Uniform	Brown	23.76	5.83	0.137
		21	40			Brown	23.93	4.91	0.204

* t- thickness of coating obtained μm; %R- average of percentage reflectance obtained in visible region (380-750nm); Ra- average roughness values in μm

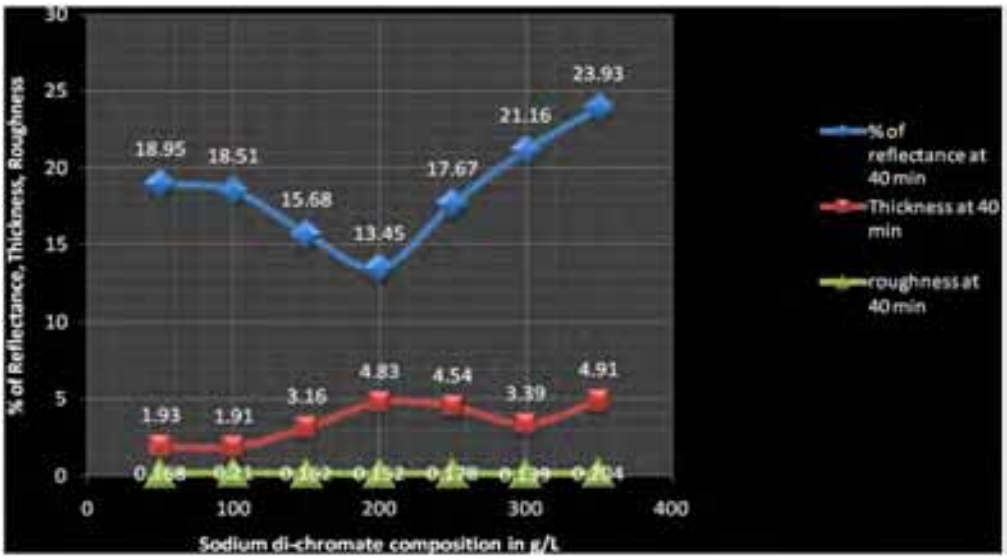


Fig. 6 Variation of % of reflectance, thickness and roughness with change in sodium dichromate composition

Since di-chromate composition is detrimental in a lower reflective coating the 410 grade samples were subjected to coating process by method 3 by varying the dichromate composition and the results are tabulated in Table III. Fig. 6 shows the variation of thickness, roughness and percentage reflectance of the samples by varying the dichromate composition. It is seen that the coating done using composition of 400 g/L of sodium hydroxide, 320 g/L sodium nitrate, and 200 g/L of sodium dichromate in 10 litres of water with a coating temperature of 1390C and coating duration of 40 minutes gave a uniform black coating with the lowest reflectance of 13.45%, thickness of 4.83 µm and a low roughness of 0.152 µm and is shown in Fig. 7 (a).The other samples in figure show lesser coating characteristics.

(a) (b) (c)

Fig. 7 Coated samples by varying sodium dichromate composition

IV. Conclusion

Conversion Black oxide coating was successfully done on grade 410 stainless steel using hot alkaline process with alkaline solution consisting of sodium nitrate, sodium hydroxide and sodium dichromate. Analysis of the coated surfaces were further made based on temperature changes, time of coating, change in composition of solution and sodium di-chromate ratio to get the coating with maximum thickness and lowest reflectance and having average roughness at the lower range. The best coating was obtained with a solution of 400 g/L of sodium hydroxide, 320g/L of sodium nitrate, 200g/L of sodium Di-chromate in water at an alkaline bath temperature of 1390C and an exposure time of 40 minutes. The minimum average percentage of reflectance in visible region obtained was 13.45% and the thickness of the coating was 4.83µm. The average roughness value is 0.152 µm. The reflectance value obtained is far less compared to base 410 grade Stainless steel samples and suggests the usage of the coating procedure for producing low reflective surgical instruments for laparoscopic and endoscopic application.

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Gene Expression Signature-Based Chemical Genomic to Identify Potential Therapeutic Compounds for Colorectal Cancer

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Abstract—There is a wide range of drugs and combinations under investigation and/or approved over the last decade to treat colorectal cancer (CRC), but the 5-year survival rate remains poor at stages II–IV. Therefore, new, more efficient drugs still need to be developed that will hopefully be included in first-line therapy or overcome resistance when it appears, as part of second- or third-line treatments in the near future. In this study, we revealed that heat shock protein 90 (Hsp90) inhibitors have high therapeutic potential in CRC according to combinative analysis of NCBI's Gene Expression Omnibus (GEO) repository and chemical genomic database of Connectivity Map (CMap). We found that second generation Hsp90 inhibitor, NVP-AUY922, significantly down regulated the activities of a broad spectrum of kinases involved in regulating cell growth arrest and death of NVPAUY922-sensitive CRC cells. To overcome NVP-AUY922-induced upregulation of survivin expression which causes drug insensitivity, we found that combining berberine (BBR), a herbal medicine with potency in inhibiting survivin expression, with NVP-AUY922 resulted in synergistic antiproliferative effects for NVP-AUY922-sensitive and -insensitive CRC cells. Furthermore, we demonstrated that treatment of NVP-AUY922-insensitive CRC cells with the combination of NVP-AUY922 and BBR caused cell growth arrest through inhibiting CDK4 expression and induction of microRNA-296-5p (miR-296-5p)-mediated suppression of Pin1–β-catenin–cyclin D1 signaling pathway. Finally, we found that the expression level of Hsp90 in tumor tissues of CRC was positively correlated with CDK4 and Pin1 expression levels. Taken together, these results indicate that combination of NVP-AUY922 and BBR therapy can inhibit multiple oncogenic signaling pathways of CRC.

Keywords—berberine, colorectal cancer, connectivity map, heat shock protein 90 inhibitor

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Hepatoprotective Effect of Oleuropein against Cisplatin-Induced Liver Damage in Rat

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Abstract**—**Cisplatin (CIS) is one of the most effective an anticancer drug and also toxic to cells by activating oxidative stress. Oleuropein (OLE) has key role against oxidative stress in mammalian cells, but the role of this antioxidant in the toxicity of CIS remains unknown. The aim of the present study was to investigate the efficacy of OLE on CIS-induced liver damages in male rats. With this aim, male Sprague Dawley rats were randomly assigned to one of eight groups: Control group; the group treated with 7 mg/kg/day CIS; the groups treated with 50, 100 and 200 mg/kg/day OLE (i.p.); and the groups treated with OLE for three days starting at 24 h following CIS injection. After 4 days of injections, serum was provided to assess the blood AST, ALT and LDH values. The liver tissues were removed for histological, biochemical (TAC, TOS and MDA) and genotoxic evaluations. In the CIS treated group, the whole liver tissue showed significant histological changes. Also, CIS significantly increased both the incidence of oxidative stress and the induction of 8-hydroxy-deoxyguanosine (8-OH-dG). Moreover, the rats taking CIS have abnormal results on liver function tests. However, these parameters reached to the normal range after administration of OLE for 3 days. Finally, OLE demonstrated an acceptable high potential and was effective in attenuating CIS-induced liver injury. In this trial, the 200 mg/kg dose of OLE firstly appeared to induce the most optimal protective response.

Keywords**—**Antioxidant response, cisplatin, histology, liver, oleuropein, 8-OhdG.

I. Introduction

CISPLATIN-based chemotherapy is widely used for the treatment of tumors including head, neck, breast, lung ovarian, testicular and bladder cancers [1]. However, the prevalence of CIS toxicity is high and liver toxicity is the most prominent side-effect of CIS [2]. The drug induces pathological signs [3], [4] and acute liver failure in laboratory animals [5], [6]. Olive leaf contains large amounts of potentially useful phytochemicals, many of the same phenolics as the olive oil but in much higher concentration. The polyphenols exhibit a range of beneficial effects including antioxidant, anti-inflammatory, antiatherogenic and anticarcinogenic properties [7]. The antioxidant activity of olive phenols has been demonstrated in several *in vitro* systems and some intervention studies have investigated the DNA protective potential of phenolical compounds [8]. OLE is the major bio-phenol component in olive leaf and fruit and the most promising bioactive natural product. OLE is commercially available as food supplement in Mediterranean countries. OLE protects against a number of pathologies, including Alzheimer's disease, type 2 diabetes, obesity, non-alcoholic hepatitis, and other natural or experimentally-induced pathological conditions. Such a protection could result, at least in part, in a remarkable improvement of the pathological signs arising from stress conditions including oxidative stress, an excessive inflammatory response, and the presence of cytotoxic aggregated material [9], [10].

The conventional chemotherapy is not adequately effective against cancer and has severe side effects. Many natural compounds isolated from plants have been found to inhibit cancer growth and to decrease adverse effects of chemotherpic drugs. CIS-induced liver toxicity involves the formation of reactive oxygen species (ROS) and decrease in antioxidant enzymes [11], [12]. Because OLE has been used to improve liver function in various regions of the world [7], the present study has been conducted to find the effects of OLE on CIS-induced liver toxicity in rat. In this regards, histopathological and biochemical evaluations of the liver tissue were performed. Additionally, the objective of this study was to evaluate the genotoxicity of CIS and to investigate antigenotoxic potential of OLE against CIS-induced oxidative DNA damage in rat liver.

II. Materials and Methods

A. Animals

Fifty-six adult male Spraque-Dawley rats (weighing 200-250 g) obtained from Medical Experimental Application and Research Center, Atatürk University were used. Animals were housed inside polycarbonate cages in an air-conditioned room (22±2 °C) with 12-h light-dark cycle. Standard rat feed and water were provided ad libitum. The rats were allowed to acclimatize to the laboratory environment for 7 days before the start of the experiment. All procedures were performed in conformity with the Institutional Ethical Committee for Animal Care and Use at Atatürk University (protocol number: B.30.2.ATA.0.23.85-11) and the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

B. Experimental Protocols

The rats were weighed and randomly allocated into eight experimental groups (n=7, each): (a) Control: the animals received 1 mL of distillated water as vehicle; (b) CIS (Sigma

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Chemical Co., St. Louis, MO): the animals received 7 mg CIS/kg b.w., diluted in distillated water (1 mL); c) OLE (HPLC grade ≥ 98%; Sigma Chemical Co., St. Louis, MO): the animals received 1 mL of OLE solution; and (d) CIS/OLE the animals received 1 mL of preparations of OLE following CIS administration.

The injections of CIS were given using a single dose, via i.p. route for 24 hours. The OLE groups received i.p. injections with a daily single dose of OLE (50, 100 and 200 mg/kg/day) for a total period of 3 days. On day 4 after injections, the rats were anesthetized with isoflurane and blood samples were collected for biochemical studies. After cervical dislocation under anesthesia, the liver specimens were gathered for further analyses. These investigations stem from the works of the researchers [13], [14].

C. Biochemical Methods

Fresh liver tissues were rinsed with ice-cold saline and immediately stored at –80°C. The tissue specimens were weighed and then homogenized in a 50-mM phosphate buffered saline (PBS) at pH 7.0. Homogenized liver tissues were then centrifuged at 10.000 rpm at 4 °C over 15 minutes to isolate the supernatant for subsequent analysis.

D. Determination of Lipid Peroxidation

Lipid peroxidation was determined by quantifying malondialdehyde (MDA) content in the tissue supernatant, which was spectrophotometrically measured by the absorbance of a red-colored product with thiobarbituric acid [15]. This reaction results in the production of a MDA-TBA2a compound that has a measurable absorbance at 532 nm [16].

E. Measurement of TAC and TOS

Total antioxidant capacity (TAC) and total oxidant status (TOS) were measured via colorimetric methods from rat liver tissue. TAC units are mmol Trolox equivalents per gram of tissue, and TOS units are mmol H2O2 equivalents per gram of tissue. The oxidative stress index (OSI) was calculated according to the following formula: OSI = TOS/TAC [17].

F. Liver Function Assessment

Blood samples were collected into serum superetor tubes (Microtainer; Becton Dickinson, Franklin Lakes, NJ, USA), allowed to stand (75-90 min), centrifuged (11,000 g, 5 min), serum harvested, and stored at -20 °C. The following parameters for liver injury were measured by an automated biochemical analyzer (Olympus AU 2700) with commercially available testing kits (Bioclinica): alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) [18].

G. Determination of 8-OH-dG Level

8-hydroxy-2'-deoxyguanosine assay kits were purchased from Cayman Chemical for determining 8-OH-dG levels in the liver samples. Since it is a competitive assay that can be used for the quantification of 8-OHdG in homogenates and recognizes both free 8-OH-dG and DNA-incorporated 8-OH-dG, many researches are being performed to use this protocol. This assay depends on the competition between 8-OH-dG and 8-OHd-G-acetylcholinesterase (AChE) conjugate (8-OH-dGTracer) for a limited amount of 8-OHd-G monoclonal antibody [19]. All procedures were carried out in accordance with the provider manual.

H. Histopathological Examination

The livers of rats were fixed in buffered 10% formalin solution for 72 h and embedded in a paraffin wax. Then tissue specimens were cut into five-micron sized pieces and stained with hematoxylin and eosin for routine histology (H&E). Reticulin stain was used for the evaluation of fibrosis [20]. In addition, glycogen storage ability of hepatocytes was assessed through periodic acid-Schiff (PAS) [21]. A semi-quantitative evaluation of liver tissue was accomplished by scoring the degree of severity according to the formerly published criteria [22]-[24]. Light microscope (X200) was used for histopathologic examination. The degree of the inflammation, hemorrhage, fibrosis, congestion, hepatocyte damage and glycogen content were scored for each group.

I. Statistical Analysis

For statistical analysis, we used SPSS for Windows 13.0 (SPSS Inc., Chicago, USA). The experimental data were analysed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test for multiple comparisons. Results are presented as mean ± standard error (SE) and values $p<0.05$ were regarded as statistically significant.

TABLE I
THE EFFECTS OF OLE ON LIVER AST, ALT, LDH, MDA, TOS, AND TAC LEVELS AFTER TREATED WITH CIS

Groups	AST(U/L) Mean±SE	ALT(U/L) Mean±SE	LDH (U/L) Mean±SE	MDA Mean±SE	TOS Mean±SE	TAC Mean±SE
Control	185.14±15.3[a]	56.70±11.10[a]	4110.33±296.53[a]	3.90±0.92[a]	15.31±4.06[a]	2.14±0.88[a]
CIS	537.23±67.73*[d]	159.80±20.05*[d]	21695.13±965.15*[d]	15.61±3.73*[c]	77.85±17.21*[c]	8.09±2.16*[d]
OLE 50 mg/kg	176.41±20.24[a]	58.13±18.10[a]	4096.17±231.25[a]	3.75±10.14[a]	16.27±3.44[a]	2.11±0.76[a]
OLE 100 mg/kg	184.47±21.23[a]	53.32±29.11[a]	4122.18±251.21[a]	3.88±0.82[a]	15.55±5.23[a]	2.24±0.53[a]
OLE 200 mg/kg	182.61±30.09[a]	55.20±23.06[a]	4165.36±210.30[a]	3.95±0.56[a]	17.48±6.38[a]	2.48±0.55[a]
CIS+OLE 50 mg/kg	522.24±40.03*[c,d]	145.23±20.15*[c]	21186.79±867.34*[c,d]	14.32±2.69*[c]	73.19±20.11*[c]	7.85±3.24*[c,d]
CIS+OLE100 mg/kg	415.52±37.21*[b]	128.76±2.07*[b]	18568.46±763.46*[b]	10.54±2.64*[b]	59.79±21.09*[b]	6.15±2.69*[b]
CIS+OLE200 mg/kg	185.14±15.3[a]	61.03±12.27[a]	4189.76±242.22[a]	4.01±0.86[a]	17.19±3.79[a]	2.54±0.69[a]

Notes: Data are presented as means ± SD (n=7). *Symbol < 0,05 represents significant difference among the groups compared to controls. [a,b,c,d] The groups in the same column with different letters are statistically significant (p0.05). For abbreviations see legend in Table I. Abbreviations used: CIS: Cisplatin; OLE: Oleuropein

III. Results

CIS group displayed remarkable elevation in the levels of liver enzymes, MDA and TOS (Table I). On the other hand, a significant decrease was found in TAC level with CIS administration. Whereas, supplementation of rat with OLE resulted in reversal of biochemical indicators. And these effects were associated with the increasing dose of OLE therapy. Notably, all parameters were reached statistical significance in the high-dose OLE group when compared to the CIS group ($p<0.05$).

As presented in Table II, the 8-OH-dG levels, a hallmark of oxidative stress-DNA base damage, were markedly increased in rat liver after CIS administration. On the contrary, there were no significant difference between the levels of 8-OH-dG in the control and all OLE treated groups. Moreover, levels of 8-OHdG caused by CIS were normalized by dose of 200 mg/kg OLE.

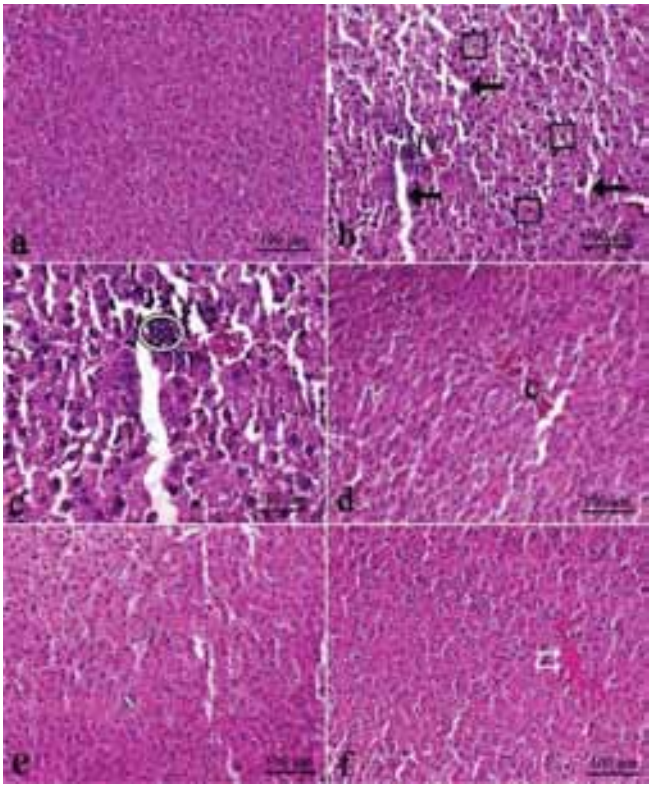

Fig. 1 Light microscopic appearance of liver from control rats (a), liver in CIS group rats (b)-(e) (b) Sinusoidal dilatation (arrows), Hepatocyte necrosis (inside of square symbols), (c) Inflammation (inside of circle symbol), (d) Congestion (C), (e) Diffuse hepatic necrosis (N), (f) Hemorrhage (double arrow) (H&E)

As compared with control group, CIS group showed significant hepatopathologic changes; hepatic necrosis, inflammation, hemorrhage, fibrosis, congestion, sinusoidal dilatations, the activation of Kupffer cells and decreased glycogen content. On the other hand, the administration of OLE was able to reduce the liver injury. Furthermore, this positive impact was notably correlated with increased OLE doses (Figs. 1-9). And the analysis of histopathological data demonstrated significant differences between the groups ($p<0.05$, Table III).

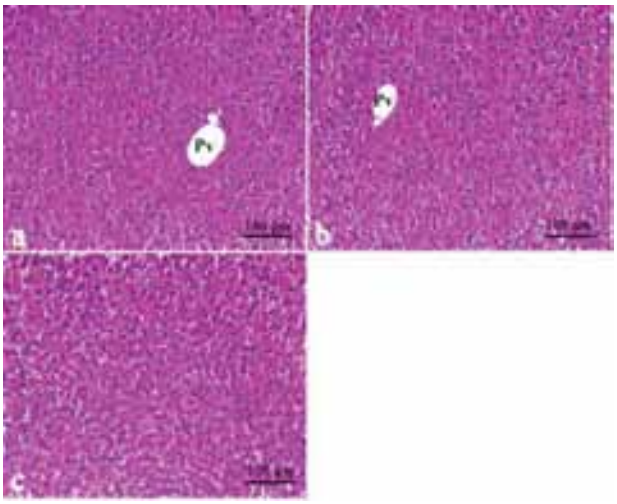

Fig. 2 Liver in OLE group rats a) 50 mg/kg OLE group, b) 100 mg/kg OLE group. Portal vein (Pv), c) 200 mg/kg OLE group, (H&E)

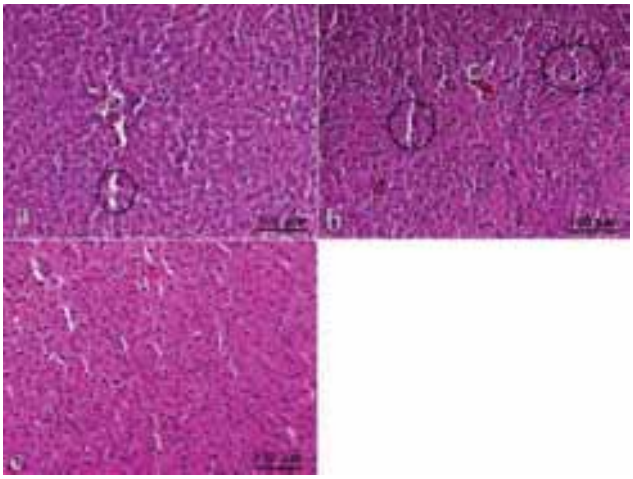

Fig. 3 (a) Decreased dilatations in CIS+50 mg/kg OLE group (inside of circle symbol), and congestion (C), (b) Decreased congestion (C) and sinusoidal dilatations as compared CIS+100 mg/kg OLE group. (c) Normal histological structure of liver in CIS+200 mg/kg OLE group, (H&E)

TABLE II
The Effect of OLE on Liver 8-OH-dG Levels after Treated with CIS

Groups	8-OH-dG level (as pg/ml); Mean±SE
Control	1.07 ± 0.9a
CIS	3.87 ± 0.31*d
OLE 50 mg/kg	0.93 ± 0.11a
OLE 100 mg/kg	0.98 ± 0.12a
OLE 200 mg/kg	1.04 ± 0.16a
CIS+OLE 50 mg/kg	2.18 ± 0.42b
CIS+OLE 100 mg/kg	1.49 ± 0.14c
CIS+OLE 200 mg/kg	1.08 ± 0.18a

Notes: Data are presented as means ± SD (n=7). *Symbol < 0,05 represents significant difference among the groups compared to controls. The groups in the same column with different letters are statistically significant (p0.05). For abbreviations see legend in Table I.

TABLE III
HISTOPATHOLOGICAL SCORES OF LIVER PATHOLOGY AFTER TREATED WITH CIS

Groups	Scores for hepatic damage (maximum score of 18) Mean±SD	Scores for hepatic glycogen content (maximum score of 3) Mean±SD	Scores for fibrous (maximum score of 3) Mean±SD	Scores for inflammation (maximum score of 18) Mean±SD	Scores for hemorrhage (maximum score of 3) Mean±SD	Scores for congestion (maximum score of 3) Mean±SD
Control	0,58±0,14[a]	3,04±0,42[a]	0,43±0,09[a]	0,12±0,03[a]	0,15±0,10[a]	0,23±0,27[a]
CIS	9,45±0,40*[c]	0,48±0,11*[c]	2,50±0,12*[c]	3,01±0,42*[d]	4,03±0,83*[c]	4,65±1,01*[d]
OLE 50 mg/kg	0,59±0,13[a]	2,91±0,33[a]	0,55±0,11[a]	0,15±0,05[a]	0,14±0,11[a]	0,22±0,33[a]
OLE 100 mg/kg	0,62±0,04[a]	2,89±0,45[a]	0,46±0,10[a]	0,13±0,04[a]	0,17±0,12[a]	0,26±0,32[a]
OLE 200 mg/kg	0,58±0,11[a]	2,93 ±0,28[a]	0,52±0,09[a]	0,12±0,08[a]	0,13 ±0,18[a]	0,21±0,28[a]
CIS+OLE 50 mg/kg	9,05±0,22*[c]	0,92±0,14*[b,c]	2,12±0,21[b]	2,45±0,44*[c,d]	3,97±0,61*[c]	3,60±0,10*[c]
CIS+OLE 100 mg/kg	7,80±0,46*[b]	1,39±0,15*[b]	1,73±0,14*[b]	1,14±0,37*[b]	2,73±0,76*[b]	1,62±0,88*[b]
CIS+OLE 200 mg/kg	0,95±0,24[a]	2,90±0,27[a]	0,66±0,09[a]	0,25±0,09[a]	0,19±0,24[a]	0,31±0,25[a]

Notes: Data are presented as means ± SD (n=7). *Symbol < 0,05 represents significant difference among the groups compared to controls. [a,b,c,d] The groups in the same column with different letters are statistically significant ($p<0.05$) by Tukey's multiple range tests. For abbreviations see legend in Table I.

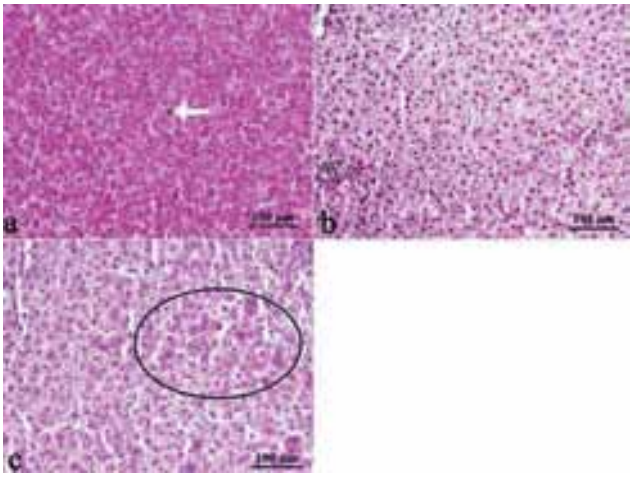

Fig. 4 Liver in control and CIS group rats; (a) Kupffer cells in control group liver (arrow) and darkly stained hepatocytes, (b) The glycogen depletion in hepatocytes of CIS group, (c) The increasing Kupffer cell activation in CIS group (inside of circle symbol), (PAS)

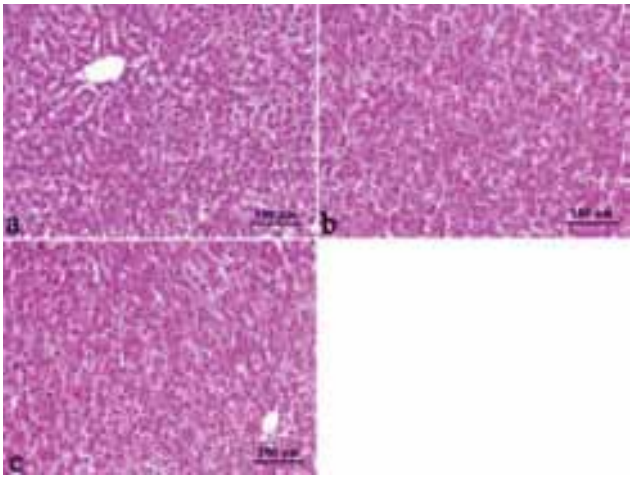

Fig. 5 The normal glycogen storage in liver of OLE group rats: (a) 50 mg/kg OLE group, (b) 100 mg/kg OLE group and (c) 200 mg/kg OLE group (PAS)

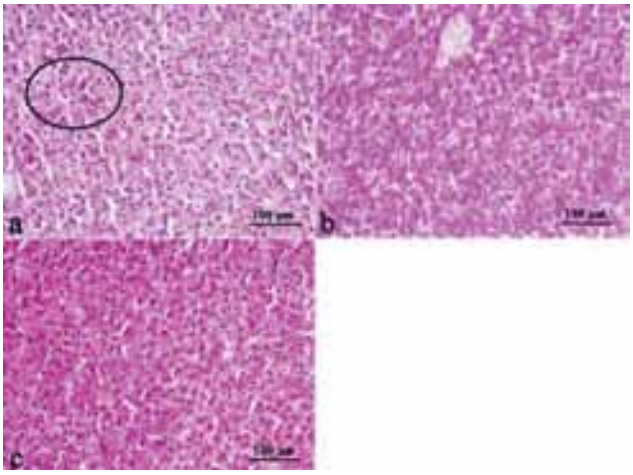

Fig. 6 The liver tissue following OLE exposure in CIS rats: a) CIS+50 mg/kg OLE group, decreased Kupffer cell activation (inside of circle symbol), b) The glycogen accumulation in liver of CIS+100 mg/kg OLE group, c) Liver histology similar to controls in CIS+200 mg/kg OLE group (PAS)

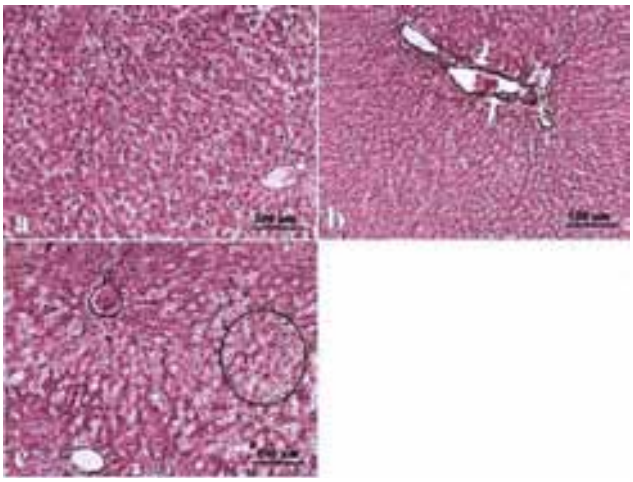

Fig. 7 Liver in control and CIS group rats: a) The normal architecture of liver from control rats, b) Increased intensity of fibrosis in portal veins of CIS group rat liver (arrows), c) Increased fibrosis in intercellular space (inside of circle symbol), (Reticulin)

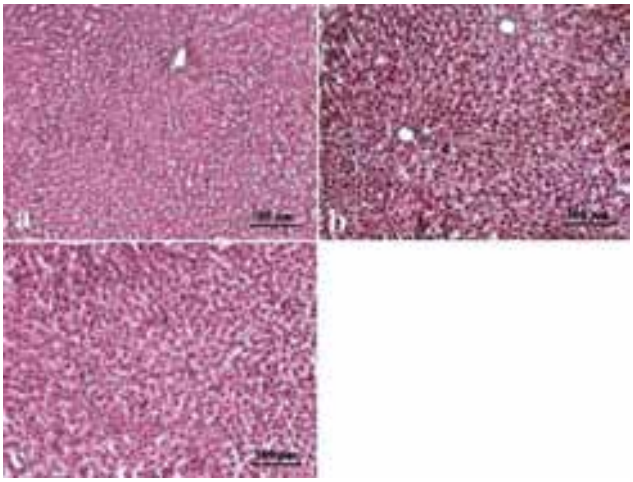

Fig. 8 The liver tissue in OLE group rats: a) CIS+50 mg/kg OLE group, b) CIS+100 mg/kg OLE group, c) CIS+200 mg/kg OLE group, (Reticulin)

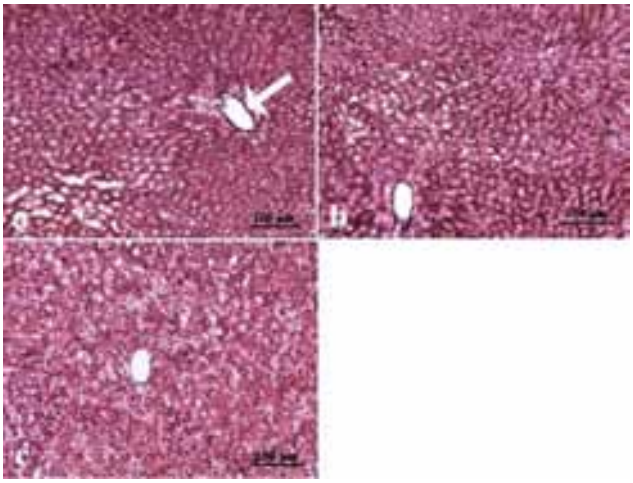

Fig. 9 a) and b) The decreased fibrosis in liver tissue following 50 mg/kg and 100 mg/kg OLE exposure in CIS rats, slightly fibrosis in portal veins (arrow) and c) The normal architecture similar to controls in CIS+200 mg/kg OLE group, (Reticulin)

IV. Discussion

Hepatotoxicity is one of the most common liver problems and occurs when a body is exposed to chemotherapy drugs [25]. Many studies have focused on the alternative approach to protect CIS-induced hepatotoxicity using natural products-derived antioxidants [26]. Our study produced two observations with important implications. CIS treatment is harmful to liver and OLE treatment is beneficial in protecting against CIS-induced hepatic injury.

Although CIS-caused nephrotoxicity has been very well documented, hepatotoxicity has been rarely characterized and is less studied. Whereas, CIS is significantly taken up in human liver and that the drug produces hepatic damages [27]. In our study, the balance between oxidant and antioxidant system seemed to be disturbed due to CIS treatment. In the present study, significant increases in MDA concentration were observed in the liver, indicating increased lipid peroxidation. The main breakdown product of oxidized lipids is the MDA and determining the concentration of MDA is one of the most popular methods for studying oxidative stress magnitude [28]. Recent studies have shown that oxidative stress and its responses are relevant to the physiological function of the liver and the development of liver injury [29]. Functional oxidative modifications of cellular proteins, both reversible and irreversible, are a causal step in cellular dysfunction. Redox-sensitive proteins with important cellular functions are important to defense in liver cells [30]. Changes in antioxidant enzymes and their protective mechanisms are early indicators of cellular susceptibility to oxidant injury caused by ROS. Modulation of oxidative stress plays an important role in the resistance to anticancer therapies [31].

Antioxidants can eliminate free oxygen radicals that appear in the body after radiation or chemotherapeutic agent exposure [32]. According to our findings, OLE could prevent membrane lipid peroxidation caused by MDA in liver and it could protect tissue from the harmful effects of CIS by increasing the level of reduced TAC. The previous studies have reported the beneficial physiological and pharmacological properties of OLE. One of its most prominent properties is its strong antioxidant activity, mainly due to the presence of hydroxyl groups in its chemical structure which could donate hydrogen to prevent oxidation [33]. The high antioxidative potential of the OLE was presented in different experimental models: in ethanol and cold stress-induced gastric ulcers [34], [35], in cerebral ischemia and reperfusion [36] and in hypertensive rats [37]. Additionally, OLE caused a significant increase of antioxidant status in erythrocytes [38], [39]. Moreover, the treatment with OLE decreased tissue MDA in aged rats [40]. Nevertheless, these comments are not based on full dose–response studies, and a more accurate investigation of the relative properties of these olive oil phenolics as biological antioxidants will be worthwhile [41], [42]. At this point, our study is one of studies contributing to dose–response studies. We established that OLE administration dose dependently increased antioxidant capacity (50 and 100 mg/kg). Especially, 200 mg/kg OLE exhibited powerful antioxidant activity in liver against CIS exposure.

A wide range of studies focus especially on substances causing oxidative DNA damage. CIS-induced oxidative DNA damage, specifically 8-OHdG lesions, has been demonstrated in both animal models and in humans [43]. Our study revealed that the highest concentration of the OLE exhibited the highest decrease in the number of cells with DNA damage in the liver samples treated with CIS. Efficiency of treatment with the OLE against CIS-induced DNA damage might be explained by the mechanisms reported by some authors that OLE increased the cells' antioxidant capacity by stimulating the synthesis of antioxidant enzymes and helped maintain their activity during oxidative stress [44]. It was shown that phenolic components such OLE had a higher antioxidant activity than vitamin C and vitamin E [45]. The olive phenolics prevented the H_2O_2-induced and phorbol myristate acetate-induced DNA damage [46]. Recently, the protective effects of OLE on sister chromatid exchange (SCE) and

chromosome aberration (CA) were found in cultured human blood cells treated with permethrin [47]. Additionally, OLE influenced the structure and stability of the DNA helix [48]. Our positive results showed that OLE dose-dependently has genoprotective feature on CIS-induced 8-OHdG lesions and altered DNA repair was a consequence of OLE exposure in liver cells. Moreover, these results were paralleled by increased anti-oxidant defense capacity and decreased TOS response in liver.

To assess the functional aspects, biochemical alterations in our study included elevated levels of AST, ALT and LDH in the blood after CIS treatment. AST and ALT are often associated with an increased release oxidative insult of CIS lead to damage in hepatocytes [49]. Cell necrosis leads to a rise in the concentration of the LDH enzyme in serum and tissue. The LDH released into the medium provides an index of cell death and membrane permeability to LDH, and an increase in LDH activity in the medium occurs as a result of cell membrane disintegration and enzyme leakage [50]. Thus, the increased activities of serum enzymes is mainly due to the leakage of these enzymes largely from the liver cytosol into the blood stream [51], which gives an indication of the abnormal function of liver. ROS can act as a molecular trigger of CIS and can attack the biological membranes directly [52]. Our results are in agreement with the commonly accepted view that serum level of transaminase returns to normal with healing of hepatic parenchyma and the regeneration of hepatocytes [53]. OLE appears to be effective in reducing the injurious effect of CIS observed in the present study. OLE is an indication of stabilization of plasma membrane, as well as repair of hepatic tissue damage caused by CIS. OLE is shown to be the effective antioxidant in protecting the 2,2'-azo-bis(2-amidinopropane) dihydrochloride-induced lipid peroxidation of the erythrocytes membranes [54]. Besides, scavenging of aqueous peroxyl radicals at the surface of membranes, as well as scavenging of lipid peroxyl radicals within the membranes, seems to play a considerable part in antioxidant activity of lipophilic antioxidants as OLE [55].

Our histopathological manifestations confirmed the biochemical changes in liver. The common findings in CIS-treated rats were fibrosis, inflammation, sinusoidal dilatations, congestion, hemorrhage, diminished glycogen content, focal necrosis and also diffuse hepatic necrosis. CIS caused an important histological sign by increasing fibrosis content in liver [56]. Current knowledge suggest that the inflammatory mediators and oxidative stress are actively involved in hepatic fibrogenesis [57], [58]. In agreement with our histological findings, CIS has been shown to activate inflammatory cells [59]. The administration of OLE decreased lymphocyte infilitration and activation of Kupffer cells in a dose-dependent manner. Thus, the suppression of them with OLE might contribute to deactivation of fibrogenic potential in our study. Because, OLE presented significant immunomodulatory properties in the liver by oxidative stress regulation. On the other hand, CIS has another critical side-effect as hemorrhage [60]. Moreover, CIS was considered in high risk for vascular disorders [61]. In present study, the OLE could save the liver from congestion, sinusoidal dilatations and also hemorrhage. It was reported that OLE reduced microvascular leakage as well as leukocyte adhesion and ROS formation, while capillary perfusion was protected [62]. Again, CIS altered the activities of gluconeogenesis enzymes in hepatocytes [63]. Our present data indicated that OLE resulted in the overall improvement of glycogenesis as evident by higher of glycogen deposits as compared to CIS group. In addition, consistent with the findings of this study, in liver of the animals treated with CIS was recorded diffuse degenerative changes and necrosis [64]. Whereas, 200 mg/kg OLE could decrease the degree and severity of necrosis after CIS therapy. Studies have suggested that oxidative stress might a causative agent of cell death in many different pathological states and in various models of toxic hepatic failure including CIS [65]. In the present study, disappearance of such morphological changes in the liver tissues of CIS-treated rats given evidence of antioxidant and anti-inflammatory properties of OLE.

In this study, OLE was tested for the first time for reducing the toxicity of anticancer drug. Our results showed that OLE dose-dependently has protective feature in the CIS-induced liver damages and might be considered a potential candidate to protect hepatocytes against the deleterious effects of cancer chemotherapy.

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Carvacrol Attenuates Lung Injury in Rats with Severe Acute Pancreatitis

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***Abstract*—**This study was designed to evaluate whether carvacrol (CAR) could provide protection against lung injury by acute pancreatitis development. The rats were randomized into groups to receive (I) no therapy; (II) 50 µg/kg cerulein at 1h intervals by four intraperitoneal injections (i.p.); (III) 50, 100 and 200 mg/kg CAR by one i.p.; and (IV) cerulein+CAR after 2h of cerulein injection. 12h later, serum samples were obtained to assess pancreatic function the lipase and amylase values. The animals were euthanized and lung samples were excised. The specimens were stained with hematoxylin-eosin (H&E), periodic acid–Schif (PAS), Mallory's trichrome and amyloid. Additionally, oxidative DNA damage was determined by measuring as increases in 8-hydroxy-deoxyguanosine (8-OH-dG) adducts. The results showed that the serum activity of lipase and amylase in AP rats were significantly reduced after the therapy ($p<0.05$). We also found that the 100 mg/kg dose of CAR significantly decreased 8-OH-dG levels. Moreover, the severe pathological findings in the lung such as necrosis, inflammation, congestion, fibrosis, and thickened alveolar septum were attenuated in the AP+CAR groups when compared with AP group. Finally, the magnitude of the protective effect on lung is certain, and CAR is an effective therapy for lung injury caused by AP.

***Keywords*—**Antioxidant activity, carvacrol, experimental acute pancreatitis, lung injury, oxidative DNA damage.

I. Introduction

ACUTE PANCREATITIS (AP) can lead to a systemic inflammatory response that often results in acute lung injury and single or multiple organ failure [1]. Of the total number of patient mortalities due to AP, >50% are ascribed to acute lung injury in the early stage [2].

Previous studies have demonstrated that pulmonary inflammation following pancreatitis-associated oxidative stress causes pulmonary swelling, which consequently produces secondary micro vascular leakage, alveolar-capillary barrier disruption, and even alveolar damage and mortality [3], [4]. In AP, pulmonary congestion poses a critical problem due to its association with acute respiratory function failure. The other pathological findings are characterized as ground a significantly wider alveolar septum, alveolar epithelial injury and fibrosis [4]. Despite the fact that there have been significant advances in the understanding of the pathogenesis of AP, the role of antioxidants in this phenomenon remain poorly understood. Antioxidant therapies are thought to improve lung morphology [5]. The lack of effective drugs to ameliorate the initiation and progression of pancreatitis-associated oxidative stress has led to increased interest in the role that antioxidants may serve in alleviating lung injury.

CAR, the most active constituent of thyme essential oils (EOs), is commonly used over a wide range of doses for various therapeutic applications [6], [7]. CAR's anti-proliferative, anti-inflammatory, and immune system modulation effects limit cancer growth and prevent leukocyte proliferation in inflammatory diseases [8]-[10]. It is also recognized as a safe food additive and used as flavoring agent in packed foods, sweets, beverages, and chewing gum [11], [12]. DNA-protective effects of CAR are assessed using different techniques. CAR shows high antioxidative effectiveness in all assays used [13].

In an effort to develop treatment modalities that reduce lung injury following AP, we studied whether CAR exerts protective effects against AP-induced oxidative lung injury in a rat model. Histological examinations were performed to determine the morphologic changes in rat lung tissues. To test oxidative DNA damage, we measured the DNA base adduct 8-hydroxy-deoxyguanosine (8-OH-dG), in the pulmonary cells. To the best of our knowledge, this is the first study that has investigated the protective effects of CAR against AP-induced pulmonary injury.

II. Materials and Methods

A. Animals

Fifty six male Spraque-Dawley rats (weighing 200-250 g) obtained from Medical Experimental Application and Research Center, Atatürk University were used. Animals were housed inside polycarbonate cages in an air-conditioned room (22±2°C) with 12-h light-dark cycle. Standard rat feed and water were provided ad libitum. The rats were allowed to acclimatize to the laboratory environment for 7 days before the start of the experiment. All procedures were performed in conformity with the Institutional Ethical Committee for Animal Care and Use at Atatürk University (protocol number: B.30.2.ATA.0.23.85-11) and the Guide for the Care and Use of Laboratory Animals [14].

B. Experimental Protocols

Animals were randomly divided into eight groups (n=7, each): (I) vehicle-treated group (control); (II) AP group; (III,

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IV and V) CAR-treated groups (at three different dose); (VI, VII and VIII) CAR-treated AP groups.

AP was induced by cerulein (Sigma-Aldrich, GmbH, Stenheim, Germany) administered i.p. 4 times with 1 h intervals at a dose of 50 µg/kg b.wt. AP was assessed after last injection of cerulein by measurement of serum amylase and lipase levels. Animals without induction of AP (control) were treated i.p. with saline at the same time as animals treated with cerulein.

To evaluate the effects of CAR, animals were treated with CAR in 10 ml saline (Peptide International Inc, Japan). The CAR groups received one i.p. injection of 50, 100 and 200 mg/kg b.wt. Therapeutic treatments were administered after 2 h of cerulein injection. The rats were anesthetized with isoflurane after 12 h taking CAR and euthanized by exsanguination with blood retained for serum harvest. These investigations stem from the works of the researchers [15], [16].

C. Biochemical Analyses

Blood for serum amylase determinations was obtained from all animals when they were anesthetized. Serum amylase and lipase levels were determined spectrophotometrically using an automated analyzer (Olympus AU 600, Diamond Diagnostic, Holliston, USA). All chemicals were obtained from Sigma (Sigma, St Louis MO, USA).

Tissue specimens were obtained from all animal groups for determination of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). SOD activity was studied on hemolysates by using commercial kits (Randox Laboratories, UK) [17]. CAT activity was measured according to Aebi's method [18]. The principle of the assay is based on determining the rate constant [k (s−1)] of hydrogen peroxide decomposition by the catalase enzyme.

Plasma thiobarbituric acid reactive substance (TBARS) levels were determined according to Schoenberg's method [19]. Lung MDA levels were determined on erythrocyte lyte obtained after centrifugation. After the reaction of thiobarbituric acid with MDA, the reaction product was extracted in butanol and was spectrofluorometrically (excitation 532 nm, emission 553 nm, slit 10 nm) evaluated. Tetramethoxypropane solution was used as standard. TBARS levels in the lung tissue were expressed as nmol/g.

GSH-Px activity in the pulmonary tissue was measured by the method described previously [20]. The reaction mixture was 50 mmol/l tris buffer (pH 7.6) containing 1 mmol/l of Na_2EDTA, 2 mmol/l of reduced glutathione (GSH), 0.2 mmol/l of NADPH, 4 mmol/l of sodium azide, and 1000 U of glutathione reductase (GR). 50 µl of plasma and 950 µl of reaction mixture, or 20 µl of erythrocyte lysate and 980 µl of reaction mixture were mixed and incubated for 5 min at 37 °C. Then the reaction was initiated with 8.8 mmol/l H2O2 and the decrease in NADPH absorbance was followed at 340 nm for 3 min. Enzyme activities were expressed as U/g in the lung tissue.

D. Histopathological Examination

The lung tissues of rats were fixed in buffered 10% formalin solution for 24 h and embedded in a paraffin wax. Tissues were then sectioned at 5 µm, stained with H & E, PAS, Mallory trichrome and amyloid methods. A semiquantitative evaluation of lung tissue was accomplished by scoring the degree of severity according to the formerly published criteria [21], [22]. For each lung section, whole slide was examined for necrosis, infiltration, congestion, fibrosis and thickened alveolar septum were observed under bright field using an Olympus BX60 microscope equipped with a digital CCD. In addition, high-resolution pictures (×200) of samples were taken under the same microscope. The lung damage was scored with maximum score of 18. The maximum score for the other pathological findings was 3.

E. Determination of 8-OHdG Level

8-hydroxy-2'-deoxyguanosine assay kits were purchased from Cayman Chemical (Ann Arbor, Michigan, USA) for determining 8-OH-dG levels in the lung samples. Since it is a competitive assay that can be used for the quantification of 8-OH-dG in homogenates and recognizes both free 8-OH-dG and DNA-incorporated 8-OH-dG, many researches are being performed to use this protocol. This assay depends on the competition between 8-OHdG and 8-OHdG-acetylcholinesterase (AChE) conjugate (8-OH-Dg Tracer) for a limited amount of 8-OH-dG monoclonal antibody. All procedures were carried out in accordance with the provider manual.

F. Statistical Analysis

For statistical analysis, we used SPSS for Windows 18.0 (SPSS Inc., Chicago, USA). The experimental data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test for multiple comparisons. Results are presented as mean ± standard deviation (SD) and values $p < 0.05$ were regarded as statistically significant.

III. RESULTS

Table I shows the effects of CAR on serum amylase and lipase levels in all experimental groups. The amylase rates in cerulein-induced AP were increased from an average 505 U/L to about 2366 U/L as compared with those of control rats. Similarly, lipase enzyme was greatly affected and increased from 23 µg/L to 126 µg/L by AP. The enzyme activation was not changed following intraperitoneal injection of CAR alone. After CAR treatments in animals with AP, positive effects on enzymes were observed and the values significantly were decreased with respect to 100mg/ kg dose of CAR ($p<0.05$).

The SOD, CAT, and GSH-PX activities in lung of AP rats markedly decreased but MDA increased compared to those found at controls ($p<0,05$). CAR groups alone showed the increased levels of antioxidant enzymes at both dosages (50 and 200 mg/kg, respectively). In these groups, MDA values were not changed. However, the best results were observed in a dose of 100 mg/kg of CAR. The activities of all enzymes were increased. Moreover, oxidative stress was significantly

decreased at this group and values returned to the control levels

TABLE I
THE EFFECT OF CAR TREATMENT ON SERUM AMYLASE AND LIPASE LEVELS IN CERULEIN-INDUCED AP

Groups	Amylase (U/L) Mean±SD	Lipase (U/L) Mean±SD
Control	505.85 ± 19.98[a]	23.52 ± 1.20[a,b]
AP	2366.56 ± 443.51*[d]	126.33 ± 9.38*[d]
CAR 50mg/kg	517.64 ± 26.01[a]	24.01 ± 1.67[a,b]
CAR 100mg/kg	492.29 ± 35.25[a]	22.67 ± 2.03[a]
CAR 200mg/kg	498.38 ± 22.85[a]	23.89 ± 0.96[a,b]
AP+CAR 50mg/kg	1334.43 ± 291.25*[b,c]	92.40 ± 11.40*[c]
AP+CAR 100mg/kg	709.68 ± 41.69[a,b]	39.14 ± 3.47[b]
AP+CAR 200mg/kg	1739.51 ± 367.98*[c,d]	110.27 ± 5.82 *[d]

Notes: Data are presented as means ± SD (n=7). *Symbol < 0.05 represents significant difference among the groups compared to controls. a, b, c, d The groups in the same column with different letters are statistically significant ($p < 0.05$). Abbreviations used: AP: Acute pancreatitis; CAR: Carvacrol

Representative images of histological examination of lung tissue in different groups of rats are presented in figures. In H&E stains in comparison to the controls (Fig. 1), the microscopic observations of lung in AP rats showed leukocyte infiltration, hemorrhage, marked alveolar thickening, congestion, smooth muscle hypertrophy, increased fibrosis and desquamated epithelial cells in bronchial lumina (Figs. 2 (a)-(d)), (Figs. 3 (a)-(d)).

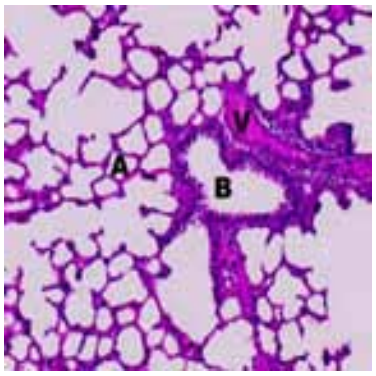

Fig. 1 Light microscopic appearances of lung from control rats. Alveoli (A), Bronchial (B), Vessel (V), (H&E)

TABLE II
EFFECT OF CAR TREATMENT ON LUNG SOD, CAT, GSH-PX ACTIVITIES AND MDA LEVELS IN CERULEIN-INDUCED AP

Groups	MDA (nmol/g) Mean±SD	SOD (U/g) Mean±SD	GSH-PX (U/mg-protein) Mean±SD	CAT (U/mg-protein) Mean±SD
Control	0.26 ± 0.08[a]	221.40 ± 45.14[a]	198.56 ± 32.25[a]	2.36 ± 0.15[a,b]
AP	0.82 ± 0.19*[b]	102.24 ± 22.29*[c]	86.79 ± 21.12*[c]	0.74 ± 0.10*[c]
CAR 50mg/kg	0.25±0.07[a]	207.16±39.32[a]	210.82±44.31[a]	2.43±0.20[a]
CAR 100mg/kg	0.23±0.06[a]	211.20±51.22[a]	201.53±27.16[a]	2.34±0.23[a,b]
CAR 200mg/kg	0.24±0.09[a]	215.21±27.36[a]	216.19±35.27[a]	2.48±0.19[a]
AP+CAR 50mg/kg	0.62±0.17*[b]	109.64±19.51*[c]	107.86±22.65*[b,c]	0.85±0.09[c]
AP+CAR 100mg/kg	0.27±0.11[a]	206.42±31.42*[a]	194.72±40.39*[a,b]	2.22±0.14[b]
AP+CAR 200mg/kg	0.77±0.16*[b]	106.31±25.54*[c]	94.45±19.53*[c]	0.81±0.11*[c]

Notes: Data are presented as means ± SD (n=7). *Symbol < 0,05 represents significant difference among the groups compared to controls. a, b, c, d, e, f The groups in the same column with different letters are statistically significant ($p<0.05$). For abbreviations see legend in Table I.

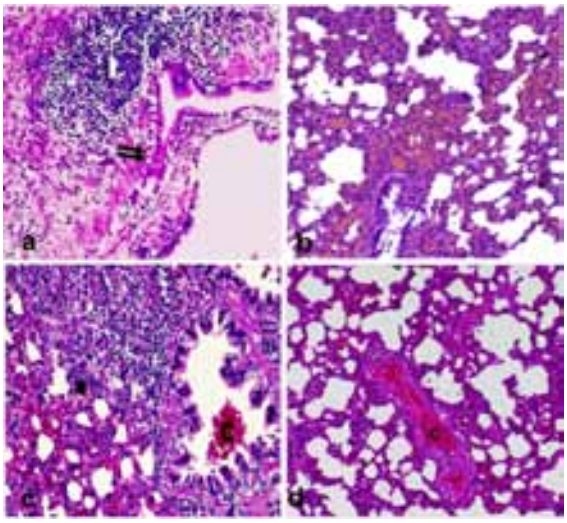

Fig. 2 The lung in the AP group (a) Vacuolisation (double arrow), Infiltration (I); (b) Diffuse haemorrhage in necrotic lung (H); (c) Congestion in bronchial (C), Prominent thickening of alveolar walls (asterix symbol); (d) Congestion in vessel (C), (H&E)

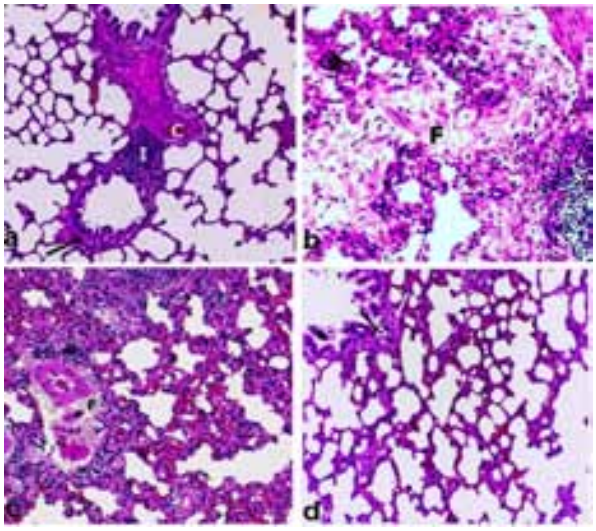

Fig. 3 The lung lesions in the AP group (a) Infiltration (I), Congestion (C), (b) Interstitial fibrosis (F), (c) Extensive fibrosis tissue around the blood vessels, (d) Desquamated epithelial cells (black arrow), Smooth muscle hypertrophy (white arrow), (H&E)

Compared with the control group, PAS staining revealed positive mucus-containing goblet cell hyperplasia and desquamated epithelial cells in bronchial sinus in lung of AP-induced rats (Figs. 4 (a)-(c)). Mallory trichrome showed increased intensity of fibrosis in bronchial and also interstitial fibrosis in animals with AP (Figs. 5 (a)-(d)). And the protein deposits in alveoli and alveolar septums were revealed by Amyloid staining method (Figs. 6 (a) and (b)).

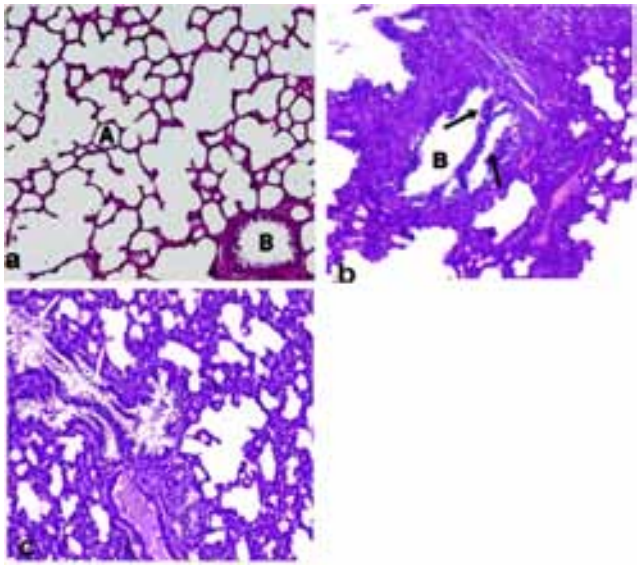

Fig. 4 The lung in control and AP rats (a) The lung in controls. The lung lesions in AP rats. Alveoli (A), (b) PAS positive mucus-containing goblet cells (black arrows), Bronchial (B), (c) Desquamated epithelial cells (white arrows), (PAS)

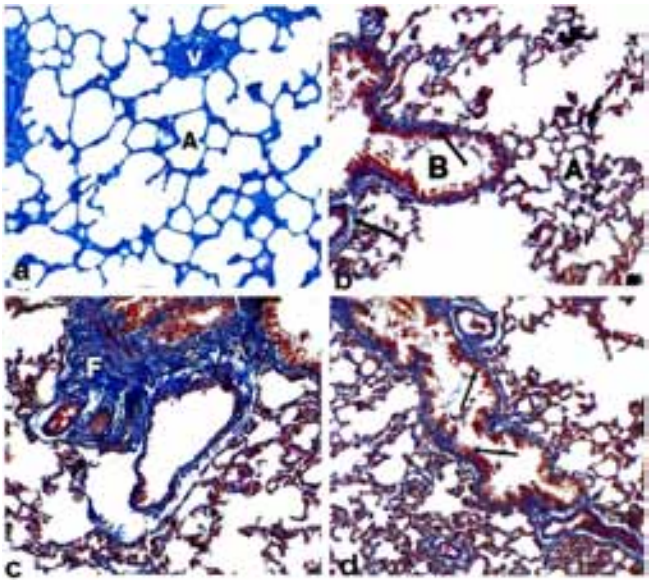

Fig. 5 The lung in control and AP rats (a) The lung in controls. The lung lesions in AP rats. Alveoli (A), Vessel (V), (b) Fibrosis tissue in bronchial walls (arrows), Bronchial (B), Alveoli (A); (c) İnterstitial fibrosis (F); (d) PAS positive mucus-containing goblet cells (arrows), (Mallory trichrome)

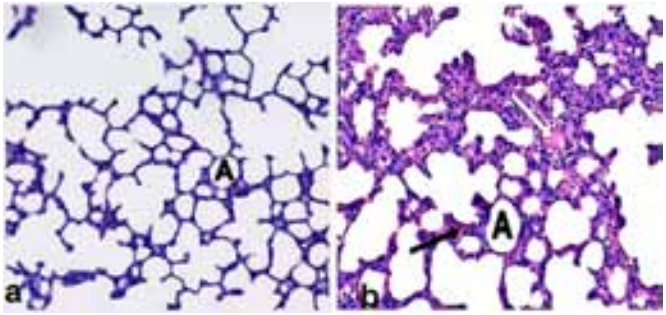

Fig. 6 The lung in control and AP rats. (a) The lung in controls. The lung lesions in AP rats. Alveoli (A), (b) The protein deposits in alveoli (white arrow) and alveoli septum (black arrow), Alveoli (A), (Amyloid)

Examination of lung sections in CAR groups revealed that the tissue retained its normal architecture (at 50, 100 and 200 mg/kg) (data not shown). In the 50 mg/kg CAR+AP groups, above-mentioned pathological findings were attenuated. There were reduction of hemorrhage and alveolar thickening. In lung of AP groups after treatment with 200 mg/kg CAR decreased congestion. However, in this group still had higher hemorrhage than those in the first. In 100 mg/kg CAR doses, the lung tissue showed a normal structure and orderly arrangement and resembled those of control rats (Figs. 7 (a)-(c)). As a matter of fact, PAS positive mucus-containing goblet cell hyperplasia and desquamated epithelial cells were not observed. Also, fibrosis and abnormal protein accumulations in tissue did not occur (Figs. 8 (a)-(c)).

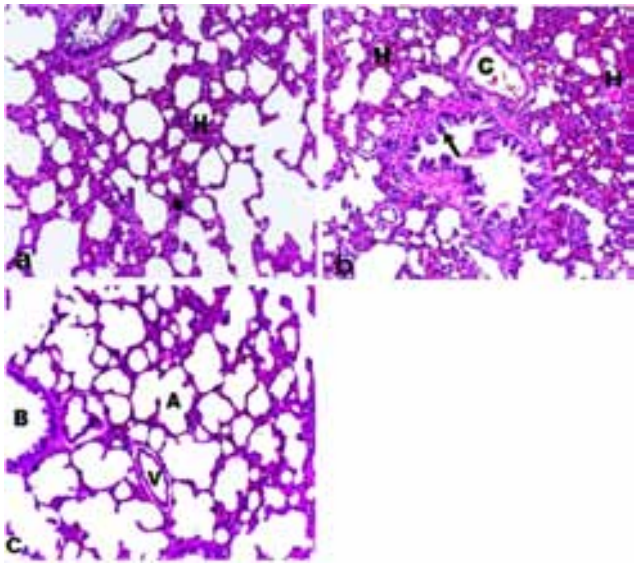

Fig. 7 The lung tissue following CAR exposure in AP rats (a) Reduction of haemorrhage and decreased alveolar thickening in AP+50 mg/kg CAR group, (b) The use of 200 mg/kg CAR decreased congestion, but AP+200 mg/kg CAR group showed more haemorrhage than AP+500 mg/kg CAR group. Branchial epithelial cells (black arrow), Diffuse haemorrhage in necrotic lung (H), Congestion in bronchial (C), (c) In AP+100 mg/kg CAR group, normal lung tissue. Alveoli (A), Bronchial (B), Vessel (V), (H&E)

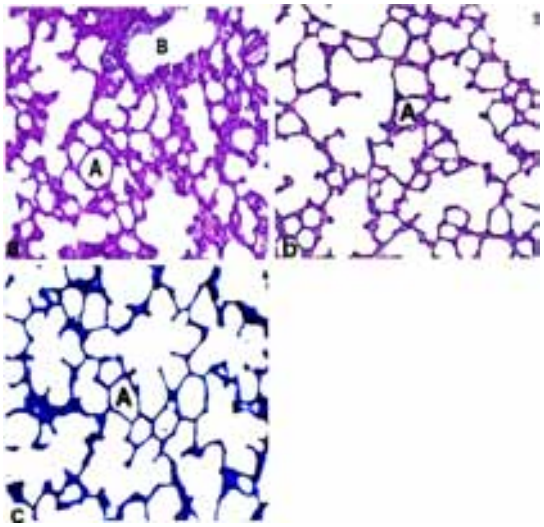

Fig. 8 The lung in AP+100 mg/kg CAR group was almost similar to that in control group (a) Alveoli (A), Bronchial (B), PAS, (b) Alveoli (A), Mallory trichrome, (c) Alveoli (A), Amyloid

Histopathological scores of the groups were summarized in Table III. The degree of pathological findings showed a significant difference between groups treated with cerulein and cerulein+CAR ($p<0.05$).

The levels of 8-OH-dG, a hallmark of oxidative stress-DNA base damage, were measured using an 8-OH-dG detection kit. There was no significant difference between the levels of 8-OH-dG in the control and all CAR treated groups (Table IV). On the contrary, the level of 8-OH-dG was significantly higher in AP as compared to control group. But treatment of CAR decreased the 8-OH-dG levels that were increased by cerulein-induced AP in a clear dose dependent manner.

IV. DISCUSSION

The most feared complication in AP is lung failure [23]. Although the exact mechanisms remain elusive, it has been reported that oxidative stress plays an important role in promoting lung damage in AP. An improved understanding of the oxidative stress mechanism operating in the lung is necessary for the development of new therapies against tissue damage in AP [24], [25]. This study documents a fundamental role of oxidative stress in the pathophysiology of AP. Inhibition of oxidative stress decreased ROS formation, leukocyte recruitment, and tissue damage in the lung. Thus, targeting oxidative stress might protect against pathological inflammation in AP.

TABLE III
HISTOPATHOLOGICAL SCORES OF LUNG IN CERULEIN-INDUCED AP

Groups	Lung necrosis (maximum score 18) Mean±SD	Infiltration (maximum score 3) Mean±SD	Alveolar Thickening (maksimum score 3) Mean±SD	Fibrosis (maximum score 3) Mean±SD	Congestion (maximum score 3) Mean±SD
Control	1.6±1.737[a]	0,400± 0,516 [a]	0,900± 0,738 [a]	0,00±0,00[a]	0,300±0,483 [a]
AP	5.94±0.77[*c]	1.37±0.15[*c]	1.94±0.11*[c]	0.7±0.16[*c]	0.8±0.18*[c]
CAR 50mg/kg	1.269±0.419[a]	0.289±0.13[a]	0.72±0.047[a]	0.02±0.01[a]	0.21±0.212[a]
CAR 100mg/kg	1.444±1.4[a]	0.364±0.42[a]	0.80±0.548[a]	0.01±0.01[a]	0.27±0.412[a]
CAR 200mg/kg	1.338±0.682[a]	0.468±0.34[a]	0.64±0.154[a]	0.04±0.01[a]	0.18±0.168[a]
AP+CAR 50mg/kg	3.29±0.64[b]	0.92±0.10[b]	1.19±0.12[b]	0.21±0.11[b]	0.39±0.21[b]
AP+CAR 100mg/kg	1.57±0.81 [a]	0.13±0.04 [a]	1.02±0.13 [a]	0.05±0.14 [a]	0.33±0.37 [a]
AP+CAR 200mg/kg	2.43±0.22 [b,c]	1.05±0.08 [b,c]	0.36±0.02 [b,c]	0.38±0.02 [b,c]	0.03±0.08 [b,c]

Notes: Data are presented as means ± SD (n=7). *Symbol < 0,05 represents significant difference among the groups compared to controls. a, b, c The groups in the same column with different letters are statistically significant ($p<0.05$) by Tukey's multiple range tests. For abbreviations, see legend in Table I.

TABLE IV
EFFECT OF CAR TREATMENT ON LUNG 8-OH-DG LEVELS IN CERULEIN-INDUCED AP

Groups	8-OH-dG level (as pg/ml) Mean±SE
Control	1.06 ± 0.09[a]
AP	4.26 ± 0.21*[,d]
CAR 50 mg/kg	0.91 ± 0.09[a]
CAR 100 mg/kg	1.03 ± 0.17[a]
CAR 200 mg/kg	1.08 ± 0.11[a]
AP+CAR 50 mg/kg	2.73 ± 0.14[c]
AP+CAR 100 mg/kg	1.78 ± 0.13[b]
AP+CAR 200 mg/kg	1.14 ± 0.12[a]

Notes: Data are presented as means ± SD (n=7). *Symbol < 0,05 represents significant difference among the groups compared to controls. The groups in the same column with different letters are statistically significant ($p<0.05$). For abbreviations see legend in Table I.

In the present study, we observed for the first time that inhibition of oxidative stress by use of CAR in the lung of rat exposed to cerulein. As mentioned earlier, a cerulein-stimulated rat model has broadly been used as an experimental model of oxidative stress organ damage, therefore we employed an AP animal model instead of using human tissue in this study, as human tissue is extremely difficult to obtain. In this model, cerulein causes lung complications with extensive lipid peroxidation, producing some metabolites such as MDA. Hence cerulein can induce systemic diseases in organs such as the pancreas, heart, kidneys, liver, and lung as well [26], [27]. In our study, lipid peroxidation was elevated in the lung of rat after exposure to cerulein, as evidenced by increased MDA production. MDA is a highly biologically active oxidative degradation product from membrane unsaturated fatty acids. As such, MDA serves as a reliable biomarker of lipid peroxidation [28]. We also analyzed the correlation of oxidative stress with the pathological lung changes and the level of the antioxidant enzymes. Here in, we

demonstrated that administration of CAR reduced levels of MDA and the activity of antioxidant enzymes significantly increased in lung of animals exposed to cerulein. Essential oils rich in CAR possess strong antioxidant properties and also increase the activity of endogenous antioxidants [29], [30] Antioxidants are ubiquitously expressed in mammalian cells and activation of enzymes such as SOD, CAT and GSH-PX promote cell defense [31]-[34]. A recent study reports that CAR prevents lipid peroxidation, cell damage, and protects the antioxidant system in tissue that also may support the results of the present study [35].

We also diagnosed AP by histopathological examination. The lung specimens of AP groups showed histopathological alterations such as leukocyte infiltration desquamated epithelial cells in bronchial lumina, congestion, hemorrhage, protein leakage, marked alveolar thickening and increased fibrosis [36]. We observed all of these histopathological alterations in our experimental AP model. As discussed below, we demonstrate that pathological changes are significantly attenuated by administration of CAR.

The AP-induced inflammatory response often causes acute lung injury with a mortality rate of up to 40% [37]. Considered together, our findings add AP to the growing list of inflammatory diseases in which ROS inhibition appears to be of beneficial value. In the present study, we found that cerulein enhanced the number of extravascular leukocyte in the lung. Convincing data have documented a key role of leukocytes in the induction of AP. For example, depletion of leukocytes markedly decreases tissue damage in AP [38]. Considering the tight relationship between leukocyte recruitment and tissue damage in AP, it is suggested that this potent inhibitory effect on infiltration at least partly help explain the protective effect of CAR in the inflamed lung.

Our presented data demonstrate that AP enhances amylase and lipase concentration in blood and also leads to major cell degenerations in the lung epithelium. The pulmonary inflammation causes lung injury, which is often characterized by apoptosis of lung epithelial cells [39], [40]. Also, the acute lung injury occurs as a consequence of markedly increased endothelial and epithelial permeability, with protein deposits into the vessels and interstitial tissues, leading to decreased gas exchange. Hence AP is closely associated with acute lung injury [37], [41]. In our study, CAR effectively prevents the leakage of protein in rats with pancreatitis. It is reported that CAR protects the cells against H_2O_2-induced cytotoxicity and membrane damage [42]. On the other hand, AP causes acute thrombus formation in the vessels of rat lung as previously reported, and congestion in the vessels is the histopathological event that preceded thrombus formation. Besides, hemorrhage is observed in the interalveolar septa as extravasated erythrocytes accompanied with congestion [37], [43]. *In vitro* studies reveal that CAR possesses therapeutic characteristic, including antithrombotic property, in both lung and breast tissues [44]. Thus, CAR in the present study does not increase, but reduces the risk for congestion in animals with AP.

The excessive collagen production results in fibrosis and thickening of alveolar septa associated with poor elasticity and exchange of respiratory gases [45]. The development of therapeutic strategies compels a better understanding of the cellular and molecular mechanisms of fibrosis, which are diverse, complex, and redundant. Although there is growing evidence that oxidative stress is related to advanced lung fibrosis, the pathophysiological mechanisms still need to be defined [46]. In this context, we think that oxidative stress mechanism in AP, by directly inducing fibrotic processes, could possibly play a significant role in the eventual outcome. It has been documented that elevated oxidative stress and diminished antioxidant defense induced pulmonary fibrosis [47]. Experimental studies on CAR have confirmed its beneficial effects on cellular immunity to oxidative stress, reduction of fibrosis, suppression of inflammation, regulation of metabolism [48]. It has been shown that CAR regulates collagen expression in fibrosis and that CAR supplementation provides significant protection against oxidative stress-mediated complications and strengthens antioxidant defenses [27]], [49]. Therefore, the activation of CAR may become a therapeutic target to improve the clinical outcome of lung diseases.

Although the number of studies on genotoxic effects of CAR is still limited, the available data discussed that the use of CAR does not pose a risk to the consumer. Regarding the safety of CAR, our findings shown that 8-OH-guanine formations were not detected at all CAR concentrations tested. In previous studies, the genotoxic potential of CAR has been suggested to be weak in the DNA-repair test and the SOS-chromotest [50] and contradictory results have been reported with the Ames mutagenicity assay [51]. On the other hand, CAR seemed to protect against peroxide and mutagen-induced DNA damage in human lymphocytes [52], [53].

8-OH-guanine has been used widely for the detection of oxidative DNA base damage [54]. In our experimental study, AP induced 8-OH-guanine formations in pulmonary cells. However, CAR showed antioxidant activity and decreased 8-OH-guanine production. The effect of CAR was not concentration depended and the effects on different parameters significantly increased with 100 mg/kg concentration of CAR. The effects of higher concentration of CAR (200 mg/kg) on all measured parameters were lower than its two lower concentrations. Incubation of mammalian cells in the presence of the whole scale of concentrations of CAR led to a significant protection of the cells studied toward DNA strand breaks induced by a potent oxidant hydrogen peroxide [55]. A study indicated a lack of clastogenic activity for CAR at biologically relevant concentrations, and a moderate antioxidant activity *in vitro* [56]. However, if the use of CAR compounds is extended to other applications that may require higher doses, the increased exposure of humans to this compound is a matter of concern [57]. Hence the concentration dependent effects of CAR are discussed [45]. At this point, we think that comprehensive studies are needed before CAR is used for the clinical application.

To the best of our knowledge, this is the first study reporting that CAR is useful in preventing AP-induced lung injury by attenuating oxidative stress and inflammation. These

results are supported by biochemical and genotoxicity studies, and also histopathological observations. Our *in vivo* data show that 100 mg/kg CAR can be considered potential candidate for the treatment of lung in AP.

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A. Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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Defective Autophagy Leads to the Resistance to PP2 in ATG5 Knockout Cells Generated by CRISPR-Cas9 Endonuclease

Sung-Hee Hwang, Michael Lee

Abstract— Upregulated Src activity has been implicated in a variety of cancers. Thus, Src family tyrosine kinase (SFK) inhibitors are often effective cancer treatments. Here, we investigate the role of autophagy in ATG5 knockout cell lines generated by the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas mediated genome editing. The CRISPR-associated protein Cas9 is an RNA-guided DNA endonuclease that uses RNA–DNA complementarity to identify target sites for sequence specific double-stranded DNA (dsDNA) cleavage. Interestingly, ATG5 KO cells clearly showed a greater proliferation rate than WT NIH 3T3 cells, implying that autophagy induction is cytotoxic. Also, the clonogenic survival of ATG5 KO cells was greater than WT cells. The MTT assay revealed that the cytotoxic effect of PP2 was weaker on ATG5 knockout cells than that WT cells. The conversion of non-autophagic LC3-I to autophagic LC3-II and RT-PCR confirmed the functional gene knockout. Furthermore, Cyto-ID autophagy assay also revealed that PP2 failed to induce autophagy in ATG5 knockout cells. Together, our findings suggest that the resistance to PP2 in ATG5 knockout cells is associated with defective autophagy.

Keywords—ATG5 knockout, Autophagy, CRISPR/Cas9, PP2

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Role of Tyrosine-Phosphorylated STAT3 in Liver Regeneration: Survival, DNA Synthesis, Inflammatory Reaction and Liver Mass Recovery.

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Abstract— In liver regeneration, quiescent hepatocytes need to be primed to fully respond to growth factors such as hepatocyte growth factor. To understand the priming process, it is necessary to analyze patterns of gene expression that occur during liver regeneration after partial hepatectomy (PHx). Recently, tyrosine phosphorylation of signal transducer and activator of transcription 3 (pYSTAT3) has been shown to play an important role in initiating liver regeneration. In order to evaluate the role of pYSTAT3 on liver regeneration after PHx, we used an intrabody which can selectively inhibit pYSTAT3. In our previous studies, an intrabody had been shown that it bound specifically to the pYSTAT3. Adenovirus-mediated expression of the intrabody in HepG2 cells as well as mouse liver blocked both accumulation of pYSTAT3 in the nucleus and downstream target of pYSTAT3. In this study, PHx was performed on intrabody-expressing mice and the expression levels of liver regeneration-related genes were analyzed. We also measured liver/body weight ratios and the related cellular signaling pathways were analyzed. Acute phase response genes were reduced in an intrabody-expressing mice during liver regeneration than in control virus-injected mice. However, the time course of liver mass restoration in intrabody-expressing mice was similar to that observed in control virus-injected mice. We also observed that the expression levels of anti-apoptotic genes, such as Bcl2 and Bcl-xL were decreased in intrabody-expressing mice whereas the expression of cell cycle related genes such as cyclin D1, and c-myc was increased. Liver regeneration after PHx was partially impaired by the selective inhibition of pYSTAT3 with a phosphorylation site-specific intrabody and these results indicated that pYSTAT3 might have limited role in liver mass recovery.

Keywords—STAT3 , pYSTAT3 , Liver Regeneration, Intrabody

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Protective Role of Peroxiredoxin V Against Ischemia/Reperfusion-Induced Acute Kidney Injury in Mice.

Eun Gyeong Lee, Ji Young Park, Hyun Ae Woo

***Abstract*—**Reactive oxygen species (ROS) production is involved in ischemia/reperfusion (I/R) injury in kidney of mice. Oxidative stress develops from an imbalance between ROS production and reduced antioxidant defenses. Many enzymatic and nonenzymatic antioxidant systems including peroxiredoxins (Prxs) are present in kidney to maintain an appropriate level of ROS and prevent oxidative damage. Prxs are a family of peroxidases that reduce peroxides, with a conserved cysteine residue serving as the site of oxidation by peroxides.

In this study, we examined the protective role of Prx V against I/R-induced acute kidney injury (AKI) using Prx V wild type (WT) and knockout (KO) mice. We compared the response of Prx V WT and KO mice in mice model of I/R injury. Renal structure, functions, oxidative stress markers, protein levels of oxidative damage marker were worse in Prx V KO mice. Ablation of Prx V enhanced susceptibility to I/R-induced oxidative stress. Prx V KO mice were seen to have more severe renal damage than Prx V WT mice in mice model of I/R injury.

Our results demonstrate that Prx V is protective against I/R-induced AKI.

***Keywords*—**peroxiredoxin, ischemia/reperfusion, kidney, oxidative stress.

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DNpro: A Deep Learning Network Approach to Predicting Protein Stability Changes Induced by Single-Site Mutations

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Abstract—A single amino acid mutation can have a significant impact on the stability of protein structure. Thus, the prediction of protein stability change induced by single site mutations is critical and useful for studying protein function and structure. Here, we presented a new deep learning network with the dropout technique for predicting protein stability changes upon single amino acid substitution. While using only protein sequence as input, the overall prediction accuracy of the method on a standard benchmark is >85%, which is higher than existing sequence-based methods and is comparable to the methods that use not only protein sequence but also tertiary structure, pH value and temperature. The results demonstrate that deep learning is a promising technique for protein stability prediction. The good performance of this sequence-based method makes it a valuable tool for predicting the impact of mutations on most proteins whose experimental structures are not available. Both the downloadable software package and the user-friendly web server (DNpro) that implement the method for predicting protein stability changes induced by amino acid mutations are freely available for the community to use.

Keywords—Bioinformatics, deep learning, protein stability prediction, biological data mining.

INTRODUCTION

Single site amino acid mutations may have a significant impact on the stability of the structure of a protein. Since experimental determination of the stability change caused by mutations on proteins is time consuming and costly, computational prediction of the stability change induced by single-site mutations is useful for screening a large number of mutations for studying the structure and function of proteins. It can be used for protein engineering, protein design, mutagenesis analysis, and the study of the relationship between phenotypes and genotypes.

Recently, a variety of methods based on physical potentials [6, 7, 8, 9, 10], statistical potentials [11, 12, 13, 14, 15, 32, 36, 37], empirical potentials [16, 17, 18, 19, 20], machine learning [1, 2, 21, 25, 26] and combined approaches [31, 33, 34] have been developed to predict protein structure stability upon single-site amino acid mutation. The physical potential approaches, statistical potential approaches, and empirical potential approaches aim to approximate either the physical or pseudo-physical energy based on physical principles or their statistical approximation. Machine learning methods are data-driven knowledge-based methods that learn a function from the data to map the input information regarding a protein and its mutation to the energy change without the need of approximating the physics underlying mutation stability. This data-driven formulation of the problem makes it possible to apply a large array of machine learning methods to tackle the problem. Therefore, a wide range of machine learning methods has been applied to the same problem. E Capriotti et al., 2004 [2] presented a neural network for predicting protein thermodynamic stability with respect to native structure; J Cheng et al., 2006 [1] developed MUpro, a support vector machine with radial basis kernel to improve mutation stability prediction; iPTREE based on C4.5 decision tree revealed temperature is an important factor in mutation stability prediction [35]; R Casadio et al., 1995 [25] proved a radial basis function neural network can predict the free energy changes induced by mutations; M Masso et al., 2008 [31] combined random forest, regression tree and ada-boost on C4.5 decision tree with statistical potential approach to predict protein stability change. This array of machine learning tools with reasonable prediction accuracy are widely used by the community, which demonstrates the effectiveness of machine learning methods for protein stability prediction.

In addition to exploring various methods to further improve the accuracy of mutation stability prediction, reducing the amount of information required to make accurate prediction is also important for making the prediction methods generally applicable to any proteins that may not have the information required by existing methods. Many previous machine learning methods [2, 21, 25, 26, 31, 35] need sequence, solvent accessibility, pH value, temperature or tertiary structure information to make good predictions, which limits their use only to a small portion of proteins whose such information are available. J Cheng et al., 2006 [1] introduced a method encoding only sequence and mutation residue information, whose accuracy is comparable to existing methods. Following this direction, we design a new method based on cutting-edge deep learning networks to further improve protein stability changes upon single-site mutations using only protein sequence information.

Deep learning [22, 23, 24, 28] is the latest development in the field of machine learning, which has shown good performance in many complex machine learning tasks such as image processing, speech recognition, and protein structure prediction. Like traditional neural networks, deep learning networks [22, 23, 24, 28] consists of multi-layer of nodes and the nodes between adjacent layers are fully connected. However, different from discriminative back-propagation supervised learning in neural networks, the weights between

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adjacent layers of deep learning networks can be learned mostly by un-supervised stochastic gradient descent or divergence-convergence algorithms in order to maximize the likelihood of input data. Therefore, deep learning networks can be trained on the almost unlimited amount of unlabeled data even though the labeled data can still be used to tune the weights of deep learning networks via back-propagation when available. This fundamental new training scheme helps avoid the vanishing gradient problem in traditional back-propagation and enables deep learning to use a large number of layers of nodes (i.e. deep networks) for complex learning tasks in contrast to typical two-layer or three-layer architecture used by traditional neural networks. And the deep architecture often makes deep learning capable of gradually transforming low-level input features into higher-level concepts from layer to layer, which often leads to better prediction performance.

As for this protein stability prediction task, a special kind of deep learning architecture - Deep Belief Network (DBN) [5] is developed, which stacks a number of layers of Restricted Boltzmann Machines [5] to predict protein stability changes upon mutations. A logistic regression [29] is added on the top layer of DBN, which allows the weights in the DNB that have been pre-trained by the unsupervised convergence-divergence algorithm to be fine-tuned by the supervised back-propagation method [22, 23]. The Dropout technique [3, 4, 27] is also applied in DBN to make the hidden layers more diverse to avoid overfitting. The deep learning model is tested in a 20-fold cross-validation on a standard protein stability benchmark. The results demonstrate this new deep learning method outperforms some widely used methods in protein stability prediction on a standard benchmark.

METHOD

A. Protein Stability Dataset

We use the S1615 dataset [1,2] in this work, which is widely used in the field of protein stability prediction [1, 2, 31, 35]. The mutations in the dataset were originally extracted from ProTherm [30] mutation energy database. The dataset has 1615 mutations obtained from 42 different proteins. PDB code of the mutated protein, secondary structure, solvent accessibility, original and mutated residues, temperature, pH and energy change are available in the dataset.

The energy change associated with each mutation in the dataset represents the change of protein stability caused by a single-site mutation. A positive energy change indicates a protein is more stable upon a mutation while a negative energy change indicates a decrease in structural stability. In a binary classification case, mutations with positive energy changes are labeled positively as well as all the others are labeled negatively. Dataset S1615 thus has 1168 negative data points and 447 positive examples, i.e. about 28% mutations increase the stability of the proteins.

Besides S1615, a redundancy-reduced subset of S1615 dataset that contains 388 unique mutations is also used to cross validate our model and optimize parameters. S388 has 340 negative examples and 48 positive ones. We carry out a 20-fold cross validation as in the previous work [1, 2, 31] to determine the good parameter values for the deep learning network. To avoid overfitting the S388 dataset during training, an independent dataset S65 is selected randomly from the S1615 dataset excluding the mutations in S388 dataset in order to blindly test the deep learning model trained on the S388 dataset. S65 has 52 negative examples and 13 positive examples. S65 is not used in any way until the final parameters and architecture of the deep learning model are determined by 20-fold cross validation on the S388 dataset

B. Encoding Scheme of Input Features

In order to make our method applicable to proteins without known tertiary structures, we only use the features that can be derived from protein sequence alone as input. Following the same feature extraction approach in [1], a window centered on the mutation site is used to capture the information regarding the mutation and its adjacent sequence environment. Each position in the window except the mutation site is represented by 20 binary numbers that represent 20 possible amino acids at the position. Only the number representing the amino acid at the position is set to 1, while all others are set to 0. For the mutation site in the window, the number representing the original residue is set to -1 and the number denoting the substitute residue is set to 1, while all other 18 numbers are set to 0. The size of the window is fixed at 7 because the previous work [1] demonstrated that the size yielded the best performance. As a result, a vector of 140 input features is generated for each mutation, which captures the information of the mutated residue and its three residue neighbors on each side.

After the encoding, the datasets consisting of both input features and binary class labels (positive or negative) are used to train and test our deep learning method - the Deep Belief Network (DBN) [22, 23] – for classifying mutations via 20-fold cross-validation and independent testing.

C. Restricted Boltzmann Machine (RBM) and Deep Belief Network (DBN) for Protein Stability Prediction

Figure 1. A Restricted Boltzmann Machine (RBM) model is comprised of a visible layer of nodes, a hidden layer of nodes, and the weighted connections between the nodes in two layers. The strength of each connection is quantified by an adjustable weight associated with it. As a simple example, in this model, there are 3 hidden units in the hidden layer and 4 visible units in the visible layer.

A Deep Belief Network (DBN) stacks several layers of Restricted Boltzmann Machines (RBMs) (**Figure 1**) [22] together to map input to output step by step. An RBM model

is composed of one visible layer of nodes representing input features and one hidden layer of nodes representing hidden / latent variables. The nodes in the two layers are fully connected and the strength of connection between two nodes (v_i in the visible layer and h_j in the hidden layer) is measured by weights (ω_{ij}) of the connection between them. The probability of activating a visible or hidden unit is defined as:

$$p(v_i = 1|h) = \sigma\left(a_i + \sum_j h_j\, \omega_{ij}\right) \quad (1)$$

$$p\left(h_j = 1\middle|v\right) = \sigma\left(b_j + \sum_i v_i\, \omega_{ij}\right) \quad (2)$$

Here, v_i denotes the i^{th} unit in the visible layer and h_j the j^{th} unit in hidden layer. ω_{ij} is the weight of the connection between them , a_i and b_j are bias for the two nodes respectively. $\sigma(x)$ is a sigmoid function as follows:

$$\sigma(x) = 1/\left(1 + e^{(-x)}\right) \quad (3)$$

The energy of a joint configuration of all the nodes of an RBM is given by:

$$E(v,h) = -\sum_{i\in v} a_i\, v_i - \sum_{j\in h} b_j\, h_j - \sum_{i\in v, j\in h} v_i\, h_j \omega_{ij} \quad (4)$$

Thus, the probability of a joint configuration of the visible and hidden nodes is defined as:

$$p(v,h) = e^{-E(v,h)}/Z \quad (5)$$

Here Z is the normalization constant – the sum of the probabilities of all joint configurations:

$$Z = \sum_{v,h} e^{-E(v,h)} \quad (6)$$

The objective of training an RBM is to adjust its weights such that the probability of the visible layer $p(v)$ (i.e. the likelihood of the data) is maximized. $p(v)$ is simply calculated as the sum of the probabilities of all possible joint configurations over all hidden units:

$$p(v) = \frac{1}{Z}\sum_h e^{-E(v,h)} \quad (7)$$

One of the most popular un-supervised methods to train RBMs is Contrastive Divergence (CD) [5] algorithm that adjusts weight ω_{ij} by the difference between the current setting ($v_i h_j^{(t)}$) at time t and the next setting ($v_i h_j^{(t+1)}$) at time t+1.

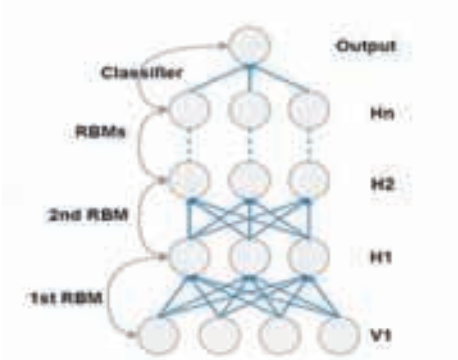

Figure 2. A Deep Belief Network (DBN) consisting of multiple RBMs with a logistic regression classifier on top of it. The output of each lower layer is used as the input for the next layer.

The same unsupervised CD algorithm can be used to pre-train all the layers of a DBN consisting of multiple RBNs step by step (see **Figure 2** for a DBN example). It iteratively trains every two adjacent layers as an RBM from bottom up while using the output of the lower layer as the visible input of the immediate upper layer. The input of the first layer is set as the original input data. Before pre-training, the weights and bias are initialized either randomly or to 0.

The unsupervised learning described above can map input features into high-level hidden features represented by the nodes in the higher layer. In order to make a DBN to classify data into different categories, a classifier needs to be added at the top of a DBN (**Figure 2**). In this work, a logistic regression is added at the top of DBN because it is easy to train. The weights between the logistic regression node and the hidden layers can be trained by the standard back-propagation algorithm - gradient descent with respect to the difference between the output of the DBN and the real labels of the data (i.e. classification error). Furthermore, the classification error is back propagated from the logistic regression node all the way down to the visible layer in order to fine tune all weights in the DBN in a supervised fashion. Although this hybrid training protocol of combining both unsupervised and supervised learning above works well in most situations, it still may be susceptible to the classic problem of overfitting data in machine learning. To reduce overfitting, we apply the dropout [3] training technique to train the deep learning network (**Figure 3**). During training, dropout randomly sets some units to 0 to temporarily drop them out of the deep network. When a unit is dropped out, all the connections associated with the unit are removed. Therefore, in each iteration of training, there is a different combination of active units. In addition to reducing overfitting, dropout also helps prevent units from co-adapting.

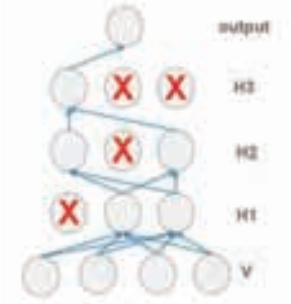

Figure 3. The dropout training protocol. Dropout stochastically sets some nodes (units) to 0 to disable them at a constant [3] or adaptive [4] rate in each epoch of training and fine-tuning. When a unit is dropped out, all the weights related to it will not be updated in this epoch.

The standard dropout approach sets the dropout rate to 0.5, which has been proved optimal for a wide range of networks and tasks. In this work, the dropout rate is applied to all layers except the input layer. In addition to the standard dropout approach, we test the adaptive dropout method. Instead of using a constant 0.5 dropout rate, the adaptive dropout (also called standout) regularizes its hidden units by selectively setting activities to 0 according to an adaptive rate determined by two user-defined parameters: α and β [4].

RESULTS

A. Parameter Estimation and Model Selection

A deep learning model has a number of parameters to determine, such as the number of hidden layers, the number of hidden nodes in each hidden layer, learning rates, and dropout rates. Choosing an appropriate deep learning model for a specific task is vital. Here, two datasets S388 and S65 are used for selecting models. We conduct a 20-fold cross-validation on the S388 dataset to test different sets of parameter values. The parameters that work well in the cross-validation are used to train deep learning models on whole S388 dataset, which are then tested on the independent test dataset S65.

Table 1. Eight sets of good parameters tested on S388 dataset according to 20-fold cross-validation. The first column lists the index of each parameter set, columns 2-6 the parameters (the number of hidden units in each hidden layer, learning rate in pre-training phase, learning rate in fine-tuning phase, dropout protocol, parameters of dropout protocol), and the last column the overall classification accuracy – the percent of correctly classified mutations among all the mutations.

Index of Parameter Set	# of Hidden Units in each Layer Separated by ","	Pre-Training Learning Rate	Fine-Tuning Learning Rate	Dropout Protocol	Dropout Parameters	Classification Accuracy
1	100,60,40	0.1	1.5	Standard	Rate = 0.5	0.8513
2	40,20	0.1	1.5	Standard	Rate = 0.5	0.8631
3	40,20	0.1	15	Standard	Rate = 0.5	0.8631
4	20,12	0.1	1.5	Standard	Rate = 0.6	0.8553
5	60,40,20	0.1	15	*Standout*	$\alpha = 1, \beta = 5$	0.8500
6	40,20,16	0.3	1.5	*Standout*	$\alpha = 2, \beta = 3$	0.8500
7	40,20	0.1	1.5	*Standout*	$\alpha = 1, \beta = 5$	0.8500
8	20,16	0.1	0.5	*Standout*	$\alpha = 2, \beta = 3$	0.8579

Table 2. The accuracy of five selected deep learning models on S65 dataset. These models are trained on S388 dataset. The index of each model corresponds to its index in Table 1, where the parameters of the model can be found.

Model Index	Model Name	Accuracy	Precision (P)	Precision (N)	Specificity	Sensitivity	Correlation Coefficient
1	DBN-1	0.877	0.857	0.879	0.981	0.462	0.571
2	DBN-2	0.831	0.600	0.873	0.923	0.462	0.426
3	DBN-3	0.846	0.800	0.850	0.981	0.308	0.433
4	DBN-4	0.846	0.615	0.903	0.903	0.612	0.519
8	DBN-8	0.862	0.750	0.877	0.962	0.462	0.515

We investigated on how different values of several key parameters (i.e. the number of hidden layers, number of hidden units in each layer, pre-train learning rate, fine-tune learning rate, and standard or standout dropout parameters) influence the performance of the deep learning models. The results of eight sets of good parameter values tested on S388 via 20-fold cross-validation are listed in **Table 1**. When different numbers of hidden layers are tested, 2 or 3 hidden layers work best. More than 3 layers of networks produce more complicated models with the similar accuracy. So we chose to use 2 or 3 layers in order to get simpler models. Among different numbers of hidden units tested, 12 to 100 hidden units work reasonably well. The fine-tuning learning rate may vary in a wide range and most of them achieve good performance. When training a Deep Belief Network without the dropout technique, the model is extremely biased toward the predominant negative class due to the highly imbalanced distribution of the two classes in the dataset. When dropout is applied to our method, the model begins to perform in a much more balanced way as reported in [3]. Both standard dropout [3, 27] and adaptive dropout [4] are used in our model. The dropout rates of 0.5 and 0.6 for the standard dropout protocol work similarly well. In the standout dropout scheme, the highest accuracy of 85.79% is achieved with alpha=2, and beta=3, which is similar to the performance of the standard dropout protocol. As shown in **Table 1**, most of models using the standard dropout have slightly better performance than standout dropout ones.

After the cross-validation on S388, top 5 models (model indices: 1, 2, 3, 4, and 8) are selected to train 5 models (DBN-1, DBN-2, DBN-3, DBN-4, DBN8) on the whole S388 dataset. The performance of these five models on the independent test dataset S65 is reported in **Table 2**. In order to assess the results, a confusion matrix is calculated for each experiment, which contains numbers of true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN). Based on the confusion matrix, the overall accuracy (the percent of correctly classified mutations), precision on positive examples, precision on negative examples, specificity, sensitivity, and correlation coefficient are calculated as follows to gauge the prediction performance of the methods:

$$Accuracy = \frac{TP + TN}{TP + FP + FN + TN}$$

$$Precision(P) = \frac{TP}{TP + FP}$$

$$Precision(N) = \frac{TN}{FN + TN}$$

$$Specificity = \frac{TN}{FP + TN}$$

$$Sensitivity = \frac{TP}{TP + FN}$$

$$Corr.Coef. = \frac{TP \times TN - FP \times FN}{\sqrt{[(TP + FN) \times (TP + FP) \times (TN + FN) \times (TN + FP)]}} \quad (8)$$

All of the 5 models have similar accuracy (i.e. percent of correctly classified mutations) on the test dataset that is comparable to the cross-validation accuracy on S388 dataset. This demonstrates that the models do not overfit the training set S388. The specificities are usually higher than the sensitivities because the dataset has many more negatives than positives. The DBN-4 model has the highest sensitivity among the 5 models. DBN-1 and DB-3 have the best specificity as well as the high precision on positive examples. So we choose the parameters of DBN-1, DBN-3 and DBN-4, respectively, to test their performance on the entire S1165 dataset through cross-validation.

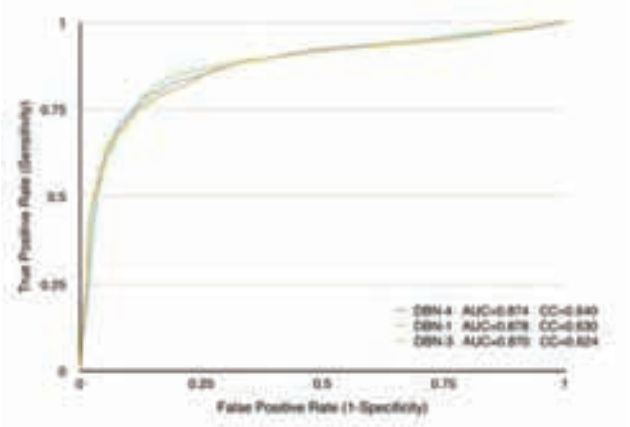

Figure 4. The ROC curves of three DBN models cross-validated on S1615. The AUC values and correlation coefficients are reported in the legend.

B. Results on the S1615 Dataset

To further investigate the performance of DBN models, the parameters of DBN-1, DBN-3, and DBN-4 are tested on the S1615 data using 20-fold cross-validation. The ROC curves of the three models are plotted and the Area Under the Curve (AUC) is calculated (**Figure 4**). The ROC curve is the receiver operating characteristic curve, which shows the relationship between true positive rate (sensitivity) and false positive rate (1 - specificity). This graphical plot can effectively illustrate a binary classifier's performance when the threshold on the false positive rate is varied.

As shown in **Figure 4**, the three ROC curves are very close and their AUCs are almost the same. DBN-1 has the best ROC curve with AUC value of 0.878, while DBN-4 has the highest correlation coefficient of 0.640. The results show that the DBN models are stable and robust.

C. Comparison with Other Mutation Stability Prediction Methods

Table 3 reports the performance of our three DBN models (DBN-1, DBN-3, and DBN-4) on the S1615 dataset in comparison with other four popular mutation stability predictors: AdaBoost/C4.5, iPTREE, NeuralNet and MUpro. Among these methods, AdaBoost/4.5, iPTREE, and NeuralNet use both sequence and structural information as input, while the three DBN models and MUpro only use sequence information as input.

Among all of the methods, AdaBoost/C4.5 has the highest overall accuracy (~87%). It applies AdaBoost algorithm on C4.5 decision tree and uses both sequence and structure information as input. The overall accuracy (~86%) of each DBN method that uses only sequence information as input is only slightly (~1%) lower than AdaBoost/4.5, and it is about 2% higher than MUpro that uses sequence information only. In particular, DBN's precision on the positive examples is around 80%, which is higher than all other methods. This makes the DBN methods very useful for identifying rare mutations that increase the stability of proteins in the real-world situation. Therefore, the DBN's good performance makes it generally applicable to the vast majority of proteins without known experimental structures.

Table 3. Results of three deep earning models (DBN-1, DBN-3 and DBN-4) on S1615 dataset in comparison with AdaBoost/C4.5, iPTREE, NeuralNet and MUpro.

Method	Input Information	Accuracy	Precision(P)	Precision(N)	Specificity	Sensitivity	Correlation Coefficient
AdaBoost/C4.5	Sequence & Structure	0.872	0.796	0.898	0.929	0.733	0.670
Random Forest	Sequence & Structure	0.862	0.771	0.894	0.919	0.713	0.650
iPTREE	Sequence & Structu	0.871	0.836	0.881	0.949	0.668	0.670

	re						
NeuralNet	Sequence & Structure	0.810	0.710	0.830	0.910	0.520	0.490
MUpro	Sequence only	0.841	0.693	0.888	0.897	0.711	0.590
DBN-3	Sequence only	0.862	0.824	0.871	0.953	0.608	0.624
DBN-1	Sequence only	0.856	0.782	0.880	0.927	0.673	0.630
DBN-4	Sequence only	0.861	0.808	0.877	0.940	0.660	0.640

D. Protein Stability Prediction Tool and Web Server

We construct a user-friendly web server (DNpro) available at [38] for users to use our deep learning methods for protein stability prediction. Users can enter a protein sequence, mutated position, and mutated residue at the web page and then click "submit" button to get prediction results. The standalone DNpro package implemented in Java can also be downloaded at the same web site. The DNpro tool has a pre-trained model that can be used to predict protein stability change upon mutation. It also has training and cross-validation function that allows users to train and test a deep learning model on any input dataset.

CONCLUSION AND FUTURE WORK

In this work, we present a deep learning method to predict the protein stability change induced by single amino acid mutations. On a standard benchmark dataset, the deep learning method that uses protein sequence as only input performs better than another popular method that uses sequence information only and similarly to the most accurate methods that use both sequence and structural information. The experiment demonstrates that deep learning is a promising approach to the protein mutation stability prediction problem and the sequence-based deep learning method can be widely useful for studying the mutations of the vast majority of proteins without known tertiary structures. In the future, we plan to test different classifiers (e.g. support vector machines) other than logistic regression on top of the deep learning networks to see if the prediction accuracy can be further improved. A larger training dataset and additional input features may be used with deep learning to improve the prediction accuracy too.

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Development of PCL/Chitosan Core-Shell Electrospun Structures

Hilal T. Sasmazel, Seda Surucu

***Abstract*—**Skin tissue engineering is a promising field for the treatment of skin defects using scaffolds. This approach involves the use of living cells and biomaterials to restore, maintain, or regenerate tissues and organs in the body by providing; (i) larger surface area for cell attachment, (ii) proper porosity for cell colonization and cell to cell interaction, and (iii) 3-dimensionality at macroscopic scale. Recent studies on this area mainly focus on fabrication of scaffolds that can closely mimic the natural extracellular matrix (ECM) for creation of tissue specific niche-like environment at the subcellular scale. Scaffolds designed as ECM-like architectures incorporating into the host with minimal scarring/pain and facilitate angiogenesis.

This study is related to combining of synthetic PCL and natural chitosan polymers to form 3D PCL/Chitosan core-shell structures for skin tissue engineering applications. Amongst the polymers used in tissue engineering, natural polymer chitosan and synthetic polymer poly(ε-caprolactone) (PCL) are widely preferred in the literature. Chitosan has been among researchers for a very long time because of its superior biocompatibility and structural resemblance to the glycosaminoglycan of bone tissue. However, the low mechanical flexibility and limited biodegradability properties reveals the necessity of using this polymer in a composite structure. On the other hand, PCL is a versatile polymer due to its low melting point (60°C), ease of processability, degradability with non-enzymatic processes (hydrolysis) and good mechanical properties. Nevertheless, there are also several disadvantages of PCL such as its hydrophobic structure, limited bio-interaction and susceptibility to bacterial biodegradation. Therefore, it became crucial to use both of these polymers together as a hybrid material in order to overcome the disadvantages of both polymers and combine advantages of those.

The scaffolds here were fabricated by using electrospinning technique and the characterizations of the samples were done by contact angle (CA) measurements, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and X-Ray Photoelectron spectroscopy (XPS). Additionally, gas permeability test, mechanical test, thickness measurement and PBS absorption and shrinkage tests were performed for all type of scaffolds (PCL, chitosan and PCL/chitosan core-shell). By using ImageJ launcher software program (USA) from SEM photographs the average inter-fiber diameter values were calculated as 0.717±0.198 µm for PCL, 0.660±0.070 µm for chitosan and 0.412±0.339 µm for PCL/chitosan core-shell structures. Additionally, the average inter-fiber pore size values exhibited decrease of 66.91% and 61.90% for the PCL and chitosan structures respectively, compare to PCL/chitosan core-shell structures. TEM images proved that homogenous and continuous bead free core-shell fibers were obtained. XPS analysis of the PCL/chitosan core-shell structures exhibited the characteristic peaks of PCL and chitosan polymers. Measured average gas permeability value of produced PCL/chitosan core-shell structure was determined 2315±3.4 $g.m^{-2}.day^{-1}$.

In the future, cell-material interactions of those developed PCL/chitosan core-shell structures will be carried out with L929 ATCC CCL-1 mouse fibroblast cell line. Standard MTT assay and microscopic imaging methods will be used for the investigation of the cell attachment, proliferation and growth capacities of the developed materials.

***Keywords*—**Chitosan, coaxial electrospinning, core-shell, PCL, tissue scaffold

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Cold Plasma Surface Modified Electrospun Microtube Array Membrane for Chitosan Immobilization and Their Properties

Ko-Shao Chen, Yun Tsao, Chia-Hsuan Tsen, Chien-Chung Chen

***Abstract*—**Electrospun microtube array membranes (MTAMs) made of PLLA (poly-L-lactic acid) have wide potential applications in tissue engineering. However, their surface hydrophobicity and poor biocompatability have limited their further usage. In this study, the surface of PLLA MTAMs were made hydrophilic by introducing extra functional groups, such as peroxide, via an acetic acid plasma (AAP). UV-graft polymerization of acrylic acid (G-AAc) was then used to produce carboxyl group on MTAMs surface, which bonded covalently with chitosan through EDC / NHS crosslinking agents. To evaluate the effects of the surface modification on PLLA MTAMs, water contact angle (WCA) measurement and cell compatibility tests were carried out. We found that AAP treated electrospun PLLA MTAMs grafted with AAc and, finally, with chitosan immobilized via crosslinking agent, exhibited improved hydrophilic and cell compatibility.

***Keywords*—**Plasma, EDC/NHS, UV grafting, chitosan, microtube array membrane.

I. Introduction

ELECTROSPINNING is a straightforward method of producing ultrafine fibers from micro- to nano-meter range diameters and with controlled surface morphology. Electrospun fibrous substrates were often used in drug delivery, artificial dressings, and cell enzyme immobilization. MTAMs made of PLLA hollow fibers have possessed wide potential applications in tissue engineering [1]-[3]. However, their hydrophobicity and poor biocompatibility have limited such usages [4].

Chitosan is a non-toxic and biodegradable material, which exhibits good antibacterial activity and cell adhesiveness, and has been widely utilized to prepare microspheres for anticancer drug delivery system [5].

Surface modification by plasma deposition and graft polymerization is often used to solve the adhesion problem between substrate surfaces and coatings. Plasma deposition can provide free radicals and peroxide groups on the substrate surfaces [6]. Plasma-deposited surface film has many advantages such as good adhesion to substrates, excellent uniformity, easy for thickness control, low porosity, and pin hole-free formation [7]. Graft polymerization on the plasma-treated surface further adds radicals to the surface for enhancing the chemical property of the surface [8]-[11].

PLLA MTAMs surface was modified by an AAP to introduce functional groups and subsequently treated by UV-graft polymerization of AAc to facilitate the immobilization of chitosan. This study employed a plasma deposition and a graft technique to modify the surface of PLLA MTAMs to facilitate the immobilization of chitosan. an oxygen plasma treatment on the PLLA MTAMs is expected to introduce peroxide groups on the substrate surface. Subsequent UV grafting with AAc on the plasma-treated PLLA MTAMs was applied to polymerize carboxylic groups on the surface. EDC/NHS crosslinking agents were used to combine the functional groups on the grafted PLLA MTAMs surface with chitosan. biocompatability of electrospun PLLA MTAMs was evaluated by MTT assay using NIH3T3 cells.

II. Experimental

A. Materials

Porous MTAMs were fabricated by co-axial electrospinning (Fig. 1) [1]-[3]. The chemicals used in this study includes AAc and ammonium peroxodisulfate (APS) from Wako Co., Japan.

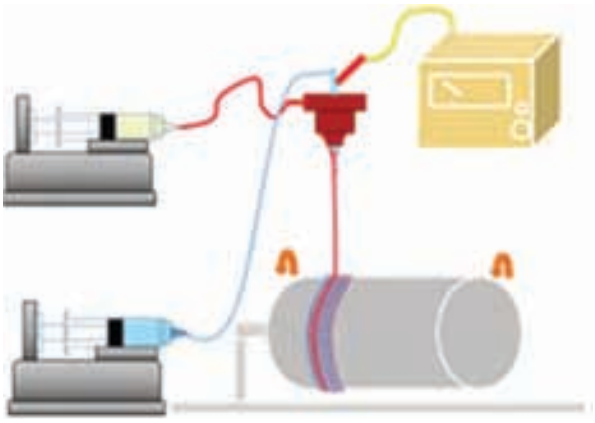

Fig. 1 Fabrication of porous PLLA MTAMs by co-axial electrospinning

B. Processes for Immobilizing Chitosan on PLLA MTAMs

The reaction steps for the immobilization of chitosan on PLLA MTAMs by plasma and grafting treatments are shown schematically in Fig. 2.

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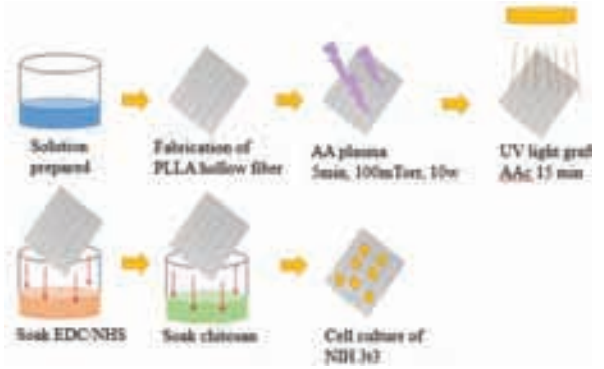


Fig. 2 Schematic reaction steps for the immobilization of chitosan on MTAMs by plasma and grafting treatments are shown

PLLA MTAMs were subjected to an AAP pretreatment (Fig. 3). The cleaned PLLA MTAMs were placed on the lower electrode of the reaction chamber before vacuumed. The reaction chamber was firstly evacuated to less than 30 mTorr. After the pressure was stable, acetic acid monomer was introduced into the reaction chamber and maintained at a constant pressure by adjusting the micro-throttle value. After the pressure has become stable, the plasma glow discharge treatment was employed. Substrate temperature was kept at room temperature during the treatment. The treatment was performed under the following conditions: AAP pretreatment at 100 mTorr, 10 W, and 5 min.

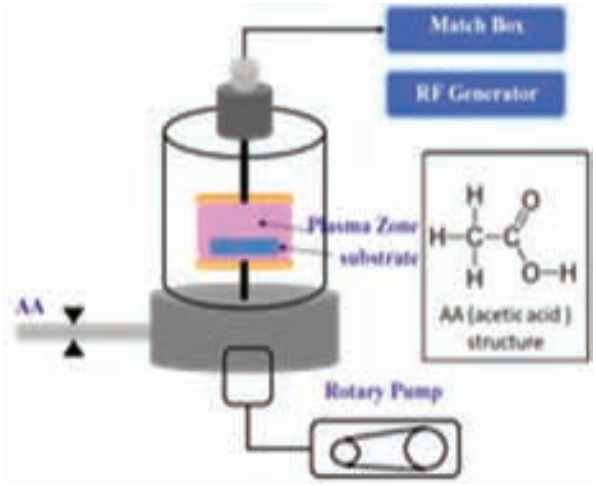


Fig. 3 Schematic diagram of AAP reactor system

Subsequently, the plasma-modified PLLA MTAMs were grafted by UV-radiation using a 10 mmol AAc solution containing 0.1 mmol APS under high-pressure mercury lamp at 1000 W for 20 min. The grafting solution of AAc was composed by: AAc 1.5ml, water 13.5ml, Vitamin B2 4ml, APS 0.0212g.

Finally, the AAc-grafted PLLA MTAMs were cross-linked with EDC/NHS to immobilize chitosan by mixing 1 wt% glutaraldehyde and 1 wt% chitosan with MTAMs at room temperature for 1 day. EDC: 5mg/ml, NHS: 1.25mg/ml, Chitosan: 2N.

Bio-compatibility: MTT assay with NIH3T3 cells. Cells were cultured on the surface of the different steps of preparation, quantitative to observe the situation NIH3T3 mouse fibroblasts grown on the surface of the material after 1,3,5, day by (MTT assay) method as mentioned previously [8], [9].

III. Results and Discussion

A. Wettability of PLLA MTAMs

Surface wettability of the modified substrate can be measured by the WCA.

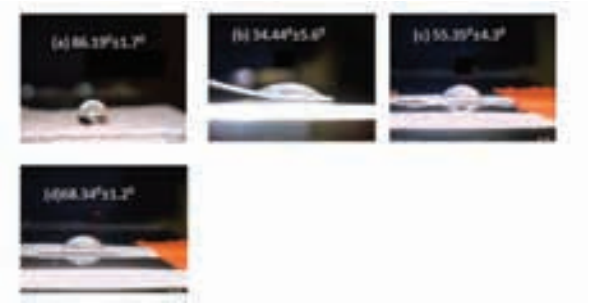


Fig. 4 WCAs of MTAMs (a) untreated, (b) P.D. acetic acid(c) S-Graft AAc (5%), and (d) chitosan Immobilized

Fig. 4 shows the WCA results of plasma treated, grafted acrylic polymer and immobilized chitosan. The WCA decreased from 59.2°±1.7° (untreated) to 34.4°±5.6° (AAP treated), then rose to 55.4°±4.3° (grafted with AAc polymer), and eventually became more hydrophobic (68.3°±1.2°) after chitosan immobilization.

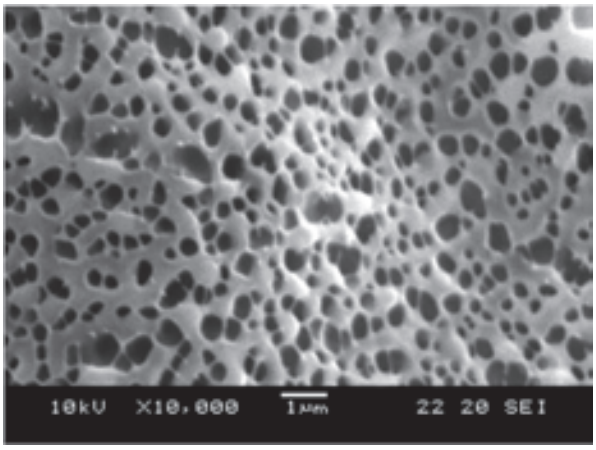
TABLE I
Hydrophobicity (WCA, Degree) of Surface Treated Electrospun PLLA MTAMs Substrates.

	Plasma	EDC/NHS + Chitosan
Untreated	86.0	75.0
PAA	48.0	46.0
PAA+G-AAc	40.0	38.0

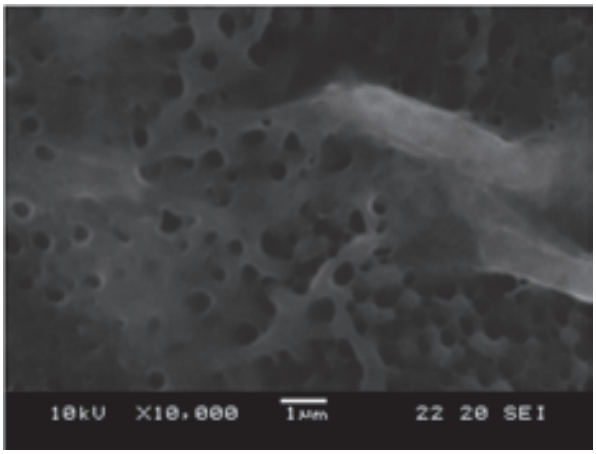
It can be observed that PLLA MTAMs have become more hydrophilic after plasma and grafting treatments however showed slightly hydrophobic than untreated PLLA MTAMs after immobilization of chitosan through EDC/NHS crosslinking agents.

B. Surface Morphology of Modified MTAMs

Surface morphology of PLLA MTAMs by scanning electron microscope (SEM) is showed in Figs. 5 and 6. A relatively thick layer of polypropylene acid and chitosan was observed.



(a)



(b)

Fig. 5 SEM of PLLA MTAMs (a) untreated, and (b) PAA+ EDC/NHS+chitosan (X10000)

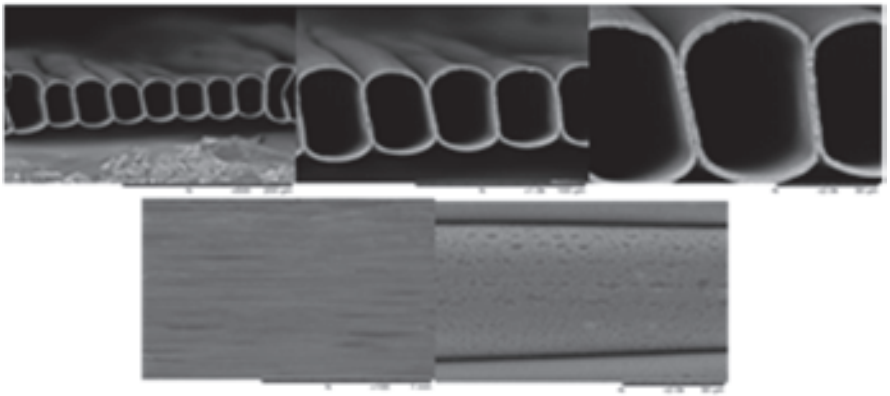


Fig. 6 Scanning electron microscopy images (surface and cross section) of electrospun PLLA MTAMs

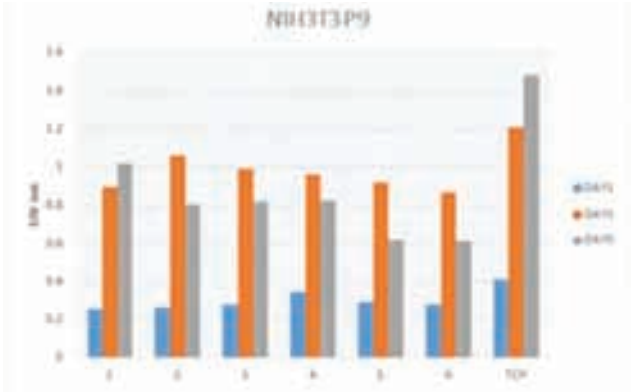


Fig. 7 Biocompatability (MTT assay) of electrospun PLLA MTAMs with different surface treatment parameters: (1) AAP+G-AAc+ EDC/NHS+chitosan, (2) AAP+G-AAc, (3) AAP+ EDC/NHS+chitosan, (4) AA, (5) untreated+ EDC/NHS+chitosan, and (6) untreated

C. MTT Assay

Fig. 7 shows the MTT assay results of electrospun PLLA MTAMs with different surface treatment parameters. The growth of NIH3T3 cells at the first to the third day is almost the same in different electrospinning process. However, cell growth on chitosan immobilized surface was observed increased. Surface immobilized with chitosan (AAP + G-AAc + EDC/NHS + chitosan) possessed improved biocompatibility, comparing with those of control and other modification parameters.

IV. Conclusions

The results from weight changes and MTT assay reveal that AAP treated electrospun PLLA MTAMs grafted with AAc and, finally, with chitosan immoblized via crosslinking agents showed improved hydrophilicity and biocompatibility, suggesting an effective and potential process to modify PLLA MTAMs for tissue engineering applications.

Acknowledgments

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Micro-Arc Oxidation Titanium and Post Treatment by Cold Plasma and Graft Polymerization of Acrylic Acid for Biomedical Application

Shu-Chuan Liao, Chia-Ti Chang, Ko-Shao Chen

***Abstract*—**Titanium and its alloy are widely used in many fields such as dentistry or orthopaedics. Dues to their high strength low elastic modulus that chemical inertness and bio inert. The micro-arc oxidation used to formation a micro porous ceramic oxide layer film on Titanium surface and also to improve the resistance corrosion. For improving the biocompatibility, micro-arc oxidation surfaces bio-inert need to introduce reactive group. We introduced boundary layer by used plasma enhanced chemical vapor deposition of hexamethyldisilazane (HMDS) and organic active layer by UV light graft reactive monomer acrylic acid (AAc) therefore we can immobilize Chondroitin sulphate on surface easily by crosslinking EDC/NHS.

The surface properties and composition of the modified layer were measured by scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD) and water contact angle. Water contact angle of the plasma-treated Ti surface decreases from 60° to 38°, which is an indication of hydrophilicity. The results of electrochemical polarization analysis showed that the sample plasma treated at micro-arc oxidation after plasma treatment has the best corrosion resistance. The result showed that we can immobilize chondroitin sulfate successful by a series of modification and MTT assay indicated the biocompatibility has been improved in this study.

***Keywords*—**MAO, Plasma, Graft Polymerization, biomedical application.

I. Introduction

Titanium alloys are widely used in the fields of dentistry or orthopedics implant material due to its good chemical stability, mechanical properties such as high specific strength ,wear resistance, corrosion resistance etc.. However, one of disadvantages is bio-inertness [1].

This material cannot form a chemical bond with living bone tissue and present poor ability to facilitate new tissue formation on the surfaces, which would delay the healing time or lead to the failure of implantation [2]. To enhance the biocompatibility of implant surface, various surface modification techniques such as electron beam-physical vapor deposition, plasma spray, plasma enhance chemical vapor deposition, magnetron sputtering and ion implantation have been developed [3]. Compared with other treatment processes, plasma deposit film has many advantages, including easy to prepared, good adhesion and uniformity and it will not restrict by surface chemistry and topography of modified material but can improve the surface properties like the wettability, roughness or electricity. Plasma modification can produce some free radicals and peroxide groups on surface to activate the surface of metal even to graft organic polymer or biomolecules [4].

Recently, micro-arc oxidation (MAO) process also call plasma electrolytic oxidation (PEO), has attracted an attention because of its easy to coat oxide ceramic layer on surface of Ti, Al and alloy with complex-shaped. it also can convenience to control the electrolyte composition, electrolyte concentration or adjust the processing condition to enhance surface properties of metal such as corrosion resistance, bioactivity and adhesion strength with the substrate [5-6].

In order to enhance the biocompatibility, corrosion resistance and surface properties, oxide ceramic layer be coated on Ti surface by micro-arc oxidation and modified the surface by plasma enhanced chemical vapor deposition of hexamethyldisilazane (HMDSZ) and oxygen to activate the surface to grafted acrylic acid (AAc) on Ti surface by UV-induced. Then we use EDC/NHS to immobilized chondroitin sulfate. The surface properties of titanium alloys are analyzed by water contact angle, SEM, XRD, ESCA, corrosion resistance and MTT assay. The experimental flow chart for this study is shown in Fig. 1.

II. Experiment details

The Substrates of 99.6% pure titanium was produced from Chun Kwang Chemical Industry Co., Ltd. (Taiwan), cutting the substrate to the size of 10 mm x 10 mm 2 mm in thickness, composition analyses were list in Table 1. Coatings were fabricated by use of a micro-arc oxidation technique. A DC power supply was used in MAO. The substrates soaked in an electrolytic solution and kept temperature below 25°C by water-cooled bath. MAO processes were carried out under the conditions selected in the range 250–440 V. The electrolyte was prepared from a solution of Sodium Metsilicate Penthydrate ($Na_2SiO_3 \cdot 5H_2O$) in distilled water with other additives.

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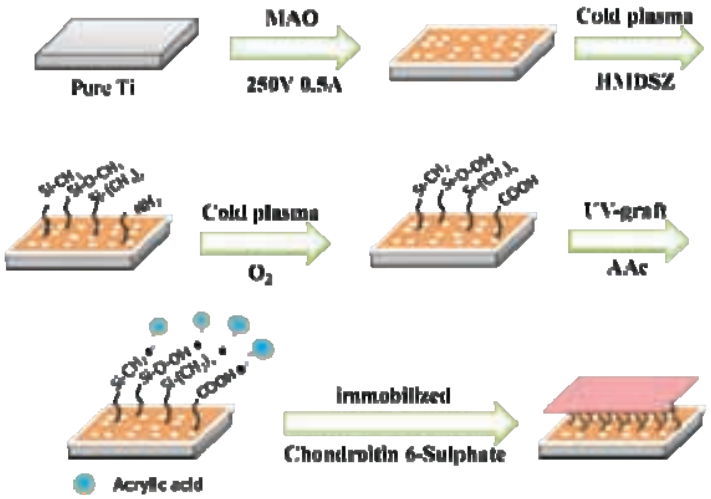

Fig. 1 Flow chart of experiment

Table 1
the concentrations of titanium steel (Wt. %)

Element	Ti	Al	V	Cr	Fe	Mn
Wt. %	99.6	0.01	0.0288	0.0115	0.084	0.0049

Post treatment by cold plasma placed titanium sheets on the site of reaction chamber, and then adjusting micro-throttle valve to introduce HMDSZ monomer after chamber was re-evacuated to a pressure less than 30 mTorr. When pressure was stable, plasma glow discharge treatment was employed. HMDSZ plasma treatment was under the conditions: Ratio frequency of 13.6 MHz was used in this study as shown in Fig. 2., input power 30 Watts, the amount of HMDSZ was 100 mTorr and treatment time 5 min. HMDSZ treated substrate was putted into the oxygen reactor, pumped to a pressure less than 30 mTorr before plasma treatment. After pressure was stable, inducing oxygen gas into chamber, and then employed oxygen plasma treatment. Oxygen plasma treatment was under the conditions: input power 100 Watts, the amount of oxygen was 40 mTorr and treatment time 1 min.

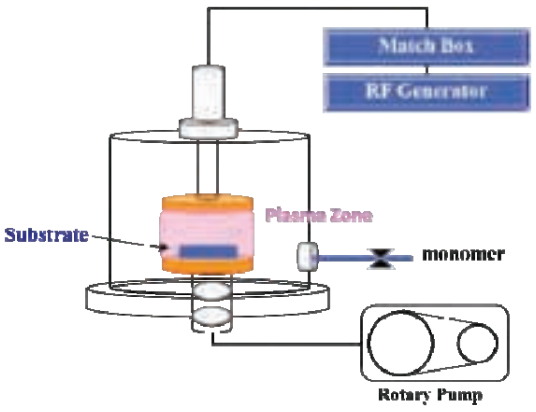

Fig. 2 Schematic diagram of plasma treatment system

After plasma treated, titanium sheets surface contained several peroxide group, they could further graft polymerization under UV light induced (Power: 1000 W; wavelength: 365 nm, Henchman Co. Inc., Taiwan) as shown in Fig. 3., After graft polymerization reaction, the grafted specimens were washed with distilled water for 24 hour to remove homopolymer and residues, and dried at room temperature. The concentration of acrylic acid solution was 10 wt. % and grafting time was fixed at 10 minutes. The substrates after UV-light induced graft polymerization with acrylic polymer was immersed in a solution which was used EDC/ NHS coupling that can bind covalently with 1 wt. % chondroitin sulphate, and kept at 4°C for 24 hour, and then distilled water was used to remove unreacted chondroitin sulphate and dried at room temperature.

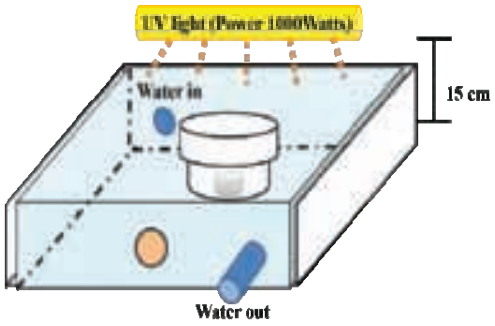

Fig. 3 Schematic diagram of UV-induced graft polymerization system

Water contact angle measurement instrument was used to measure the surface contact angle. Each sample data were measured three times to take the average. Surface morphologies of substrates were observed by scanning electron microscope (HITCHI S-3400N). The electron spectroscopy (ESCA, ESCA PHI 1600) was used to characterize chemical composition of surface. The X-ray diffraction measurement (PANalytical-X'Pert PRO MPD) is taken to analyze crystal phase of ceramic oxide layer after micro-arc oxidation and corrosion resistance was tested by electrochemical analyzer (EG&G Model 236). Before the cell culture, the substrate was sterilized by UV-light for 24 hour. Dental pulp stem cells were cultured in well plate with medium. Cells were seeded at a

density of 3×10^4 cells/well, incubated in a humidified atmosphere with 5% CO_2 at 37 °C. After cultured for 3 days, MTT were added in well and placed for 2~4 hour, and then used 50μL DMSO (100%) to mix medium. The OD value was obtained by ELISA, spectra range is 570~650 nm.

III. Results and Discussion

Phase analysis

X-ray diffraction (XRD) of treatment under micro-arc oxidation treated for 5 min. It can be seen that the oxide coating is rutile phase and have no anatase phase are presented in Fig. 4, which are different from other study [1~2].

Corrosion resistance property

Using 3.5wt% NaCl solution as vehicle to carry out the polarization curves tests as shown in Fig.5. From the results, it can be known corrosion potential distribute between -0.17 ~-0.35V, the corrosion current distribute between $3.09E^{-8}$ ~ $6.32E^{-9}$ (Amps/cm^2). To compare to each step of modified, we can find micro-arc oxidation and MAO/p-HMDSZ /p-O_2 have the better corrosion resistance.

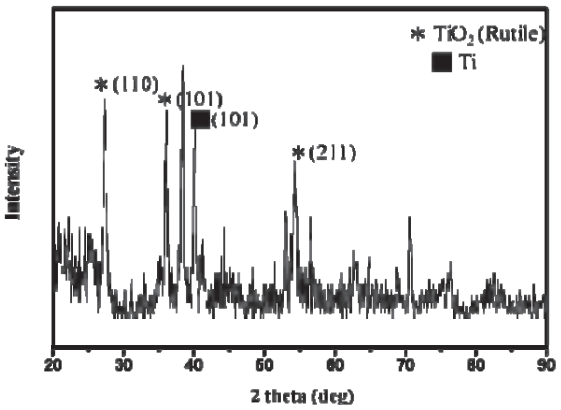

Fig. 4 XRD patterns of micro-arc oxidation Ti less than 5 min

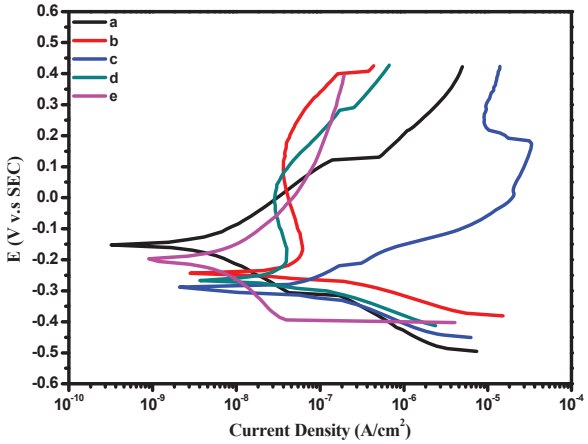

Fig.5 Corrosion characteristics of (a) untreated(b) MAO (c) MAO/p-HMDSZ(d) MAO/p-HMDSZ/p-O_2(e) MAO/p-HMDSZ/p-O_2/UV-AAc

The wettability of modified surface

In table2, the water contact angle of titanium sheets with different treatment. The water contact angle of untreated titanium is 63.4°. After micro-arc oxidation treated, water contact angle of titanium sheets surface decrease less than 10°. The surface of titanium sheets become hydrophobic after plasma deposited HMDSZ film. Use oxygen plasma to treat substrate, the water contact angle is 18.2° because of the treatment of oxygen plasma create a hydrophilic functional group on titanium sheets surface. After substrates grafted AAc and immobilized chondroitin sulphate, water contact angle is 71.2° and 38.8°.

Chemical composition analysis

Infrared spectroscopy was used to characterize the chemical structure of the modified films by the reflection mode [7].In table 3, the element composition of sample with untreated, micro-arc oxidation treated, deposited HMDSZ film, oxygen plasma modified, grafted polymerization of AAc and immobilized chondroitin sulphate, respectively. Table 3a showed is untreated Ti the surface also having other element including 45% O1s and 37% C1s, it may be an oxide on surface. Table 3b is micro-arc oxidation treatment on the Ti surface. Because oxide layer form on surface, O1s content increase to 54%. After deposited HMDSZ film, surface contains 54% C 1s, 11% O 1s and 27% Si 2p was showed in table 3c. The sources of high carbon content are methyl from HMDSZ chemical structure. Table 3d is the surface activation by oxygen plasma treatment, the O 1s content was observed significantly increased from 11% to 41%. Table 3e is surface after grafted AAc polymer. AAc is carbon hydroxide, thus the result of analysis also indicate the surface contain C and O elements. After immobilized chondroitin sulphate on the surface, it can be found 0.39% S 2p, this results show success immobilized chondroitin sulphate on the surface.

Table 2
Wettability of step modified Ti

Modified	Water contact angle(degree)
Untreatment	63.4±1.2
MAO	< 10
MAO/p-HMDSZ	110±11.6
MAO/p-HMDSZp-O_2	18.2±0.8
MAO/p-HMDSZp-O_2/UV-AAc	71.2±1.3
MAO/p-HMDSZp-O_2/UV-AAc/immobilized-CS	38.8±1.3

Table 3
ESCA analysis of modified Ti

Sample	Chemical composition (%)							
	C1s	N1s	O1s	Na1s	Si2p	K2p	Ti2p	S2p
a	37.40	1.87	45.74	-	6.46	-	8.54	-
b	10.13	0.36	54.57	1.27	31.60	1.50	0.58	-
c	54.03	6.49	11.69	-	27.79	-	-	-
d	34.00	-	41.72	0.85	23.43	-	-	-
e	22.95	-	49.23	0.18	26.47	-	1.17	-
f	68.86	1.00	25.20	0.82	3.73	-	-	0.39

Surface morphology

Fig. 6 is SEM micrographs of titanium under different treatment. Fig. 6 (a) shows the untreated surface under SEM. It can be seen that scratches exist on untreated titanium sheets. Fig. 6 (b) shows the morphologies of surface coating at micro-arc oxidation treating for 5 min. Oxide ceramic layer of TiO2 is coated on titanium sheet surface by micro-arc oxidation and spots can be seen on the surface of titanium sheet. After plasma treatment, it can be seen that the thin film be deposited on surface that show in Fig. 6 (c) and Fig. 6(d). Fig. 6 (e) is the morphologies of surface after grafted AAc polymer; it indicated that AAc polymer is network structure without water. Fig. 6(f) can be found that surface is covered by chondroitin sulphate, obviously.

In vitro Cytotoxicity Measurements

In vitro test employed a colorimetric cell-viability (MTT) assay, as shown in Fig. 7. Form MTT assay of dental pulp stem cells after culturing for 3 days on untreated titanium and sample with different treatment. It can be seen the cell viability increase from 14.3% to 26.3% after micro-arc oxidation and plasma treatment. However, it decrease to 17.2% after grafted AAc polymer on titanium surface, it indicate that AAc polymer presented electronegativity on surface and reject cell. After immobilized chondroitin sulphate, the cell viability become 50.3%, it is significantly better than pervious treatment. Therefore, the biocompatibility of titanium can be improved by immobilized chondroitin sulphate surely.

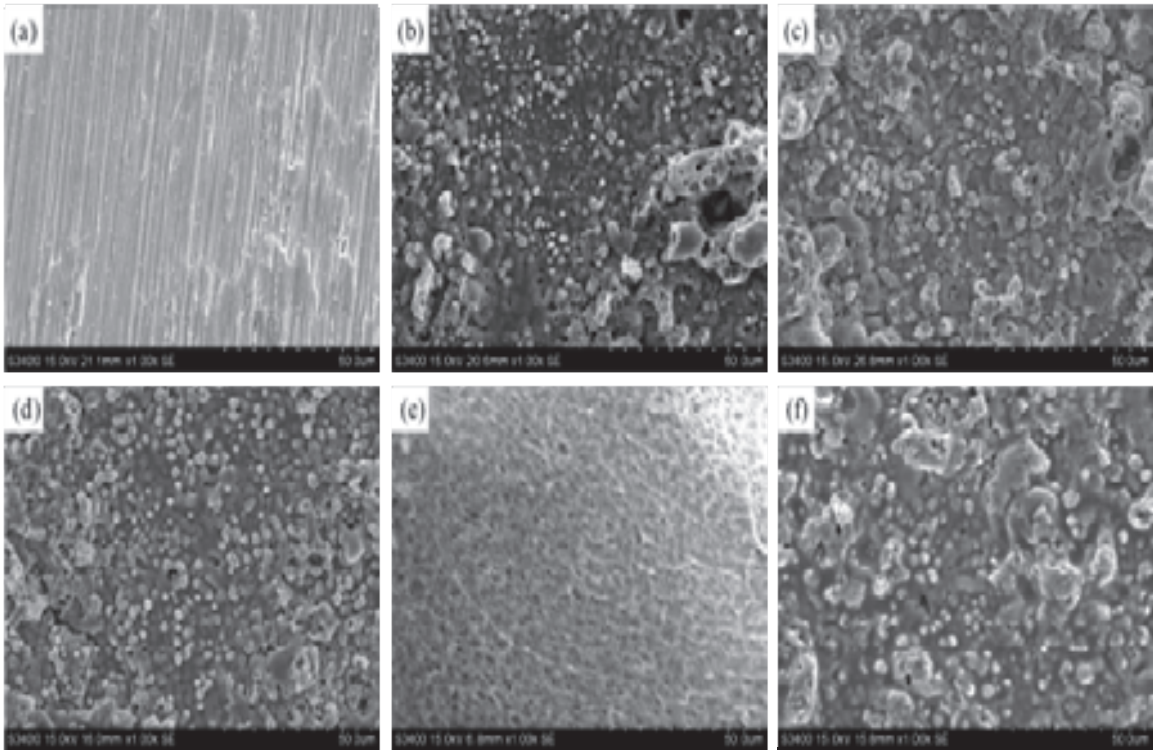

Fig. 6 SEM of Ti surface (a) untreated (b) MAO (c)MAO/p-HMDSZ (d) MAO/p-HMDSZ/p-O_2 (e) MAO/p-HMDSZ/p-O_2/UV-AAc (f) MAO/p-HMDSZ/p-O_2/UV- AAc/immobilized CS

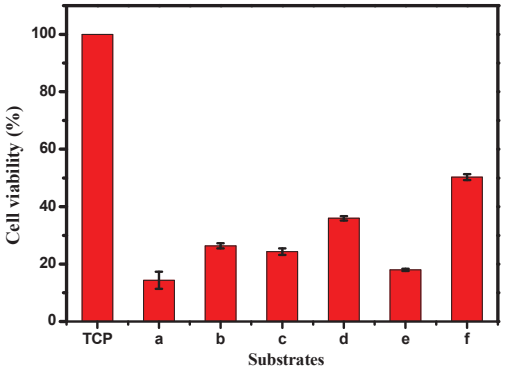

Fig. 7 MTT assay of (a) untreated (b) MAO (c)MAO/p-HMDSZ (d) MAO/p-HMDSZ/p-O2 (e)MAO/p-HMDSZ/p-O2/UV-AAc (f) MAO/p-HMDSZ/ p-O2/UV-AAc/immobilized CS

IV. Conclusion

In this study, the wettability results indicate the substrate following of Ti substrate transformed the hydrophilicity from 63.4° to 0.0°, 110.0° and 71.2° after micro-arc oxidation, plasma treatment and grafted polymerization of AAc, respectively. From the ESCA analysis confirm the success of immobilization of chondroitin sulfate on Ti substrates surely. Corrosion characteristics indicated that the corrosion resistance of Ti surface can be improved by micro-arc oxidation and plasma modification. The chondroitin sulfate was successfully immobilized on Ti substrate surface by plasma modification and UV graft polymerization, furthermore, the MTT assay results shown that the cell viability was improved efficiently from 14.3% to 50.3% by surface modification.

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Auxin and Siderophore Fast Detection by Microplate Technique

Anguiano Cabello Julia Cecilia, Flores Olivas Alberto, Aguilar González Cristóbal Noé, Arredondo Valdés Roberto, Laredo Alcalá Elan Iñaky

Abstract—Plant-growth promotion is able by exogenous addition of compounds as auxins, siderophore, inorganic acids and antibiotics, produced by plant-growth-promoting rhizobacterias (PGPR) as some Bacillus species. Those bacterias have to be isolated from soil and then evaluated for metabolites growth promoter production. Some techniques have been developed for metabolite quantification or detection, especially chromatographic techniques and colorimetric methods. For auxin quantification or detection the salkowski reagent technique is the most used, and for siderophores detection the CAS media. Even though, those techniques are expensive or have to be evaluated sample by sample, and it makes the process slow. For these reason, the aim of the present research was developed a fast microplate technique for auxin and siderophore detection, using salkowski reagent for auxin and CAS media reagents for siderophore, using different solvents and incubation time. Finally, were evaluated by those microplate technique the auxin and siderophores production in Bacillus subtilis filtrates.

Keywords—auxin, siderophore, microplate, bacillus

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Flood Risk Assessment and Adapted to the Climate Change by a Trade-Off Process in Land Use Planning

Nien-Ming Hong, Kuei-Fang Huang

Abstract—Climate change is an important issue in future, which for a long term planning and management in water resource. Flood assessment is highly related with climate and land use. For adapting the impacts of climate change, a land use planning is a good strategy for flood reduce. The study is to build a trade-off process with different land use types. The Ta-Liao watershed is the study area with three types of land use that are build-up, farm and forest. The build-up area is concentrated in the downstream of the watershed. Different rainfall amounts are applied for assessing the land use in 1996, 2005 and 2013. The adapted strategy is based on retarding the development of urban and a trade-off process. When a land use change is from farm areas to built-up areas in downstream, searching for a farm area change to built-up area or building a retention areas in the upstream. For assessing the effects of the strategy, the inundation area are simulated by the Flo-2D model with different rainfall conditions. The results show that inundation map of several cases of land use change planning. The results also show the trade-off strategy can decrease the inundation area and divide the concentrated inundation area, which are better than retarding urban development. The land use change usually is non-reverse, the planning should be done before the climate change.

Keywords—climate change, land use change, Flood risk assessment, land use planning

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Quantification of Enzymatic Activities of Proteins, Peroxidase and Phenylalanine Ammonia Lyase, in Growing Phaseolus vulgaris L, with Application Bacterial Consortium to Control Fusarium and Rhizoctonia

Arredondo Valdés Roberto, Hernández Castillo Francisco Daniel, Laredo Alcalá Elan Iñaky, Gonzalez Gallegos Esmeralda, Castro Del Angel Epifanio

Abstract—The common bean or Phaseolus vulgaris L. is the most important food legume for direct consumption in the world. Fusarium dry rot in the major fungus disease affects Phaseolus vulgaris L, after planting. In another hand, Rhizoctonia can be found on all underground parts of the plant and various times during the growing season. In recent years, the world has conducted studies about the use of natural products as substitutes for herbicides and pesticides, because of possible ecological and economic benefits. Plants respond to fungal invasion by activating defense responses associated with accumulation of several enzymes and inhibitors, which prevent pathogen infection. This study focused on the role of proteins, peroxidase (POD), phenylalanine ammonia lyase (PAL), in imparting resistance to soft rot pathogens by applied different bacterial consortium, formulated and provided by Biofertilizantes de Méxicanos industries, analyzing the enzyme activity at different times of application (6 h, 12 h and 24 h). The resistance of these treatments was correlated with high POD and PAL enzyme activity as well as increased concentrations of proteins. These findings show that PAL, POD and synthesis of proteins play a role in imparting resistance to Phaseolus vulgaris L. soft rot infection by Fusarium and Rhizoctonia.

Keywords—fusarium, peroxidase, phenylalanine ammonia lyase, rhizoctonia

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附錄二、電子海報資料

The Taiwan Environmental Impact Assessment Act Contributes to the Water Resources Saving

Reporter： Feng-Ming Fan

Bureau of Environmental Inspection,
Environmental Protection Administration, Taiwan
May 26, 2016

Outline

Introduction

Methodologies

Major findings

Conclusions

Introduction

Reservoir in Taiwan Face Water Scarcity Crisis

Introduction

Water resources scarcity in Taiwan become a major concern

Face water scarcity crisis

- The limits of climate and geographic condition
- Lack of effective and mandatory regulation
- Lack of effective inspection methods

Break through plight

- **Legislation** By EIA Act regulation
- **Innovation** Research effective inspection methods
- **Vision** Water resources sustainability

First, the effective and suitable methods were developed by Taiwan EPA, such as calculation formulas and factors. We expect to enhance water resources management by inspection which based on the suitable methods .

Methodologies

By EIA Act regulation

Environmental promise

Ex. For high-tech industries

- **Process water recovery rate would be reach 85%.**
- **Total water recovery rate would be reach 75%.**

Legislation ?
How to do ?
Methods and Formulas ?

Methodologies-Environmental promise

We have accomplished the inspection of 65 high-tech companies that daily water consumption is over 1,000 tons individually located at 3 science parks in Taiwan last year.

Enterprises		Environmental Promise	
		Process water recovery rate	Total water rate
Hsinchu Science Park	Hsinchu zone 1	After 1999：85%	—
		Between 1994～1999：70%	
		Before 1994：50%	
	Hsinchu zone 2	85%	—
	Hsinchu zone 3	85%	60%
	Hsinchu zone 4	85%	75%
Taichung Science Park	Taichung zone1	Semiconductor Industries：85%	—
		Semiconductor Industries：80%	
	Taichung zone2	Semiconductor Industries：85%	—
		Photoelectron Industries：85%	
	Taichung zone3	Total water rate：85%	
Tainan Science Park	Tainan zone1	Semiconductor Industries：85%	75%
		Photoelectron Industries：85%	75%
	Tainan zone2	85%	75%

Methodologies

finalized the manual

Professional training

Invited leading experts and some related officials to discuss the project

EIA Inspection Plan

Action

Training before action

Inspection process

8

Inspection process



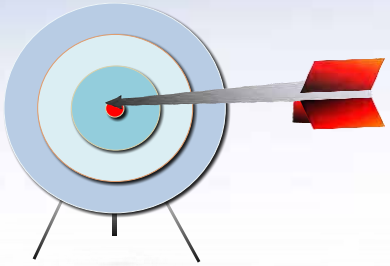





Inspection process

Major findings

3 science parks, including 65 enterprises were inspected.
As a result, all of the enterprises met the request of their environmental promise.

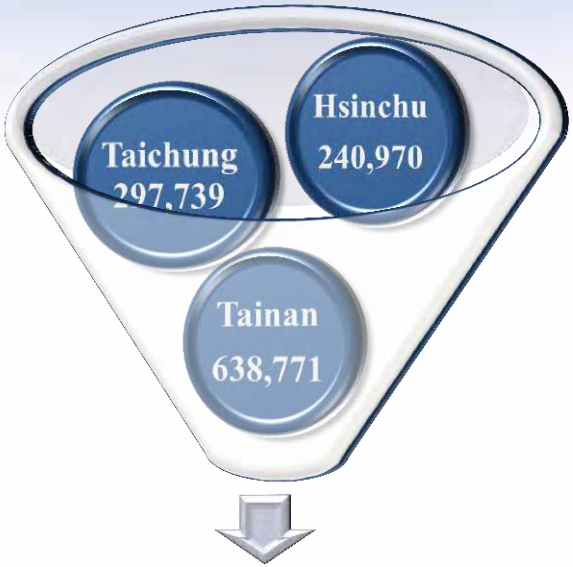


Great achievement on water recycling was achieved at an amount of 429 million tons per year.

Environmental promise

An amount of 395 million tons water has to reuse per year at least.

Major findings



Great achievement on water recycling

Bottles of 600 ml cola

International standard swimming pools of 2,500 tons

Some 1,000 tons irrigation water for per hectares of rice fields

Daily water consumption for 1 person is about 250 liters

The reservoir storage of Taipei Feitsui Reservoir is about 250 million tons

Conclusions

THANK YOU FOR YOUR ATTENTION

Bureau of Environmental Inspection,
Environmental Protection Administration, Taiwan